Nanotechnology in Dental Implantology

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Materials Horizons: From Nature to Nanomaterials

Sabu Thomas
R. M. Baiju *Editors*

Nanomaterials in Dental Medicine

Springer

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Sabu Thomas · R. M. Baiju
Editors

Nanomaterials in Dental Medicine

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To the students of dentistry, medicine and technology

Preface

The Covid-19 pandemic has been an eye opener for mankind in more ways than one. It made humans believe that health is the most important of all. Health care is a very rapidly expanding field with rigorous research happening across the world. Emerging evidence reconfirms the fact that oral health is an integral part of general health. Nanotechnology as a branch of science is advancing in a tremendous pace and it has made its mark in daily human life through industrial medicinal and energy applications. Nanomedicine has become an established interdisciplinary branch with a plethora of applications ranging from disease prevention to effective management of killer diseases including cancer. Due to the ever-growing importance of the potential applications of nanotechnology in dentistry, a rapidly growing branch of dentistry—nanodentistry—is emerging. It is poised to become the future of dentistry, thanks to the huge demand for newer and better solutions to many dental health issues that affect humans. The application of nanostructures and nanoparticles in dentistry ranges from providing better solutions for daily oral hygiene care to advanced regenerative medicine to restore lost tissues or parts due to disease. The book has been conceived and written to include all aspects of the scope of nanostructured materials for dental applications ranging from diagnostics to therapeutics. It discusses the emerging area of dental medicine through genomics, proteomics and microbial robots. The key clinical applications including nanostructured composites and other restorative materials, glass fibre splints, nanorobots for dental biofilm removal and sustained local drug delivery are discussed at length. There are thirteen chapters each focusing on the core research areas based on current understanding and evidence. Chapter contributions are from key opinion leaders and international experts in the field of nanostructured materials as well as from eminent dental researchers making this book a true interdisciplinary collaboration.

The topics are chosen based on the existing research evidence, current clinical applications and future needs. The first chapter is an overview of the emergence of the field of nanomedicine and dentistry. The important landmark events and contributions by the pioneers in the field are discussed in an interesting readable manner. The second chapter highlights the applications of nanotechnology in the diagnosis of

various oral and dental diseases and conditions. The third chapter is about the potential role of nanotechnology-based solutions in dental disease prevention. One of the most promising applications of nanomaterials is in the development of nanocomposite dental restorative materials and Chap. 4 reviews the existing literature in this regard. Sustained targeted release of drugs in itself is a huge area in therapeutics and its role in the treatment of several oral conditions including periodontal disease is widely studied. Chapter 5 reviews the current role of nanomaterials in the field of controlled drug delivery. Chapter 6 analyses the potential of nanotechnology-based solutions in pain management including anaesthesia. Nanorobotics has received wide attention in health care and dentistry is no exception. Chapter 7 explores the current as well as the future scope of nanorobotics in dentistry. Regenerative medicine is a rapidly growing field. Chapter 8 discusses the recent developments in the field of regenerative medicine with special emphasis on dental medicine. One of the fast-growing branches of the dental industry is dental implantology and nanotechnology has revolutionised the development of improved dental implant inserts. Chapter 9 is about this most exciting branch of dentistry. Oral cancer is a killer disease and more people die due to oral cancer than cervical cancer or skin cancer. Chapter 10 tries to review the therapeutic potential of nanotechnology in the treatment of oral cancer. Chapter 11 analyses the opportunities, challenges and risks of this rapidly emerging interdisciplinary field of science. Chapter 12 is dedicated to bioceramic inserts, and the role of nanotechnology in the development of such inserts is described in depth. The final chapter throws some light on the potential hazards of this highly promising branch of medicine and dentistry.

This book is envisaged as a primary reference source for dental graduates, post-graduates, research scholars, industrial engineers and technicians working in the field of dental materials, materials science, polymer chemistry and nanoscience and nanotechnology. We have taken sincere efforts to review and compile all relevant information based on currently available evidence. We wish that this book will be extremely useful for students, researchers and industrialists working in the area of dental science and technology.

Kottayam, India

Sabu Thomas
R. M. Baiju

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We express our sincere thanks to our colleagues in the International and Inter University centre of nanosciences, Mahatma Gandhi university and the Department of Periodontics, Government Dental College, Kottayam. The research scholars and postgraduate students of both centres have put in their valuable time and effort to the successful completion of this book.

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Sabu Thomas
R. M. Baiju

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About the Editors

Prof. Sabu Thomas He is currently the Vice Chancellor of Mahatma Gandhi University. He is also a full time professor of Polymer Science and Engineering at the School of Chemical Sciences of Mahatma Gandhi University, Kottayam, Kerala, India and the Founder Director and Professor of the International and Interuniversity Centre for Nanoscience and Nanotechnology. Prof. Thomas is an outstanding leader with sustained international acclaims for his work in Nanoscience, Polymer Science and Engineering, Polymer Nanocomposites, Elastomers, Polymer Blends, Interpenetrating Polymer Networks, Polymer Membranes, Green Composites and Nanocomposites, Nanomedicine and Green Nanotechnology. Dr. Thomas's groundbreaking inventions in polymer nanocomposites, polymer blends, green bio nano technological and nano-biomedical sciences, have made transformative differences in the development of new materials for automotive, space, housing and biomedical fields. In collaboration with Apollo Tyres, Professor Thomas's group invented new high performance barrier rubber nano composite membranes for inner tubes and inner liners for tyres. Professor Thomas has received several international awards including: Fellowship of the Royal Society of Chemistry, London FRSC, Distinguished Professorship from Jozef Stefan Institute, Slovenia, MRSI medal, Nano Tech Medal, CRSI medal, Distinguished Faculty Award, Dr. APJ Abdul Kalam Award for Scientific Excellence—2016, Mahatma Gandhi University- Award for Outstanding Contribution—Nov. 2016, Lifetime Achievement Award of the Malaysian Polymer Group, Indian Nano Biologists award 2017. He is in the list of most productive researchers in India and holds a position of No.5. Because of the outstanding contributions to the field of Nanoscience and Polymer Science and Engineering, Prof. Thomas has been conferred HonorisCausa (D.Sc) Doctorate by the University of South Brittany, Lorient, France and University of Lorraine, Nancy, France. Recently he received the National Education Leadership Award—2017 for Excellence in Education. Professor Thomas has published over 1400 peer reviewed research papers, reviews and book chapters. He has co-edited 137 books published by Royal Society, Wiley, Wood head,

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Prof. R. M. Baiju He is currently working as Professor of Periodontics, Government Dental College. He is also the post graduate Guide since 2011. He passed BDS with first rank from the Tamilnadu Dr. MGR Medical University and MDS in Periodontics from University of Kerala. He received Post Graduate Diploma in Clinical Research (PGDCR) from Annamalai University and Ph.D. in Dentistry (University of Kerala). He was awarded an international Fellowship in Dentistry by the prestigious Royal College of Physicians and Surgeons of Glasgow (FDS RCPS Glasg) in March 2021. Since 2020 he is an approved Ph.D. Guide of the Kerala University of Health Scineces (KUHS). He is a hugely popular teacher with more than 21 years of teaching experience. He has supervised more than 14 post graduate dissertations. He has contributed two chapters to text books in periodontology. Prof. Baiju has delivered over 150 Key note and Invited lectures in national/international meetings. He is an Associate member of the British Society of Periodontology and Implant Dentistry (BSP) since 2019. He is the Editor in Chief of Journal of Clinical Dentistry and former editorial board member of Health Sciences, official e journal of KUHS. Prof. Baiju was a member of Board of studies (UG) of University of Kerala. He recieved several awards and recogntitions—Excellence in Dentistry award for contribution to the field of peridontics and Interdisciplinary dentistry by International Association of Interdesciplinary Dentistry (AIOI)—SPIK President's appreciation award for in 2011–2012, 2012–2013 and in 2017–2019—Best Clinical paper award at 32nd Kerala Dental Conference, Kannur 1999 (Co author)—Runner up-Young Scientist award (Kerala Science Congress 2000). Dr. Baiju is an invited reviewer of several peer reviewed International scientific journals including Clinical Case Reports (Wiley-Blackwell), International Journal of Dental Hygiene, Journal of Indian Society of Periodontology Journal of Clinical and Diagnostic Research BMJ Case reports, Hindawi Journals, Paediatric Health, Medicine and Therapuetics, Dovepress Interdesciplinary Journal of Healthcare, Clinical, Cosmetic and Investigational Dentistry and Clinical Epidemiology. He has more than 30 international peer reviewed publications with over 180 citations.

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Chapter 1
Nanotechnology and Medicine: The Interphase

Sabu Thomas and R. M. Baiju

1 Introduction

Medicine and dentistry have advanced at a fast pace in the last couple of decades, thanks to the integration with other disciplines including nanotechnology. The refinement with which scientists are manipulating materials on the nanoscale has opened newer avenues in the fields of medicine and dentistry. The field of nanotechnology, commonly referred to as molecular nanotechnology or molecular engineering, allows for total control of materials at nanoscale levels. One thousand millionth of a metre (10^{-9}) is described by the Greek prefix 'nano' which means 'dwarf'.

Nanotechnology is defined as the direct manipulation of materials at the nanoscale [1]. This scale comprises the development of materials or devices comprising of components that are at least one dimension less than 100 nm in size. The fundamental component of nanotechnology is the nanoparticle. Better understanding and control of nanoparticles have paved the way towards many discoveries that have influenced human life significantly. The daily life applications of this branch of science range from cooking vessels, electronic appliances, and renewable energy to the medicine and aerospace industry.

Early and timely detection and diagnosis of diseases [2–4], targeted drug delivery [5, 6] and pharmacological development, detection of protein [7] and DNA structure, photothermal tumour ablation [8, 9], separation and purification of biologic molecules, tissue engineering [10, 11], management of SARS CoV 2 [12, 13], and fabrication of artificial organs [14] are few applications made feasible with nanocharacterized tools. This section tries to establish the link between the extreme worlds of

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nanoscopic particles and macroscopy, highlighting the influence of nanotechnology in dentistry.

2 Pioneers in Nanotechnology

The concept of nanotechnology was introduced by American physicist and Nobel Prize laureate Richard Feynman. In his famous lecture, he discussed the concept of employing machines to build smaller machines and further down to the molecular level [15]. When one delves deep into anything, one can discover the endless possibilities and potential of basic principles. Feynman is regarded by the fraternity as the father of nanotechnology.

The year 1986 witnessed another milestone in nanotechnology with the publication of the first book on nanotechnology. This led to the popularization of the theory of molecular engineering [16]. For the benefit of the reader, a compilation of significant milestones in the history of nanotechnology is given in Table 1 [17].

2.1 Properties of Nanoparticles

- General Characteristics

In contrast to the bulk material, nanoparticles exhibit unique chemical, magnetic, thermal, optical, and electro-optical properties. For instance, using an aluminium can is completely safe, but when it is converted to its nanosized counterpart, the general characteristics undergo a drastic transformation and converts itself into an explosive hence used in manufacturing bomb.

Nanoparticles' superior surface, size, and quantum effects can be attributed to their improved properties. Enhanced toughness, rigidity, transparency, scratch and abrasion resistance, heat resistance, and reduced gas permeability are some of the improved qualities [31].

- Self-assembly of Nanoparticles

One method used to create nanoparticles is self-assembly, which is viewed as an autonomous organizing of parts into patterns or structures avoiding human intervention [32].

Two types of self-assembly are static and dynamic. The system is categorized according to whether it dissipates or absorbs energy. While dynamic self-assembly involves energy dissipation during component interaction, static self-assembly utilizes energy to create an ordered structure.

- Antibacterial and Antiviral Properties

Table 1 Milestones in nanotechnology

Sl. no.	Pioneer	Milestone
1	Richard Feynman, 1959	Concept of nanotechnology [15]
2	Charles Plank and Edward Rosinski	Patented the process of zeolite catalytic cracking of hydrocarbons
3	Norio Taniguchi, 1974	First use of the term nanotechnology
4	Jacop Sagiv, 1980	Discovery of self-assembly monolayers (SAMs)
5	Gerd Binnig and Heinrich Rohrer, 1981	Invention of scanning tunnelling microscope (STM) [18]
6	Alexey Ekimov, 1981	Discovery of nanocrystalline quantum dots in a glass matrix
7	Eric Drexler, 1981	Molecular engineering [16]
8	Nadrian Seeman, 1981	Development of the concept of DNA Nanotechnology [19]
9	Louis Brus, 1983	Discovery of colloidal quantum dots [20]
10	Richard Smalley, Robert Curl, and Harold Kroto, 1985	Discovery of Buckminsterfullerene C60 [21]
11	Gerd Binnig, Christoph Gerber, and Calvin F. Quate, 1986	Invention of atomic force microscope (AFM), 1986 [22]
12	Dimitri Averin and Konstantin Likharev, 1987	Single-electron tunnelling (SET) transistor [23]
13	Sumio Iijima, 1991	Discovery of multi-wall carbon nanotubes [24]
14	Cees Dekker, 2002	Carbon nanotubes functionalized with DNA [25]
15	Naomi Halas, 2003	Development of gold nanoshells [26]
16	Andre Geim and Konstantin Novoselov, 2004	Discovery of graphene [27]
17	James Tour, 2005	Nanocar with turning buckyball wheels
18	Leonhard Grill, 2011	Scanning tunnelling microscope (STM) describes the electronic and mechanical properties of individual molecules and the polymer chains
19	Jean-Pierre Sauvage, Sir J. Fraser Stoddart, and Bernard L. Feringa, 2016	Nobel prize in chemistry for the design and synthesis of molecular machines [28]
20	Rainer Weiss, Barry C. Barish, and Kip S, 2017	Nobel prize in physics—gravitational waves [29]
21	Anthonius H. J. Engwerda, 2020	Molecular assembler that produces polymers [30]

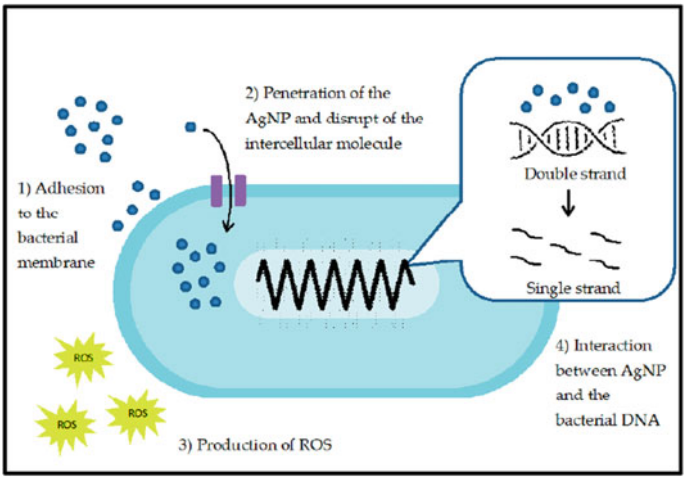

Fig. 1 Antibacterial activity of AgNPs (adapted with permission from 'The potential of silver nanoparticles for antiviral and antibacterial applications: a mechanism of action' Salleh et al. [34])

Some metallic nanoparticles show antibacterial activity. Copper nanoparticles (CuNPs) and silver nanoparticles (AgNPs) are potent antibacterial agents. Gold nanoparticles (AuNPs) are weak inhibitors of bacterial growth when compared to AgNPs. The antimicrobial actions of AgNPs can be summarized as follows [33]:

(1) Adherence to a microbe's surface membrane.
(2) AgNPs' property of cell penetration, which disrupts biomolecules and destroys intracellular matrix.
(3) Ability to cause cellular toxicity by producing reactive oxygen species (ROS), with resultant oxidative stress.
(4) Interfere with the cells' signal transduction pathways.

The antimicrobial property of nanoparticles is illustrated in Fig. 1.

Silver nanoparticles (AgNPs) are also one of the best candidates for antiviral action. Their mechanism of action is linked to the interaction of AgNPs with the outer coat of the virus as well as with the viral DNA/RNA. This is interesting and worth more exploration in the backdrop of the ongoing pandemic. The antiviral properties of AgNP are shown in Fig. 2.

For a technology to be categorized as nanotechnology, the following criteria should be satisfied [35]:

1. The technology should be capable of modifying the matter at the nanoscale level.
2. Periodic repetition must be present in the formed structure (i.e., Nanoparticle should periodically repeat itself along one or more directions).
3. In spite of being nanometric, their unique properties and functions should resemble parent matter or be better than parent matter.

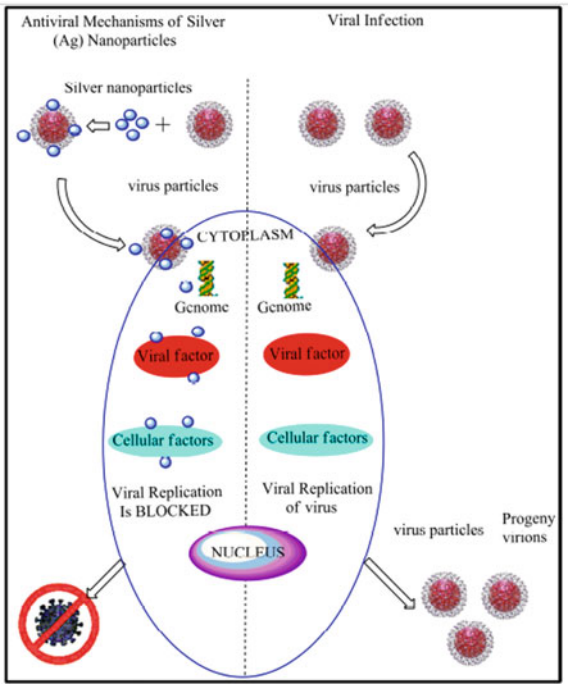

Fig. 2 Antiviral activity of AgNPs (adapted with permission from 'The potential of silver nanoparticles for antiviral and antibacterial applications: a mechanism of action' Salleh et al. [34])

The three primary, highly overlapping subfields of nanotechnology are nanomaterials, nanoelectronics, and nanobiotechnology all having applications in healthcare [36].

3 Manufacturing Approaches in Nanotechnology

The techniques for molecular manufacturing can be classified into two approaches namely the 'top-down' and the 'bottom-up' approaches (Fig. 3).

Top-down approach: The majority of modern industrial production methods rely on 'top-down' technologies, in which undesirable material is removed from larger objects to create small objects [38]. This method allows for precise patterning and the reduction of bigger materials to nanoparticles. Materials that have been scaled down to the nanoscale suddenly display extremely different characteristics, opening up new uses. The ratio of surface area to volume increases as particle size decreases, improving the effects of quantum mechanics.

Bottom-up approach: It involves organizing smaller parts into a complex assembly. 'Assembler' machines will build the desired products molecule by molecule, making larger objects with atomic precision [38]. These molecules are

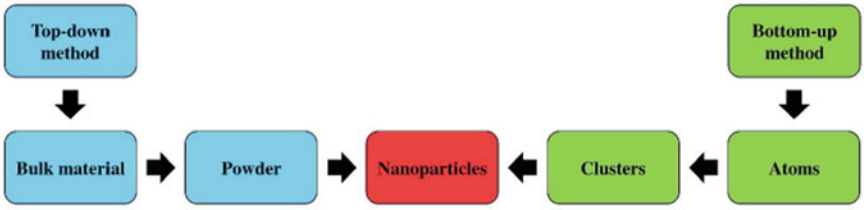

Fig. 3 Approaches for the synthesis of nanoparticles (adapted with permission from 'A review on the classification, characterization, synthesis of nanoparticles and their application' Mary Ealias et al. [37])

capable of self-assembly or higher order self-organization. Assemblers only add material where it is needed, minimizing waste during the process.

Nanoassemblers are computer-controlled devices made to do certain specified tasks. These nanoassemblers might be smaller than a cell nucleus so they can fit in spaces that are difficult to access with a human hand or any other kind of technology.

4 Types of Nanomaterials

Based on Origin

Nanoparticles originating from natural resources are categorized as natural nanoparticles. Mineralized materials like natural colloids and clays can be considered under natural nanoparticles. Those particles prepared deliberately through various physical, chemical, or biological procedures belong to the class of synthetic nanoparticles. Quantum dots, graphene, and nanotubes are a few examples of synthetic nanoparticles (Fig. 4).

Based on Composition

Biodegradable and nontoxic particles are called as organic nanomaterials. Ferritin, micelle, dendrimers, and liposomes are examples of well-known organic nanoparticles. Inorganic nanoparticles are generally described as those made of metal or metal oxide. They contain no carbon.

Based on Dimension

Low-dimensional nanostructures can be categorized as zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) [40]. This classification is based on the number of dimensions of a material, which are outside the nanoscale (<100 nm) range. When all the dimensions fall within the nanoscale, these materials are called zero-dimensional nanomaterials. Likewise, when one dimension is outside nanoscale, they are called one-dimensional nanomaterial. They include nanotubes, nanowires, and nanorods. When two dimensions are outside the

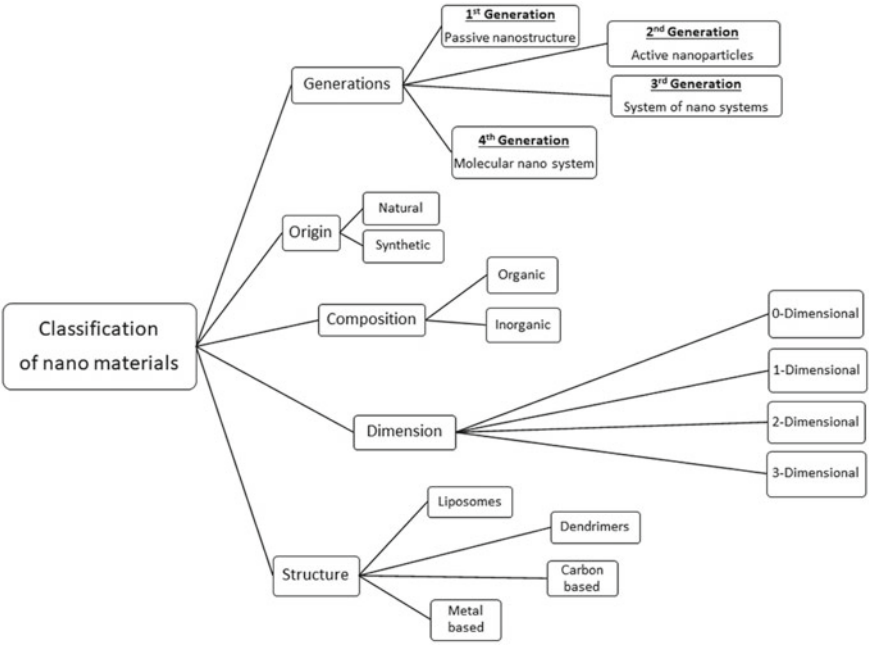

Fig. 4 Classification of nanomaterials (adapted from Nanotechnology in periodontics: an overview. Medico-Legal Updat. Thenappan et al. 2020 [39])

nanoscale, it is known as 2D. Nanosheets, nanoplates, and nanoflakes are included in this class. Three-dimensional nanomaterials are substances that do not fit within any dimension of the nanoscale (Fig. 5).

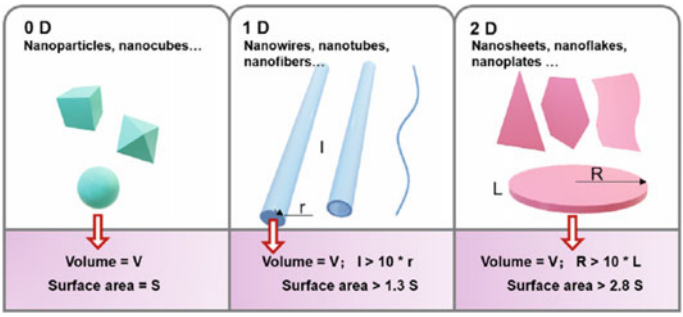

Fig. 5 Representation of low-dimensional nanoparticles. For a constant volume, the surface area of 1D and 2D nanomaterials is larger than 0D counterparts (adapted from 'One-dimensional and two-dimensional synergized nanostructures for high-performing energy storage and conversion' Li et al. [41])

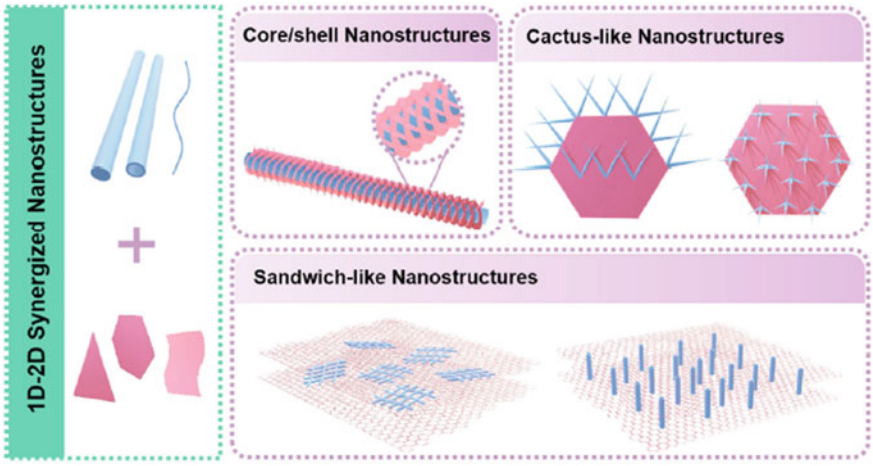

Fig. 6 Categories of 1D-2D synergized nanostructure (adapted from 'One-dimensional and two-dimensional synergized nanostructures for high-performing energy storage and conversion' Li et al. [41])

ID-2D Synergized Nanostructure: 1D and 2D nanostructures are coupled to produce synergized nanostructures. Different types are depicted in Fig. 6.

Based on Structure

Based on structure, nanomaterials are classified into liposomes, dendrimers, carbon-based, and metal-based. Carbon-based nanomaterials are those which are primarily composed of carbon. Cylindrical nanoparticles are known as nanotubes, whereas spherical and ellipsoid ones are called fullerenes. Other carbon-based nanomaterials include graphenes, carbon nanofibers, carbon black, and nanosized activated carbon [42].

Metal-based nanoparticles are those nanoparticles that are produced from metals down to nanometric sizes by either destructive or constructive methods. Aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn) are the most often used metals for nanoparticle synthesis [37].

Based on Generation

Roco M. C. has described various generations of nanotechnology development [43]. Passive nanostructures include the creation and addition of nanoscale particles that change the property of existing material whereas active nanostructures make changes to other materials. Active nanostructures have a different profile of benefits and potential risks when compared to passive structures. Examples of active nanostructures include targeted drugs and chemicals, sensors, and energy storage devices. Third-generation nanotechnology includes various machines working together. Molecular nanosystems include complete control of the actual molecules that make up the nanosystem (Fig. 7).

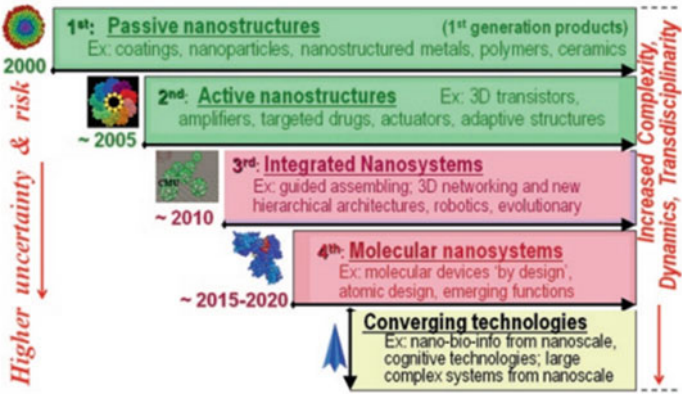

Fig. 7 Introduction to a new generation of products and productive processes from 2000 to 2020 (adapted from 'The long view of nanotechnology development: the national nanotechnology initiative at 10 years' Roco [43])

5 Uses of Nanostructures

5.1 Nanopores

A nanoparticle with nanoscale-level hole or channel in a membrane is called as a nanopore. Nanopore analysis is the process of employing voltage to drive molecules through a nanoscale pore in a membrane placed between two electrolytes. As the molecule moves through the pore, changes in the ionic current generated are monitored. This method does not require labelling or amplification and enables sub-nanometer resolution analysis of charged polymers (including single-stranded DNA, double-stranded DNA, and RNA) [44]. Encoded information, including flaws in the code known to be linked to cancer, can be decoded by passing DNA through a nanopore.

The pore size, pore distribution, porosity, and chemical characteristics of the pores in these materials may now be accurately controlled owing to developments in nanofabrication. As a result, they have a wide range of applications, including in implanted drug delivery systems, artificial organs, novel medical equipment, and controlling and sensing molecular transport [14].

5.2 Nanotubes

Carbon nanotubes are strong and electrically conductive structures created from carbon. Nanotubes have a number of extremely potential uses in biology, ranging from their use as growth substrates or tissue scaffolds. They may also be employed

as intracellular transporters for various diagnostic and therapeutic purposes. Carbon nanotubes have a substantial optical absorption in the near-infrared range, where tissue looks translucent, highlighting its use in photothermal tumour ablation and biological imaging applications. But there are still questions that need to be clarified regarding carbon nanotube toxicity [45].

5.3 Quantom Dots

According to Lee K.H. et al., quantum dots (QDs) are spherical nanocrystals with a diameter of 1–10 nm that illuminate when UV light is activated [46]. Unique photochemical and photophysical properties that are unattainable from either isolated molecules or bulk solids are seen in semiconductor quantum dots. These nanometer-sized semiconductor particles can act as biological labels by covalently attaching to biorecognition molecules such as peptides, antibodies, nucleic acids, or small-molecule ligands. QD photoluminescence can be manipulated by changing the particle size. It is thought that their application as fluorescent markers for biological macromolecules is a ground-breaking discovery [6] (Fig. 8).

Each crystal produces light when triggered by UV light and this light acts as a spectral bar code to identify a specific section of DNA. These crystals can be made to connect to certain DNA sequences, making it possible to use latex beads packed with these crystals to detect cancer by binding to the DNA sequences linked to the disease. Other uses include checking blood samples for virus presence and identifying particular proteins linked to illness.

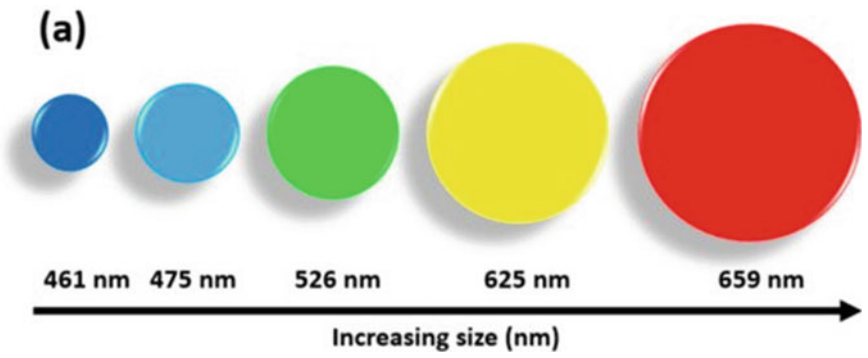

Fig. 8 Emission colour and wavelength of QDs corresponding to their sizes (adapted with permission from 'Recent advances in two-dimensional quantum dots and their applications' James Singh et al. [47])

5.4 Nanoshell

For the past few years, the development of photothermal cancer therapy and diagnostics has been largely dependent on nanoshells, namely dielectric core, spherical, and gold shell nanoparticles. By changing the form of the nanoparticle, the optical resonance of the nanoshell can be tailored to the appropriate wavelength. Maximum blood and tissue transmission occurs when the wavelength is in the near-infrared range. Nanoshell resonances can function as helpful contrast agents in the imaging of malignancies when they are tuned to this part of the spectrum. The absorption of light by the nanoshell generates intense heat making them nanoscale heat sources. This heat can trigger cell death by photothermal means and lead to tumour remission [8].

5.5 Dendrimers

Dendrimers are radially symmetric, nanoscale molecules having well-defined, homogenous, and monodisperse structures made up of arms or branches that resemble trees [48]. Built around a tiny molecule or linear polymer core, these hyperbranched synthetic macromolecules are distinguished by a mix of functional groups [49]. Large surface areas provided by this form allow for the attachment of therapeutic agents or other biologically active substances (Fig. 9). Under adverse pH conditions, the dendrimer's entire structure can be pulled apart. Dendrimer has many uses in the delivery of drugs, diagnostic imaging, and anticancer therapy.

Dendrimer-drug association may occur either through covalent or non-covalent interactions.

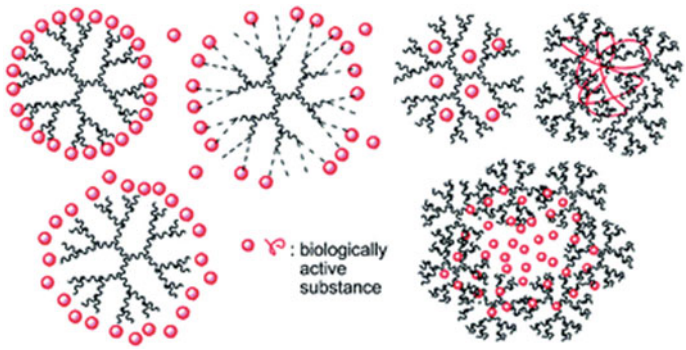

Fig. 9 Structure of dendrimers used for drug delivery (adapted with permission from 'Dendrimers for drug delivery' Caminade et al. [50])

Simple drug encapsulation inside dendrimers or electrostatic interactions between charged pharmaceuticals and surfaces are examples of non-covalent interactions. The covalent relationship can occur through stable bonds or through cleavable bonds.

5.6 Nanobelts

Nanobelts are belt-like nanostructures made from semiconducting oxides of zinc, tin, indium, cadmium, and gallium. Metal oxide powders are vapourized at high temperatures to produce nanobelts that are flawless, uniformly structured, and devoid of flaws and dislocations. This nanostructure's cross section is rectangular, measuring a few millimetres in length and 30–300 nm in width.

By simply evaporating the necessary commercial metal oxide powders at high temperature, ultralong belt-like (or ribbon-like) nanostructures (referred to as nanobelts) were successfully created from semiconducting oxides of zinc, tin, indium, cadmium, and gallium. For a thorough understanding of dimensionally constrained transport phenomena in functional oxides, the nanobelts may be the perfect device [51]. Structurally modified nanobelts find application in nanocantilever-based technology used in scanning probe microscopy and sensor applications [52].

5.7 Liposomes

Liposomes are microscopic closed lipid vesicles that are made of a phospholipid bilayer [53] (Fig. 10). Drugs that are soluble in water can be incorporated into their aqueous phase, while those that are soluble in lipids can be incorporated into their lipid phase. It is simple to adjust their lipid composition, size, membrane permeability, and electric charge. Being biodegradable, nontoxic, non-antigenic, and easily metabolized, they find a multitude of applications in drug delivery and cancer therapeutics [9]. The surface of the liposome can be modified by adding hydrophilic polymers like polyethylene glycol (PEG) which are called as PEGylated liposomes or sheath liposomes.

6 Nanomedicine and Nanodentistry

Robert A. Freitas Jr. first suggested the idea of nanomedicine in 1993. He has defined it as 'observing, controlling, and treating the biological system of the human body at molecular level using nanostructures and nanodevices' [38]. In other terms, nanomedicine is the application in medicine of matter manipulation at the nanoscale.

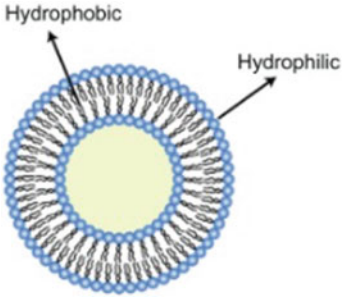

Fig. 10 Liposome structure (adapted from 'Liposomes and nanotechnology in drug development: focus on ocular targets' Honda et al. [54])

With its diverse array of uses, including targeted medication delivery to tissue scaffolds, and the employment of nanorobots for diagnostic and therapeutic purposes, nanomedicine has certainly revolutionized the medical industry [55]. The treatment of cancer has advanced significantly with the advent of nanotechnology, particularly with the use of targeted medication delivery that selectively kills cancerous cells while sparing healthy cell populations.

Nanodentistry is a brand-new discipline that has emerged as a result of the notion of nanotechnology gradually influencing the dental industry. With the application of nanomaterials, nanobiotechnology, tissue engineering, and nanorobots, nanodental technology is anticipated to enable the repair and maintenance of excellent oral health [56]. Various approaches in nanodentistry have opened up new vistas for disease control and are mentioned in subsequent pages.

7 Approaches in Nanodentistry

- **Nanodiagnostics**

Nanodevices may be used for early detection and diagnosis. Apart from offering improvements in available diagnostic techniques, nanodiagnostics also contribute to developing new diagnostic techniques. Development of devices that can work inside the human body assisting in the early detection and diagnosis of tumour cells and quantification of levels of protein and toxins are made possible by the intervention of nanotechnology [2]. Nanopores, nanotubes, quantum dots, and nanocantilevers are used in this regard.

Nanoelectromechanical Systems (NEMS)

It is being developed to create NEMS biosensors based on nanotechnology which have exceptional sensitivity and specificity for analyte detection down to the single molecule level. Chemical signals are converted to electrical signals by them. Individuals' oral fluids are used as a diagnostic tool to examine their health and/or disease status [3].

Oral Fluid Nanosensor Test (OFNASET)

For the purpose of identifying salivary biomarkers for oral cancer, the Oral Fluid Nanosensor Test (OFNASET) technology integrates self-assembled monolayers (SAM), bionanotechnology, cyclic enzymatic amplification, and microfluidics. Four salivary mRNA biomarkers (SAT, ODZ, IL-8, and IL-1b) as well as two salivary proteomic biomarkers (thioredoxin and IL-8) have been shown to work together to detect oral cancer with excellent specificity and sensitivity [4]. The capture and detector probes of this electrochemical sensor are designed to either target or bind with cancer-related antibodies. The detector probe detects the presence of the target by sending a reporter molecule, whereas the capture probe binds the target to the sensor [57].

Optical Nanobiosensor

The nanobiosensor is a special fiberoptics-based technology that enables the minimally invasive study of intracellular components like cytochrome C, a crucial molecule involved in both apoptosis, or programmed cell death, and the production of cellular energy [7].

Lab-on-a-Chip Methods

A device called a lab-on-a-chip (LOC) combines a number of laboratory operations on a single chip. LOCs handle extremely small fluid quantities, down to picoliters or smaller. Chemically sensitized beads that are populated into etched silicon wafers with embedded fluid management and optical detection capabilities are used for the assays. Small sample volumes, rapid analysis, and significantly lower reagent costs enable the execution of complex tests. Interleukin-1beta (IL-1beta), C-reactive protein (CRP), and matrix metalloproteinase-8 (MMP-8) are potential biomarkers for detecting and classifying the degree and extent of periodontitis [58]. The levels of these biomarkers can be assessed non-invasively in saliva using LOC methods.

Atomic Force Microscopy (AFM)

Another non-invasive method for viewing surface structures at subatomic resolution is atomic force microscopy (AFM). AFM, a subset of the scanning probe microscope, has developed into a popular diagnostic instrument that offers fine-grained topographical 3D images with 0.1 nm of lateral and vertical resolution [59]. AFM uses tip-sample interaction as the foundation for its scanning technique. A cantilever connects to the sample. A laser beam focused on the rear of the cantilever allows for the measurement of its bending as it scans a surface. A photodiode captures the reflected light and measures positional change. The feedback panel receives the measured data, process them, and then converts them into a voltage that is utilized to retract or extend the piezo. Either the experimental object or the cantilever can be moved by moving the position of the piezo [60]. AFM can be used as a nanodiagnostic tool to find dental cavities. They aid in the ultrasensitive detection of demineralization caused by bacteria [61].

The future detection of cancer, bacteria, fungal, and viral infections may be done with the aid of cantilever array sensors and nanoelectromechanical systems [62] (Fig. 11).

- **Nanoanaesthesia and Nanoneedles**

With the help of nanotechnology, local anaesthetic techniques can be used without any pain. The patient's gingiva will be injected with a colloidal suspension that contains millions of active, analgesic, micron-sized dental robots. The ambulating nanorobots would make contact with the surface of the crown or mucosa and then travel to the pulp via the gingival sulcus, lamina propria, and dentinal tubules under the dentist's supervision and with the assistance of a nanocomputer [56, 62]. Once inside the pulp, these nanorobots eliminate any tooth sensitivity that calls for treatment. These nanorobots can be controlled to restore sensation and exit the tooth using the same techniques as for entry after the process is finished. Nanosized stainless steel crystals that can be used as suture needles are developed. Additionally, nanotweezers are being developed. More discussion on nanorobots is given in Chap. 7.

- **Nanotechnology in Orthodontics**

Nanoparticle-Coated Arch Wires

The routine arch wire used for orthodontic movement can potentially induce frictional forces that can ultimately result in root resorption and anchorage loss. Reduction in frictional forces was observed on coating inactive fullerene-like tungsten disulfide nanoparticles on orthodontic wires [64]. It is suggested that the wires coated with these nanoparticles can present a novel way to significantly lessen friction while moving teeth.

Nanorobots in Tooth Movement

Future nanorobots may be able to directly control periodontal tissues, enabling quick, painless tooth rotation, repositioning, and straightening within minutes to hours.

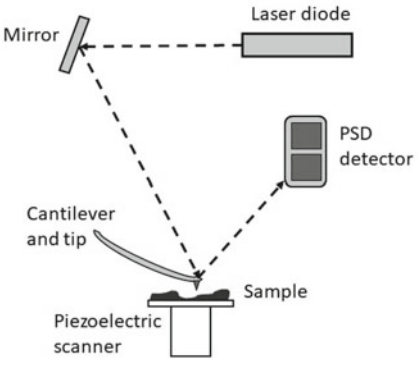

Fig. 11 Working principle of atomic force microscope (adapted from 'Atomic force microscopy study of tooth surfaces' Farina et al. [63])

Titanium nanotubular layer-coated temporary anchorage devices (TAD) are another application of nanotechnology in orthodontics. These biocompatible coatings can facilitate early osseointegration and act as an interface between the TAD and freshly produced bone [56].

- **Nanotechnology in Periodontics**

Anti-hypersensitivity Agents

Hypersensitive teeth have higher surface density and diameter of dentinal tubules compared to non-sensitive teeth. Nanorobots can be used to alleviate hypersensitivity by selectively occluding dentinal tubules thereby offering a rapid relief. Nanocomputers direct nanorobots as they enter the dentinal tubules and move towards the pulp. They provide instant sensitivity alleviation since they can get to the pulp in about 100 s [65].

Drug Delivery

Drug delivery uses three different types of nanoparticles. Among them are (a) monolithic nanoparticles, also known as nanospheres, (loaded active drug is adsorbed, dissolved, or dispersed within the matrix) (b) nanocapsules, (the loaded active drug is trapped, dissolved, or dispersed in a hydrophilic or lipophilic medium and surrounded by a shell-like wall) and (c) nanoparticles, where the medication is the primary ingredient in the pharmaceutical composition (Fig. 12). Diffusion, erosion, degradation, or a combination mechanism of release are the typical ways that the medicine included in the nanoparticles is released [5].

The benefits of utilizing nanoparticles as drug carriers include increased drug stability, high carrier capacity, the ability to incorporate both hydrophilic and hydrophobic compounds, and a variety of delivery methods. These drug carriers

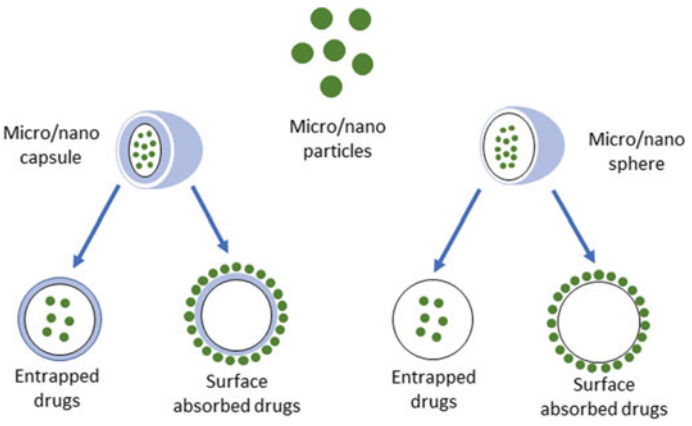

Fig. 12 Micro/nanoparticles for the purpose of periodontal drug delivery (adapted from 'Advanced biomaterials and their potential applications in the treatment of periodontal disease' Chen et al. [66])

may also be made to allow for the gradual and controlled release of the medication from the matrix.

Drug penetration into the biofilm layers is a significant barrier to biofilm removal. Nanoparticles' physicochemical properties might enable improved penetration and retention in the biofilm structure. The potential micro-localized dosage would enhance if the nanoparticles were trapped in the biofilm, acting initially directly on the immobilized bacteria followed by rupturing the biofilm matrix [5].

Tests on oral biofilm have shown that nanoparticles of bismuth subsalicylate, nanochlorhexidine, polymeric PolymPn active nanoparticles with silver and doxycycline, nanoparticles incorporating triclosan, and nanoparticles doped with zinc, calcium, silver, and doxycycline are all effective [67–70].

Photodynamic Therapy

In order to kill cells, antimicrobial photodynamic therapy (aPDT) uses a light source and a photosensitizer medication (such as methylene blue). A novel nanoplatform design for improved medication delivery and photodestructive elimination of oral biofilms is provided by the use of nanoparticles in aPDT. An antibacterial effect on planktonic P. gingivalis was observed when nanospheres from indocyanine green were used as photosensitizers [71]. High adsorption of the nanospheres on the surface of the bacteria helps in localizing the antibacterial effect.

Nanovectors in Gene Therapy

The goal of gene therapy is to correct disease-causing genes through repair or replacement. There are now three types of gene transfer techniques: chemical, physical, and viral. Gene therapy uses nanocarriers as vectors, such as calcium phosphates, lipids, and cationic polymers like chitosan, polyamidoamine dendrimers polyethylenimine, and poly(lactide-co-glycolide) [72]. Nanosized calcium phosphate particle was found to be an efficient nanovector used to deliver target genes (Platelet-Derived Growth Factor plasmids) to fibroblasts for periodontal regeneration in vitro [73].

Nanotoothbrush and Dentifrices

Incorporation of colloidal silver and gold particles onto the bristles of the toothbrush is found to reduce gingival inflammation. The affinity of silver particles towards the negative molecules leads to the disruption of the bacterial cell wall and thus eradication of plaque biofilm.

Due to the particle size, nanotoothpaste fills the voids between hydroxyapatite crystals [74]. These porosities in the enamel prism are sites for the agglomeration of bacteria. Obturation of these porosities with nanoparticles leads to the elimination of biofilm [75].

Implants

One of the areas of dentistry that is currently expanding quickly is dental implants. Insufficient osseointegration around the implant biomaterial soon after implantation is the most common reason for dental implants to fail. Implant surface improvements have improved dental implant survival and success. As the properties of

surfaces, such as chemistry and roughness, have a decisive role in osseointegration and preserving the long-term stability of implants in bone tissue, nanotechnology principles are increasingly being applied for surface modifications of dental implants [76]. Researchers with the advent of novel nanotopographies have demonstrated that nanostructured ceramics, carbon fibres, polymers, metals, and composites improve osteoblast adhesion and calcium/phosphate mineral deposition. Nanoscale mechanical modification by the creation of nanogrooves and nanopillars as well as a chemical coating using nanoparticles of diamond, hydroxyapatite, graphene, titanium dioxide, etc. are found to improve implant success [77, 78]. Nono Tite BIOMET 3i, a commercially available nanohydroxyapatite-coated implant, has around 50% of this material [39]. According to some studies, nanophase titanium dioxide and zinc oxide may boost osteoblast functions needed to support the effectiveness of orthopaedic implants [79].

Self-assembling Implants

Self-assembled Monolayer (SAM) is a nanoscale titanium surface modification technique used for dental implants. They are a quick, precise, and accurate method of changing surface characteristics. SAMs are organic assemblages created when molecules from solutions or the gas phase are adsorbed in predictable patterns on the surface of solids or liquids [80]. In a study by Li et al. on the performance of nanostructured self-assembling dental implants in type-II diabetic patients, they found that these implants performed better in terms of osseointegration and marginal bone loss than traditional dental implants [81].

Subgingival Irrigation

Nanobubble technology can be employed in the production of ozone nanobubble water that can be used for subgingival irrigation. These have a high level of safety and storage stability. The antibacterial action of nanobubble water can be used as an adjunct to periodontal therapy [82].

Laser and Nanoparticles

It has been demonstrated that laser irradiating surfaces covered with nanotitanium particles increase collagen synthesis. This idea can be used to effectively carry out periodontal operations like gingival depigmentation. Nanoparticles and a diode laser can also be used to clean the surface of the dentin [83].

Host Immunomodulation Therapy

Numerous methods for controlling the host immune response have been suggested in order to enhance care for patients with susceptible periodontitis. Periodontal therapy has expanded thanks to the immunomodulatory effects of host-modifying drugs administered using a system based on nanotechnology. Different nanocarriers' adaptability enables the development of their loading and release capabilities, which may be exploited for immunomodulation, periodontal regeneration, and/or microbial control [84]. The observed outcomes included a reduction in the activity of Th-1,

Th-22, and Th-17 proinflammatory and bone-resorbing T-cells and an increase in the development of Th-2 and Treg cells.

Bone Grafts and Nanomembranes

Root conditioning, the application of growth factors, the use of barrier membranes (GTR), bone grafting, or a combination of these are among the therapy techniques intended to promote periodontal regeneration. Numerous materials have been introduced for the treatment of bone abnormalities since the development of nanotechnology, with encouraging results. Because of their microscopic dimensions, which resemble the natural bone particles, nanoscale-based transplants produce better results. They are effective for treating intrabony defects, socket preservation, and procedures involving sinus augmentation [85, 86].

A new membrane for guided bone regeneration made of silk fibroin (SF) nanofiber was created by electrospinning native silk nanofibril solution [87]. With the usage of SF membrane, there is a noticeable improvement in cell adhesion and proliferation in vitro as well as bone defect repair in vivo. The SF nanofibrous membrane demonstrated adequate mechanical stability, good biocompatibility, delayed degradation, and enhanced new bone regeneration without any adverse inflammatory effects. Nanomembranes are also created using polymers such as polycaprolactone (PCL) or polylactic acid/cellulose acetate (PLA/CA). To improve the antibacterial and bone regeneration activity, silver nanoparticles (AgNPs, 1–2% w/v) and hydroxyapatite nanoparticles (HANPs, 10–20% w/v) were added to the scaffolds [88] (Fig. 13).

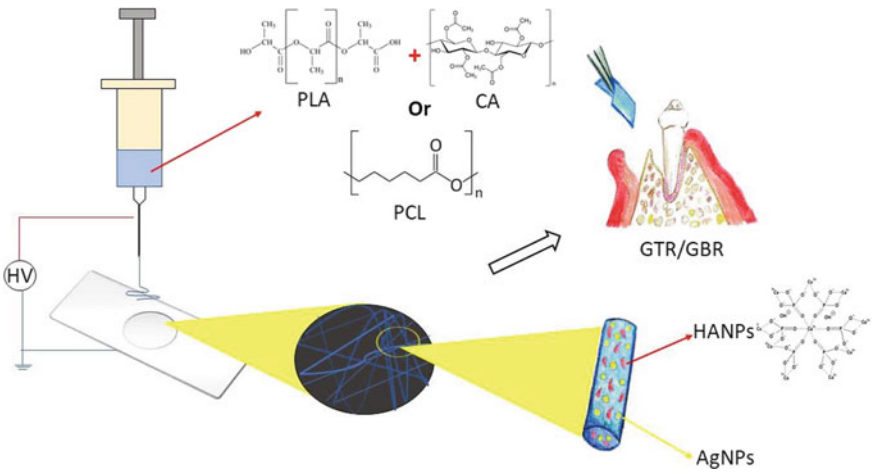

Fig. 13 Nanofiber-based membranes for periodontal therapy (adapted from 'New biodegradable nanoparticles-in-nanofibers based membranes for guided periodontal tissue and bone regeneration with enhanced antibacterial activity' Abdelaziz et al. [88])

Dental Nanorobots/Dentifrobots for Halitosis

Dentifrobots are imperceptibly small mechanical objects that move over the tooth surfaces at a speed of 1–10 μm per second. They are capable of actuation, sensing, signalling, information processing, intelligence, manipulation, and exhibiting swarm behaviour at the nanoscale. This nanorobotic dentifrice can patrol all supragingival and subgingival surfaces at least once per day, metabolizing trapped organic matter into flavourless, harmless vapours and conducting continuous calculus debridement. They are supplied via mouthwash or toothpaste. Nanorobotic operations can be managed by nanocomputers that have been previously programmed using ultrasonographic acoustic signals [56].

They can safely disable themselves if ingested. Dentifrobots with the right configuration can recognize and eliminate bacteria that are harmful to the oral cavity. Dentifrobots will also operate as a constant deterrent to halitosis because bacterial putrefaction is the main metabolic mechanism causing oral malodor [89].

Periodontal Dressing

A post-surgical periodontal dressing is employed for wound protection and patient's comfort in the post-operative period after periodontal surgery. Periodontal dressing generally does not contribute to the healing. However, it supports the wound healing by protecting the surgical site from mechanical trauma. However, the periodontal dressing containing silver nanoparticle was found to accelerate the gingival wound healing histologically in animal models [90].

7.1 Nanotechnology in Aesthetic Dentistry

Nanosolution

Nanosolutions produce unique and dispersible nanoparticles, which can be added to various solvents, paints, and polymers in which they are homogenously dispersed. Bonding agents are produced using nanotechnology, which guarantees uniformity and provides better properties than the parent material. One-bottle dentin adhesive may benefit mechanically from the addition of hydrophilic nanofillers, which would subsequently strengthen the bond [91]. Nanoparticles have also been used as sterilizing solutions in the form of nanosized emulsified oil droplets that bombard pathogens.

Nanocomposites

The increased interest in aesthetic restorations has led to the development of nanocomposites.

Nanocomposites are prepared by homogeneously distributing non-agglomerated discrete nanoparticles in resins or coatings (Fig. 14). The aluminosilicate powder employed as a nanofiller has the advantages of being very hard, flexible, strong,

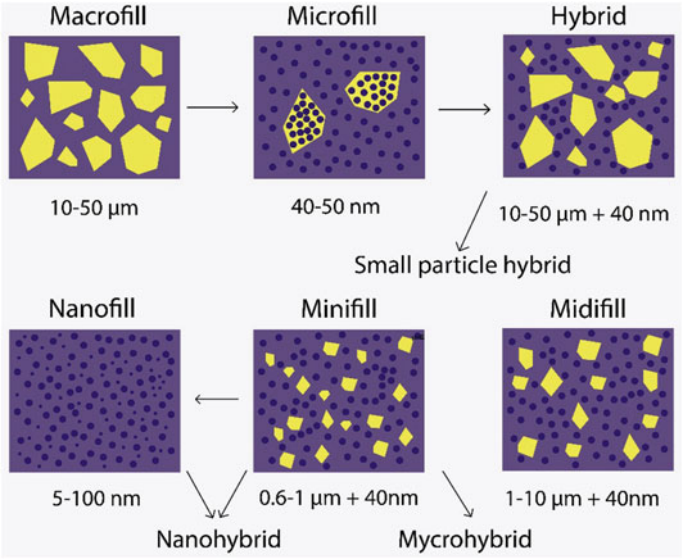

Fig. 14 Chronologic development of dental composites based on filler particle modification (adapted from 'Resin composite—state of the art' Jack L. Ferracane [92])

elastic, attractive, polished for a long time, and having less shrinkage during polymerization.

Since the fillers in nanocomposites are smaller than the wavelength of light, they are more translucent and permit more aesthetically pleasing restorations with a wide variety of colour options [93].

Nanoceramics

The easiest way to describe nanoceramic particles is as hybrids of inorganic and organic materials, with the organic component being methacrylic and the inorganic component being siloxane. Nanofillers are used to improve polishing capabilities and reduce wear, nanopigments are used to match the restoration's colour to the neighbouring teeth, and nanomodifiers boost material stability and prevent sticking to instrument [94]. Better resistance to microcrack propagation is considered as a superior property of nanoceramics.

Nanofilled Dental Cement

There is improvement in aesthetics and polishability of the restoration when nanomers and nanoclusters are added to a fluoroalumino-silicate glass of glass ionomer cement. The addition of nanohydroxyapatite and nanofluoroapatite has improved the compressive strength, bond strength, diametral tensile strength, and biaxial flexural strength and has also resulted in the reduction of porosity [95].

Dental Durability and Cosmetics

Replacement of outer enamel layers with covalently bonded synthetic materials, such as sapphire or diamond, which have 20–100 times the hardness and failure strength of natural enamel or modern ceramic veneers and superior biocompatibility, can increase tooth durability and aesthetics. Despite sapphire's susceptibility to acid corrosion, it can be produced in almost any colour, providing interesting cosmetic alternatives to common whitening and sealing techniques. It is possible to increase the fracture resistance of pure sapphire and diamond by including them into a nanostructured composite material that may also contain embedded carbon nanotubes [96].

Tooth Repair

Nanotechnology principles are used to create the tooth's biological and mineral components. The toughest tissue in the human body, dental enamel, was created by simulating the natural biomineralization process using highly ordered microarchitectural units of calcium hydroxyapatite crystals that resemble nanorods and are generally parallel to one another. At the water or air interface, freshly created and modified hydroxyapatite nanorods can self-assemble into an enamel prism-like structure [97].

Renaturalization Procedures

The development of aesthetic dentistry will make dentition renaturalization techniques a potentially well-liked addition to dental practises. This is primarily applicable to individuals who want their old dental amalgams removed and their teeth repaired using organic elements from their own bodies. All crowns and restorations are removed during full coronal renaturalization treatments, and the affected teeth are then aesthetically restored to seem identical to their natural counterparts.

- **Nanotechnology in Prosthodontics**

Impression Materials

Vinyl poly siloxanes are combined with nanofillers to create novel silicone impression materials. It is claimed that this material has superior flow and adhesion characteristics. Additionally, it has superior hydrophilic qualities, leading to less margin voids, better model pouring, and increased detail precision.

Nanofilled Composite Denture Teeth

In nanocomposite dentures, polymethylmethacrylate (PMMA) and filler particles of nanoscale are distributed. High polishability, impact and stain resistance, a dynamic surface structure, improved surface hardness, and wear resistance are a few of the benefits [56].

CAD CAM Blocks

New innovative CAD/CAM materials that provide superior aesthetic results are based on nanotechnology such as Lava Ultimate Resin Nanoceramic (RNC) blocks. They

basically are blocks that are made up of highly cured resin matrix with embedded nanoceramic particles [98].

- **Nanotechnology in Endodontics**

Nanoparticulate-Based Disinfection of Root Canal

Remnant bacteria entrapped within the dentin of human teeth may be responsible for the failure of root canal treatment. Due to the complex anatomy of the tubules, currently available techniques based on passive diffusion of antimicrobial agents are not adequate. The broad-spectrum antibacterial activity of nanoparticles can be harnessed in disinfecting root canals [99]. The nanoparticles of chitosan, zinc oxide, and silver are found to be effective in disrupting the cell wall of the most important endodontic pathogen—Enterococcus fecalis [100]. Additionally, these nanoparticles are also capable of disintegrating the biofilm found within the root canal system.

Endodontic Sealers/Nanosealers

The success of endodontic treatment largely relies on the impermeable seal achieved by root canal obturation. The sealer application fills shortcomings in root canal biomechanical preparation and increases adaptation of the filling to the root canal walls [101]. Newer nanosealers (nanocalcium hydroxide and nanobioactive glass) possess the ability to inhibit biofilm formation within the sealer dentin interface, improve sealability, and reduce cytotoxicity [102]. Improvement in the flowability of the nanosealer offers advantages in improving the sealer penetration into the minute dentinal tubules and resulting in better sealability.

- **Tissue Engineering**

Tissue engineering aims to repair damaged tissues in the body with functionally engineered tissue substitutes. Potential applications of stem cells are highly promising in regenerative medicine and dentistry. Treatment of bone augmentation, orofacial fractures, cartilage regeneration of the temporomandibular joint, periodontal regeneration, pulp repair, and implant osseointegration are potential areas of application of the principles of tissue engineering in dentistry. In tissue engineering, nanotechnology has allowed substantial improvement of scaffolding materials to the present unique 3D matrix for cells and tissues [10]. Conventional technologies do not possess the intrinsic mechanical properties that nanoengineered scaffolds have.

Nanoparticles are capable of enhancing the mechanical properties of existing biodegradable polymers or ceramic materials used in tissue engineering. Nanocrystalline hydroxyapatite can be used to create bone grafts with improved properties. Furthermore, it was demonstrated that periodontal tissue regeneration can be achieved with nanocrystalline hydroxyapatite-stimulated cell proliferation [103].

Stem cells of non-dental origin, like those from adipose tissue, bone marrow, and induced pluripotent stem cells, were used for dental tissue reconstruction. Owing to their affinity with target tissues, stem cells of dental and periodontal origin appear more promising [11]. The migration, survival, and regenerative impact of stem cells can be assessed by using labelling technique. This phenomenon called stem cell

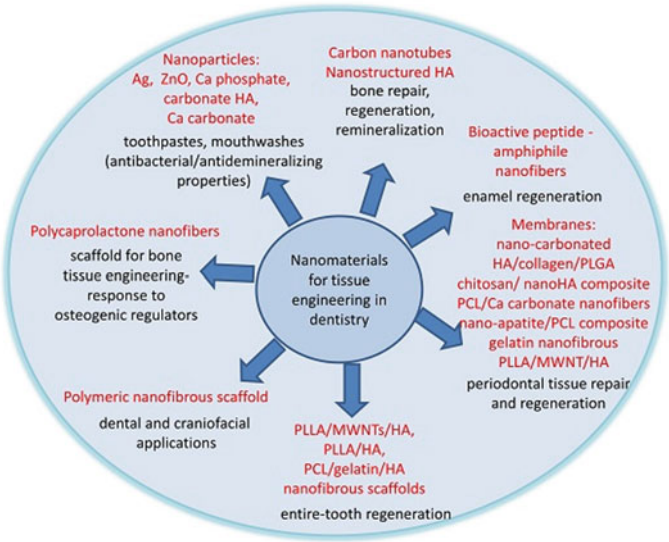

Fig. 15 Nanomaterials used for tissue engineering in dentistry (adapted with permission from 'Nanomaterials for tissue engineering in dentistry' Chieruzzi et al. [11])

imaging or tracking can be done with the help of nanoparticles like supermagnetic iron oxide [104].

The nanostructured materials used in tissue engineering range from simple nanopowders, to nanocarriers, to the establishment of complex scaffolds of different compositions and structures (Fig. 15).

- **Nano-Based Products in COVID-19 Pandemic**

Nanotechnology has made promising strides towards the management of the COVID pandemic. Nano-based antiviral and antimicrobial formulations, nanoparticle-incorporated facial masks, gloves, hand sanitizers, PPE kits, and nano-based vaccines have added new dimensions in managing viral infection [12] (Fig. 16). Recent developments in this area include very effective nano-based antibacterial and antiviral compositions that can be used to reinforce personal protection equipment like face masks in addition to cleansing surfaces and air. Recent evidence pointed out that special facial masks with silver nanocluster/silica composite coating had virucidal effects against SARS-CoV-2.

New nano-based sensors that allow early detection of COVID-19 are sensitive and accurate. Generally, testing kits operate on principles of enzyme-linked immunosorbent assay or polymerase chain reaction. Newer developments include a colloidal gold-based test kit that easily facilitates the conjugation of gold nanoparticles to IgM/IgG antibodies in human serum/blood [105]. Yet another development is the colourimetric assay based on gold nanoparticles for viral detection [13].

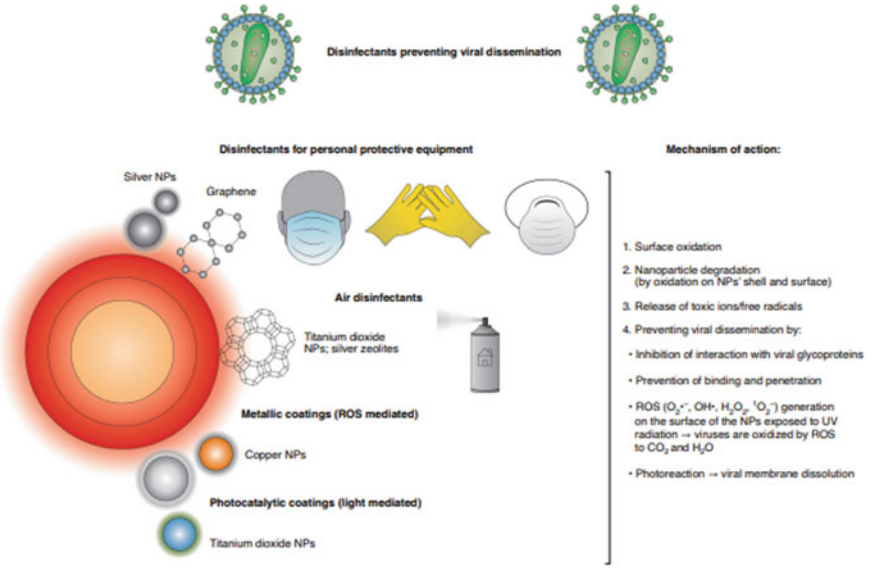

Fig. 16 Mechanism of action of nanotechnology-based viral disinfectants against SARS CoV-2 (adapted from 'Nanotechnology-based disinfectants and sensors for SARS-CoV-2' Talebian et al. [12])

8 Challenges Faced by Nanodentistry

Although nanotechnology offers novel and innovative techniques and armamentariums in dental science, there are a few concerns as well. These include concerns in the commercially viable production of nanorobots, bioethical issues, biocompatibility issues including human safety, and the need for expertise in precise positioning and assembly of molecular scale parts [99].

According to the American Heart Association research report, short-term exposure to elevated particulate matter in outdoor air predisposes to acute cardiovascular mortality. Leaching out of components from restorative dental materials could potentially lead to embryotoxicity. A study observed that cell culturing in culture media experimentally conditioned by composites caused blastocyst degeneration and apoptosis [106].

9 Conclusion

The advent of nanotechnology is set to revolutionize dentistry, healthcare, and human life in the years to come. They not only offer alternatives for existing treatment but also provides superior approaches in the prevention of diseases. With refinement in gene therapy, targeted drug delivery, tissue engineering, and biomaterials, improved

and personalized dental care could be possible. The use of nanotubes, nanoshells, dendrimers, and core–shell structures will expand realms in material development for the dental industry. Nanotechnology holds the key to an upright and sustainable future. However, like any other technology, nanotechnology too carries the potential for misuse and overuse if not properly regulated.

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Chapter 2
Nanotechnology in Oral and Dental Diagnosis

Betsy Joseph

1 Introduction

Nanotechnology is a multidisciplinary field that uses different nanomaterials and nanodevices to improve diagnosis and treatment. It involves the manipulation of matter at molecular and atomic levels. Richard P Feynman, in 1958, introduced the concept of nanotechnology, and the term was first used in 1974 by a scientist from Tokyo University of Science. The use of nanomaterials in the biomedical field has revolutionized the way diseases are diagnosed and treated. Over the past few decades, contributions arising from nanotechnology in dentistry have been made primarily in three fields: atomic force microscopy, imaging contrast enhancers, and biochips. Nanodiagnostics is the concept of using the principles of nanotechnology for the diagnosis of diseases with increased sensitivity and early detection. Nanomaterials have been widely researched in oral diseases such as oral cancer, dental caries, dentinal hypersensitivity, and oral cancer [1]. Some of the exciting applications of nanotechnology include drug delivery systems, the development of new medical devices, biofiltration systems, regeneration of lost tissues, etc. Nanomaterials have a significantly increased surface area for unit mass due to their tiny size. This leads to altered electrical, optical, and magnetic properties of these materials. Some of the practical examples of nanomaterials include nanosized liposome vesicles for non-invasive drug delivery; nanoparticle-delivered collagenases which have the ability of remodelling periodontal fibres; nanocomposites and nanofillers in restorative nano-dentistry, tooth, and dental tissue regeneration, osseointegration, nano-anaesthesia,

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management of tooth hypersensitivity, nanorobotic dentifrices, orthodontic tooth movements using nanorobots artificial teeth with nanocomposites, etc. [2].

Since nanomaterials vary on different levels, various classifications are used to classify them based on their dimensions, shapes, sizes, and compositions. Based on the function of their origin, they are also grouped as naturally occurring and engineered nanomaterials. Nanomaterials are classified as zero-, one-, two-, and three-dimension nanomaterials based on dimensions [3]. **Zero-dimension (0D)** nanomaterials are less than 100 nm in size. These include nanoparticles, nanoclusters, quantum dots, graphene, fullerenes, magnetic nanoparticles, up-conversion nanoparticles, and polymer nanoparticles. Their superior optical stability, wavelength-dependent photoluminescence, and biocompatibility make zero-dimensional nanomaterials significant for biomedical uses. **One-dimension (1D)** nanomaterials have more than 100 nm particle size. Metal, metal oxides, and carbon-based nanomaterials such as nanotubes, nanowires, and nanofibers are examples of one-dimensional nanomaterials. They emit electrons in a low electric field and have a large surface-to-volume ratio with high porosity. They are often used in tissue engineering and wound healing as scaffolds. **Two-dimension (2D)** nanomaterials are plate-like structures that are bigger than 100 nm and at least one atomic layer thick. Some examples include graphene/graphene oxide, silicate clays, layered double hydroxides, boron nanosheets, and tin telluride nanosheets. They have uniform shapes, a high surface-to-volume ratio (in contrast with the bulk material), and a surface charge that offers them superior chemical, optical, and biological properties. Among these, graphene exhibits excellent electronic and photonic characteristics due to π-orbitals orthogonal in the hexagonal plane. **Three-dimension (3D)** nanomaterials also have a size of more than 100 nm and include structures such as graphene nanostructures, bundles of nanowires and nanotubes. They have high mechanical strength and form an integral part of micro-electromechanical systems, biomedical devices, robotics, and 3D printing technologies.

Based on their chemical composition, they are classified into carbon, inorganic, organic, and hybrid nanomaterials. **Carbon nanomaterials** are composed of sp^2-bonded carbon atoms. These include nanodiamonds, fullerenes, graphene, single- and multi-walled carbon nanotubes, carbon nanofibers, nano horns, nano-onions, and nano-graphite. The variety of allotropies makes carbon-based nanomaterials a group of significance. **Organic nanomaterials** include lipid and polymer nanoparticles such as dendrimers, micelles, liposomes, and ferritin. The presence of carbon as their primary constituent offers it specific functionalities and reactivity. Carbon nanotubes, nanospheres, nanocapsules, quantum dots, super magnetic nanoparticles, liposomes, solid lipid particles, nanocrystals, dendrimers, fullerenes, and nanosponges are among the other nanomaterials which are being studied for the detection of oral and dental diseases.

Inorganic nanomaterials are formed by non-carbon elements, such as metals, metal oxides, and metal salts. The high surface reactivity and sensitivity are reduced by functionalization. The magnetic nanoparticles have exciting properties due to their superparamagnetic behaviour at the reduced nanoscale. Among the metals, gold NPs are most stable and are prepared in various shapes such as nanospheres, nanorods,

nanocubes and nanoshells. Silver NPs are being used widely in biosensing markers in cancer diagnosis, as surface-enhanced Raman scattering (SERS) substrate to detect severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and in the identification of pathogenic bacteria. Superparamagnetic nanoparticles (MNPs) are often preferred as contrast agents in cancer cell detection and aptasensors for the identification of pathogenic bacteria by acting as conjugating locations for proteins, aptamers, and fluorescent dyes. These MNPs have the advantage of excellent physicochemical stability, cheaper production, environment friendly, and biocompatible [4]. Other than these, there is another group called **hybrid nanocomposites** that are made up of different phases of materials, at least one of which is nanosized (1–100 nm) and the matrix is made up of either polymer, ceramic, hydrogel, or metal. Among various the nanomaterials available, nanoparticles have been researched most extensively for biomedical imaging, such as the detection of oral cancer, employing nanomaterials such as graphene, carbon nanotubes, and nanoparticles containing gold, silver, and platinum-palladium, nanostructures based on quantum dots, and magnetic and up-conversion composite NPs.

Nanomaterials are being widely researched for the early detection and diagnosis of oral diseases such as oral cancer, dental caries, periodontal disease, halitosis, salivary biomarkers of many systemic conditions such as COVID-19, diabetes, etc. Nanomaterials that find use in this area include metal/metal oxide nanoparticles (carriers or agents for MRI and ultrasound image), carbon nanotubes (diagnosis in DNA transformation biomarker for changes in protein structure), nanocore shells (contrast imaging for tumours), 1D, 2D nanostructures (scanning, detector for protein diseases, detection of DNA mutation, diagnosis of gene mutation), and quantum dots (diagnosis of gene and protein structures due to optical properties, detection of tumour, and lymph nodes) [5]. It is noteworthy that gold nanoparticles have unique optical and electrical properties dependent on the property of surface plasmon resonance (SPR) which is a highly popular nanomaterial in nanodiagnostics. It has been widely used for in vitro studies and as loading and releasing agents for drugs.

2 Detection and Diagnosis of Oral Cancer and Premalignant Lesions

Oral cancer is one of the most common type of cancer with a mortality rate. Various risk factors such as smoking, alcohol, and spicy food have been associated with oral cancer. Oral squamous cell carcinoma is the most common type of oral cancer, which affects the tongue buccal mucosa floor of the mouth, alveolar bone, and tongue. It is multifactorial in aetiology and often develops from premalignant lesions such as oral leukoplakia, oral lichen planus, and submucous fibrosis. Therefore, early detection of oral premalignant lesions and oral cancer is crucial for successful management and a better prognosis of the disease.

Biopsy using a scalpel and subsequent histopathologic examination is still considered the gold standard for diagnosing oral cancer and other potential oral malignant lesions. This technique has some limitations as it is invasive and causes patient discomfort. The results of this histopathological examination depend on the margin of the resected tissue selected. The outcome can be influenced by the nature of the specimen and the pathologist's expertise. Moreover, the results would vary even if the genetically abnormal cells at the margins are very small in number. Due to these reasons, many novel diagnostic techniques for the detection of oral cancer have been explored in the past few years. Toluidine blue staining, chemiluminescence, and light-induced autofluorescence technique have been used in conjunction with traditional techniques to diagnose premalignant and malignant lesions of the oral cavity. These techniques have shown good sensitivity but questionable specificity in identifying such lesions. This makes nanotechnology a potential candidate for early detection with good sensitivity.

3 Molecular Imaging of Oral Cancer

Molecular imaging techniques such as magnetic resonance imaging (MRI), optical coherence tomography (OCT), photoacoustic imaging, Raman spectroscopy, and diffuse reflectance imaging have demonstrated the application of nanoparticles in detecting the presence of oral cancer cells. Nanoparticles such as liposomes, dendrimers, polymeric NPs, gold NPs, magnetic NPs, quantum dots, and carbon nanotubes are being widely used to detect oral cancer cells due to their ultrasmall size, and high reactivity. In **MRI**, super magnetic nanoparticles have been used as contrast agents for cancer screening [6]. Contrast agents have the advantage of recognizing the unique surface markers on the cell and exhibit a longer blood circulation half-life. Folate preconjugated chitosan, magnetic poly (lactide-*co*-glycolide) NPs, and magnetic nano-contrast agents based on Gd3+ doped amorphous TiO_2 are some of the commonly researched supermagnetic NPs. Folic acid-conjugated nanoparticles stained the surface of folate receptor-positive oral cancer KB cells without affecting the normal L929 cells and were found to be biocompatible for MRI [7]. Magnetic NPs, when used as contrast agents in MRI, help to refine proton relaxation and can be selectively injected into the tumour site without penetrating other organs. Such newer active targeting MNPs can improve tumour detection possibilities based on the specific molecular signatures of these pathologies [6]. Table 1 shows various applications of nanomaterials in oral cancer diagnosis.

Optical coherence tomography (OCT) is another non-invasive technique for oral cancer detection that simulates ultrasound and presents cross-sectional images of deeper tissues of about 2 mm with the help of infrared light. The most successfully used contrast agent in this technique is gold NPs, as they are biocompatible and do not show significant absorption in tissues [7]. Gold NPs cause localization of surface plasmon resonances (SPR) at near-infrared wavelengths such

Table 1 Application of various nanomaterials in oral cancer diagnosis

Diagnostic technique	Nanomaterials	Features	References
Magnetic resonance imaging (MRI)	Magnetic NPs	Contrast agents; help to refine proton relaxation; passive targeting	[6, 7]
Optical coherence tomography (OCT)	Gold NPs; quantum dots; fluorescent NPs probes	Biocompatible; localization of surface plasmon resonances; no predominant absorption in tissues	[5, 7]
Photoacoustic imaging	Polymeric NPs; gold NPs; carbon nanotubes	Better sensitivity; real-time screening; economical	[5, 6]
Raman spectroscopy	Gold NPs	Superior optical properties; effective light scattering; surface plasmon resonance (SPR), high selectivity to intracellular organelles	[6, 8]
Diffuse reflectance imaging	Gold NPs	Helps in the identification of tumour margins accurately in surgical sites	[6]
Air scanning electron microscope	Gold NPs	Helps in improved tumour margin determination during surgery	[6]
Nanobiosensors salivary biomarkers	Nanostructured zirconia decorated reduced graphene oxide	Cytokeratin-19	[9]
Nanobiosensors salivary biomarkers	Silicon nanowire	Selective detection of TNF-α and IL-8	[10]

that predominant absorption in tissues is avoided. Monoclonal antibodies conjugated gold nanoparticles have been an effective contrast agent in the hamster model that was delivered with the help of microneedles and ultrasound [7]. Near-infrared fluorochrome-labelled NPs, quantum dots, and fluorescent NPs probes are also being researched for the early detection of cancer [5].

Another NP-based contrast agent is polymeric NPs which find use in **photoacoustic imaging** [5]. They are formed by combining natural and synthetic polymers and are classified as nanocapsules and nanospheres. Polymeric NPs are simple in design, biocompatible, and have a wide structural variety. They have also been used for fluorescent endoscopic detection of oral cancer where folic-acid-conjugated chitosan NPs facilitate endocytosis by targeting folate receptors on oral cancer cells. Negatively charged N-succinyl chitosan polymer reduces the intensity between chitosan and the drug, enhancing the 5-aminolevulinic acid released in oral cancer cells. Gold NPs, gold nanorods, carbon nanotubes, and fluorescent-loaded NPs are also used in photoacoustic imaging of oral cancer [5].

Similarly, gold NPs have also been used as an exogenous contrast agent in **Raman spectroscopy** to enable Raman signals with increased speed and resolution. Gold

NPs have high selectivity to intracellular organelles and the ability to specifically distribute in organelles such as cytoplasm, mitochondria, and nuclei. Gold NPs have superior optical properties and can effectively scatter near-infrared (NIR) and visible light when irradiated with their surface plasmon resonance (SPR) [8]. This is significant because this light scattering is much more prominent when compared with chemical fluorophores under dark-field microscopy [6]. These nanoparticles are available in different shapes, such as nanospheres, nanorods, nanocubes, and nanobranches. Among these, gold nanorods are extensively used for molecular imaging due to their higher index sensitivity over other forms of gold NPs. Gold nanorods conjugated with rose bengal have been used to monitor optical absorption in the near-infrared region for specific and quantitative oral cancer cell lysates analysis with a good detection sensitivity of 2000 cells per ml [6].

Gold NPs are finding their use in **diffuse reflection imaging** techniques also. In this technique, the portion of the light that gets diffusely reflected is significantly affected by cytologic and morphologic changes during cancer development, such as the size of the nucleus, proportion of collagen, thickness of the epithelium, and changes in blood flow. This technique can identify tumour margins accurately so that diseased regions can be surgically resected completely. In vitro studies show that gold nanorods are categorically attached to oral cancer cells with good sensitivity and specificity. The reflectance spectrum at 780 nm was prominent in areas of carcinoma in situ and gave good discrimination [6]. The quantum dots imaging technique is another technique for in vitro and in vivo identification of oral squamous cell carcinoma. It uses nanometer-sized semiconductor crystals to generate signals with high tissue permeability, good fluorescence intensity, and stability against photobleaching than traditional organic fluorescence materials due to their unique quantum size and surface effects [6].

A sufficient amount of gold NPs in the tumour site is critical for the accurate diagnosis of oral cancer. This is achieved by the enhanced permeability and retention effect, a form of passive targeting of gold nanoparticles in the tumour site as they enter the cells by the endocytosis process. Gold nanoparticles conjugated to antibodies also enter these cells by means of active targeting. Discrimination of benign and malignant premalignant oral lesions has also been done using gold nanorods conjugated to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies [6].

Gold nanoparticles are also used in an **air scanning electron microscope** that helps in improved tumour margin determination. Acid transformation gold nanoclusters are used along with optical coherence tomography for the early detection of oral cancer. Gold nanoparticles can also increase the intensity of Raman spectroscopy signals when added to the blood sample. Immuno sensing of oral cancer cells has been made possible by customized gold nanoparticle-reduced graphene oxide-based bioelectrode [6]. Dendrimers (DNA-dendrimer and polypyrrole (DDPpy) sensors also have been used to detect various interleukin proteins from oral cancer cells. Furthermore, discrimination between benign and malignant tumours was possible

using gold NPs bio-conjugated to anti-EGFR monoclonal antibodies as dysregulated Epidermal growth factor receptors within the malignant salivary gland tumour suitable targets for nanoparticle-based contrast agents [6].

4 Application of Nanomaterials in the Detection of Oral Biofilm

Detection of microorganisms is essential in clinical diagnosis and correct treatment planning. Traditional methods of culturing, immunological assays, and polymerase chain reaction have some limitations, are time-consuming, and depend on the skill of the laboratory personnel. Nanobiosensing is an analytical technique that combines a biologically active element with a suitable physical transducer to produce a recordable signal comparable to the concentration of chemical species in any type of sample [1]. Nanomaterials such as nanotubes, nanowires, and nano-dots have been used as nanobioreceptors to enhance this process. The micro-sized particles of conventional biosensors are replaced with nanosized ones to form nanobiosensors that can identify analytes at a very-low molecular level. Piezoelectric, electrochemical, optical, and colourimetric changes due to NPs are used for such detections. This has made analytical biosensors a popular technique for rapidly detecting pathogens, even at a lower concentration [11]. Various biosensors have been used with aptamer, antibodies, bacteriophage, enzymes and were found to rapidly identify and quantify many pathogens with good sensitivity and specificity. Nanoparticles are preferred in developing biosensors due to the surface plasmon resonance properties of gold and silver NPs [11]. Once these nanoparticles are modified with recognition elements, they demonstrate colour changes that are visible to naked eyes. This colour change is due to the plasmon peak shift exhibited by the nanoparticles.

5 Aptamer

Aptamer, single-stranded nucleic acid strands, is now being used instead of traditional antibodies in identifying pathogenic bacteria. They are stable, smaller in size, easily modified, and lack immunological reaction [4]. These aptamers are combined with different nanoparticles to amplify the signals from the microbes and biochemical analytes [12]. Specific nanomaterials with good electronic, optical, and magnetic properties are used to combine with aptosensors. The tiny nanomaterial size enhances the sensor's performance without changing its properties. It also results in a large surface area that increases the interaction between the sensor and analyte, which is critical in the sensitive detection of the analytes [12].

Gold NPs have excellent electronic and optical properties and are highly stable, making them suitable for bioanalytical uses. The nanoparticles exhibited good

magnetic properties, which prevented sample sedimentation instead of repeated centrifugation. Similarly, silver-coated magnetic NPs were also used as an aptasensor to identify pathogens such as *Staphylococcus aureus*. DNA aptamer can be combined with gold nanoparticles to detect *Staphylococcus aureus* using direct detection and bead amplification techniques. This technology can be helpful for the rapid detection of different types of pathological bacteria in the clinical scenario [2]. Aptamers are also used to detect *Pseudomonas aeruginosa, Campylobacter jejuni, Shigella dysenteriae, Mycobacterium tuberculosis,* etc. which are serious pathogens causing severe infections. Localized surface plasmon resonance-based nanosensor technology rapidly detects numerous pathogenic bacteria in a short duration ranging from 20 min to one hour. Silver nanoparticles, platinum nanoparticles, magnetic NP, and molybdenum NPs are also being used to detect bacteria rapidly [4].

Carbon nanomaterials, especially carbon nanotubes and graphene, are becoming popular materials for nanosensors in detecting bacteria. Carbon nanotubes are preferred due to their specific chemical, thermal, and electrical properties. The binding of the aptamer microorganism changes its configuration and surface charge which results in a potential difference. Detection of *Mycobacterium tuberculosis* has been made possible using ssDNA aptamer [13]. Multi-walled carbon nanotubes are helpful in the detection of Salmonella sp. as the presence of this pathogen increases the impedance of the electrode surface used. Graphene is made of a layer of sp^2 hybridized carbon atoms and has unique electronic properties due to graphene having the longest mean free path among all nanomaterials. This makes graphene a suitable nanomaterial as it encounters only eligible resistance and hence, a very high charge carrier mobility of 15,000 cm^2/V s at room temperature.

These properties vary according to the number of graphene layers. Graphene has an extremely high specific surface area, a relatively high tensile strength, high elasticity, and is a very good thermal conductor. Similarly, graphene oxides are being used for developing highly sensitive detection systems due to their biocompatibility, variations in oxidation level, ability to control the size, and mechanical and electrical properties. Robust and early detection of pathogens is possible with sensory nanoparticles' help. These can detect and discriminate pathogens at low concentrations, which is of great clinical significance. Plasmonic nanomaterials like gold and silver are widely studied for detecting bacterial presence. Once these nanoparticles are coated with recognition elements like antibodies or aptamer, they can detect particular pathogens.

6 Immune-Based Sensors

Particles are coated with antibodies to attach to the surface of the bacteria. This ligand and receptor reaction causes nanoparticle aggregation of the nearby targeted bacteria, resulting in colour change. This property is characteristic of metallic nanoparticles. The reaction oscillates electron clouds within the nanoparticles that allow careful monitoring of the nanoparticle [7]. For example, gold nanoparticles modified with

an antibody specific to *E. coli* produce a colour change of red to purple in the presence of these bacteria. The sensitivity and performance of these colourimetric nanoparticle-based sensors depend on the size, shape, binding sites, and aggregation status of net metal nanoparticles. The smaller particle size of gold nanoparticles showed greater sensitivity and selectivity. Calorimetric sensors have also been used to detect bacterial phages. The detection of bacteria also depends on the type of antibodies modified on the nanoparticle [7].

7 Aptasensors

Despite the good specificity and sensitivity of monoclonal antibodies, they have limitations, such as the cost and time involved, solubility, and stability, that make aptasensors an emerging alternative option for the biorecognition of bacteria. Aptamers use surface-enhanced-Raman-scattering (SERS)- and fluorescent-based sensors for bacterial detection [4, 14]. Metal and metal oxide NPs (gold, silver, palladium, and iron oxide MPs), carbon-based nanomaterials (carbon nanotubes, graphene oxide and reduced graphene oxide, carbon nanowires), silica NPs, quantum dots, and polymer NPs have been studied for the detection of pathogenic bacteria.

8 Bacteriophage-Based Sensors

Aptamers had limitations of low stability and reduced half-life in biological media and cross-reactivity. Bacteriophages are economical, readily available, infect only bacteria, identify live and dead cells, and proliferate in living cells. These are also stable during pH and temperature changes. The calorimetric method has been used to detect *E. coli, Pseudomonas aeruginosa,* and *Vibrio cholera* with high diagnostic accuracy. Similarly, gold nanoparticles coated with bacteriophages specifically identified *E. coli* and *Methicillin-resistant Staphylococcus aureus* (MRSA) based on SPR changes [12].

9 Array-Based Sensors

The earlier-mentioned sensors require complicated procedures to ensure reproducibility and reliability. Array-based sensing does not require any recognition element to detect bacteria from the analytes. The affinity of bacterial species to different nanoparticles varies based on size, composition, and surface energy. This results in light scattering at different levels and emitting different fluorescence emission intensities. Therefore, gold–silver alloy nanoparticles have been developed to

detect sulphur-emitting bacteria. As the bacteria have a particular affinity for gold–silver alloy NPs, they produce different optical signals at very low concentrations [12].

10 Optoelectronic Nose

This is a method of detecting bacteria using cross-reactive sensors and forms a typical pattern for different bacteria. Nanoparticles with the characteristic molecule were used to identify bacteria based on this mechanism. When the bacteria are not present, gold nanoparticles bind to the light base and prevent fragrance production. In contrast, in the presence of bacteria, the nanoparticles selectively bind to the bacterial surface, and the free lipases produced are rose fragrance. The technique has been currently used for identifying contamination of drinking water. This strategy was also used to detect different types of bacteria galactosidase enzyme instead of lipase [12].

Nanotechnology-based sensors have the potential for identifying different types of oral bacteria. This technique allows rapid detection of bacteria. More studies are needed to develop nanosensors that can identify the presence of different types of bacteria in biological fluids in real-time accurately. The challenges that need to be addressed include their inability to correctly identify low concentrations of bacteria in biological fluids like blood plasma and cerebrospinal fluid. There is also a need to evaluate the specificity of nano-based sensors in identifying specific types of bacteria from other bacteria in a clinical sample. Drug resistance and susceptibility of bacteria also need to be identified using nanotechnology sensor methods. Table 2 shows applications of various nanomaterials in oral biofilm-induced diseases.

11 Early Diagnosis of Periodontal Disease

Magnetic nano-inclusions such as ferroferric nanoparticles can be used along with polymers to reinforce or convert them into sensing materials. Modifying the hydroxyl groups of the nanoparticles with a silane-type coupling agent can result in novel sensing material [16]. Calix arenes (synthetic macrocycles) act as ionophores and binding hosts for various molecules and are part of the macrocyclic molecular receptors. These are added in several polymeric ion-selective membranes that provide various analytes' fast and robust sensing methods. Substances like calixarenes are significant as they cross membranes and help gradually release drugs. The sensing of periodontal pockets has been studied with the help of membranes with magnetic nanoparticles and ionophore inclusions in the polymeric matrix. These can be remote-controlled due to the magnetic behaviour of magnetic nanoparticles. Such a sensor can identify increased sodium cation levels from unstimulated whole human saliva.

Table 2 Application of various nanomaterials in oral biofilm-induced diseases

Oral disease	Diagnostic technique	Nanomaterial	Properties	References
Dental biofilm	Aptamer	Gold and silver NPs; carbon nanotubes; graphene	Large surface area; localized surface plasmon resonance; configuration and surface charge changes help in identifying the organism	[4, 11, 12]
	Immune-based sensors	Gold NPs	Nanoparticle aggregation of the nearby targeted bacteria result in colour change	[4, 7, 14]
	Aptasensors	Metal and metal oxide NPs; carbon-based nanomaterials	Surface-enhanced-Raman-scattering and fluorescent-based sensors	[4, 14]
	Bacteriophage-based sensors	Gold NPs	Economical; readily available; identify live and dead cells, proliferate in living cells; stable during pH and temperature changes; high diagnostic accuracy; based on SPR changes	[12]
	Array-based sensors	Gold–silver alloy NPs	Bacteria produce different optical signals at very low concentrations	[12]
	Optoelectronic nose	Gold NPs	Uses cross-reactive sensors; forms a typical pattern for different bacteria	[15]
Periodontal disease	New sensor-based membranes	Magnetic nano-inclusions with polymer matrix	Detects increased levels of sodium in saliva that can be possibly due to alveolar bone destruction	[16]
	Photoacoustic imaging	Melanin NPs	Contrast agent that enables broad photoacoustic absorption between 680 and 970 nm; non-invasive measuring of probing pocket depth	[17]

(continued)

Table 2 (continued)

Oral disease	Diagnostic technique	Nanomaterial	Properties	References
	Photoacoustic imaging	Melanin NPs	Novel hockey stick-shaped transducer detects the periodontal pockets with a photoacoustic contrast agent (cuttlefish ink)	[18]
	Nanobiosensors-based salivary biomarker detection	Platinum nanocluster with immobilization of a cholesterol enzyme	Non-invasive electrochemical biosensing of salivary cholesterol	[19]
Caries	Nanobiosensors-based salivary biomarker detection	Organic electrochemical transistors coated with platinum NPs	Selectively detect the presence of glucose and lactate in saliva	[19]
Inflammatory diseases	Nanobiosensors-based salivary biomarker detection	Graphenes; carbon nanotubes	Interleukin-1; interleukin-6; interleukin-8; biomarkers for DNA oxidative damage; cortisol	[11]
Periodontal disease	Lateral flow immunoassay	Up-conversion nanoparticles (G-UCNPs) as luminescence probe	Detect matrix metalloproteinases-8; Interleukin-1 beta; tumour necrosis factor-alpha	[20]
Oral Malodor	Fluorescent mouthguard	Zinc oxide–poly(dimethylsiloxane) (ZnO-PDMS) nanocomposite	Visualization of dental lesions using a fluorescent probe; identification of areas that release volatile sulphur compounds	[21]
COVID-19	Electrochemical nanobiosensors	Combination of graphene and carbon nanotubes	Better electrical property; chemical stability; increased surface area; better sensitivity and specificity to detect the virus was increased due to surface modification	[22]
COVID-19	Electrochemical nanobiosensors	Gold NPs conjugated with iron oxide NPs; graphene functionalized with p-sulfocalix arene	Detecting ultrasensitive RNA from SARS-CoV-2; reduced time and cost	[23]

This can be useful in identifying cases of severe periodontitis as increased levels of sodium in saliva can be due to alveolar bone destruction [16].

12 Photoacoustic Imaging of Periodontium

Melanin nanoparticles have been used in photoacoustic imaging as a contrast agent that enables broad photoacoustic absorption between 680 and 970 nm. This technique can be used for non-invasive measuring of probing pocket depth in patients with dental disease. Spherical melanin nanoparticles within the contrast agent that are capable of dynamic light scattering properties were used along with an ultrasound frequency of 40 MHz used to image the periodontal anatomy [17]. The imaging included the teeth, gingiva, and gingiva thickness with cuttlefish ink as a contrast medium. The results were highly precise (0.01 mm) than the traditional periodontal probes used by the clinician. The entire shape of the pockets was visualized with a standard deviation of 10% when done in a small sample size of 5 patients [17]. Another technique of photoacoustic imaging that used a novel hockey stick-shaped transducer showed highly correlated results in identifying periodontal pockets when it was validated with human subjects. Simultaneous imaging of up to four teeth per quadrant was possible with a bias of ~0.3 mm as compared to routine periodontal probing [18]. Similar to the previous study, the periodontal pockets were highlighted with the help of melanin nanoparticles that were within the photoacoustic contrast agent (cuttlefish ink).

13 Nanobiosensors for Salivary Biomarker Detection

Saliva can reflect the entire spectrum of the body's health and disease states. Several research have been done to develop microfluidics and micro-electromechanical systems for salivary diagnostics. This system utilizes small salivary samples and integrated detection methods to perform salivary diagnostics. High blood glucose levels result in defective chemotaxis of leucocytes and increase the risk of periodontal tissue. High glucose levels in saliva result in excess lactic acid in the plaque metabolism that can lead to an increased risk of dental caries. Organic electrochemical transistors coated with platinum nanoparticles [19] can selectively detect the presence of glucose and lactate in saliva. UV–ozone posttreatment enhances the catalytic ability of the Platinum NPs. High cholesterol levels can also increase the risks of the occurrence of periodontitis by increasing the alveolar bone resorption. A platinum nanocluster with immobilization of a cholesterol enzyme can be used for non-invasive electrochemical biosensing of salivary cholesterol [19].

Various types of salivary biomarkers can be detected using nanoparticle-based biosensors. Functionalization and chemical modifications of these NPs, such as graphenes and carbon nanotubes, create a hybrid compound that can detect various

substances in saliva. Presence of uric acid, interleukin-1, interleukin-6, interleukin-8, biomarkers for DNA oxidative damage, therapeutic drugs such as analgesic antipyrine and the anaesthetic benzocaine, ketamines, cardiac troponin I, cortisol, hunger hormone such as ghrelin and peptide YY, etc., and around half of these biosensors have been validated using the gold standard test of ELISA [11]. Potential salivary biomarkers linked to oral cancer, such as those belonging to the cytokeratin family, such as cytokeratin-19, have also been extensively studied. Silicon nanowire transistors have shown selectivity towards biomarkers such as tumour necrosis factor-alpha and interleukin-8, is emerging as a potential tool for the early detection of oral squamous cell carcinoma [10]. Up-conversion nanoparticle-based lateral flow immunoassay using a luminescence probe was developed to detect matrix metalloproteinases-8, interleukin-1 beta, and tumour necrosis factor-alpha in the gingival crevicular fluid, which are biomarkers of periodontitis. It showed high sensitivity and specificity in GCF and artificial saliva [20]. It showed high correlations when compared with clinical techniques and detected it in a very short duration of 30 min.

14 Oral Malodor

Another development is a fluorescent mouthguard for visualizing dental lesions [21]. Here, zinc oxide–poly(dimethylsiloxane) (ZnO-PDMS) nanocomposite was used as the fluorescent probe to identify the release of VSCs from sites with the carious lesion. This technique is highly sensitive to VSC levels and has high fluorescent stability in standard physiological conditions. The ZnO QDs with a wavelength of 565 nm were chosen for the mouthguards, as it has a high quantum yield of 3.5% with wavelength within the sensitive region of the cone cells of our eyes (550–570 nm). The nanoporous structures of the cured ZnO-PDMS nanocomposite exposed ZnO QDs and interacted based on the presence of gas molecules. These mouthguards emitted strong yellow fluorescence when viewed under ultraviolet (UV) light while transparent under natural light. CH_3SCH_3, CH_3SH, and H_2S quenched the fluorescence emissions in a time-dependent pattern among which H_2S was the most prominent. This device was also able to localize otherwise inaccessible sites of oral biofilm-induced lesions.

15 Viral Infections

The need to detect and prevent the spread of viral infections that can escalate into pandemics is increasing. Detection of SARS-CoV-2 (COVID-19) has been improved by including lightweight graphene and carbon nanotubes in electrochemical nanobiosensors. They result in better electrical properties, chemical stability, and increased surface area. The sensitivity and specificity to detect the virus increased

due to surface modification with certain functional groups or hybrid nanostructures. The ability of graphene to quench photoluminescence makes it helpful in designing optical sensor devices. Carbon nanotubes are also suitable nanoscale electrode biosensor transducers. An economical electrochemical biosensor with graphene integration has been used to quickly detect salivary and serum biomarkers for COVID-19 [22]. Another study showed the application of the modified electrochemical method for detecting RNA from SARS-CoV-2 reduced the time and cost as it did not need to go through the amplification and reverse-transcription steps. Gold nanoparticles conjugated with iron oxide NPs and graphene functionalized with p-sulfocalix arene enhanced the detection of ultrasensitive RNA from COVID-19 samples [23]. Gold NPs were immobilized on a graphene surface with the help of chitosan, and silver nanoparticle–graphene composite has also been researched to detect viral infections such as H1N1 viral infections and influenza-A viruses [22].

16 Potential Cytotoxicity

Nanocomposite materials that can seep through tissues can result in some degree of cytotoxicity. Their biocompatibility varies according to the constituting materials/particles. The large surface area-to-volume ratio of NPs results in bioaccumulation in distant organs. The number of nanoparticles that remains within the body depends on their concentration and the duration of exposure. The size of the nanoparticles also influences toxicity. Small silver NPs (~10 nm) exhibit greater cell penetration and toxicity than larger Ag NPs (20–100 nm). Similarly, the aspect ratio and surface area, crystal structure, and surface functionalization also play a part in toxicity [3]. Legislative, ethical, and regulatory issues related to nanodiagnostics need to be focused on in future [24].

17 Future Perspective

So far, we have briefly summarized the application of nanomaterials in diagnosing oral and dental diseases. But there are still several places that could be improved. The NPs help to visualize diseases, but most methods use artificial samples instead of clinical samples that are infectious in nature. Many of the findings we have are still at the proof-of-principle stage. Their validation and clinical application need to be taken forward. In detecting oral cancer, more focus should be on hybrid systems that can form a flexible platform for the NPs to diagnose and treat cancerous tissues. The detection techniques could be combined with artificial intelligence and machine learning algorithms to identify multi-bacteria and validate detection techniques. The use of NPs in fluorescence probes to detect bacteria, and point-of-care devices also need to be studied further extensively. These techniques will help translate laboratory procedures into point-of-care and home applications. Developing

new detection sensors for pathogenic bacteria using optical methods with the help of noble metal nanoparticles would be helpful in the early detection of biofilm-induced diseases. Materials like graphene and carbon nanotubes should be further explored for producing miniatures of electrochemical transducers into wearable, flexible biosensors to constantly monitor disease and offer personalized care. The potential to develop hand-held devices to check biomarkers of diseases using NPs should also be explored such that signal read-out circuits can be integrated into a smartphone. The most common hand-held electrochemical device for checking biomarkers is glucometers. The use of carbon-based nanostructures such as graphene and carbon nanotubes can be an opportunity to improve this technique to measure various biomarkers that could bring down the healthcare cost and enable early monitoring of diseases, especially during times of the COVID-19 pandemic. Electrochemical transducers interfaced with graphene and carbon nanotube serve as powerful tools for detecting various disease biomarkers. A combination of cutting-edge technologies such as nanodiagnostics, electrochemical biosensors, and 3D printing holds great potential for developing point-of-care devices that can help clinicians rapidly and efficiently diagnose oral and dental diseases.

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Chapter 3
Nanotechnology for Oral Disease Prevention

R. M. Baiju and Sabu Thomas

1 Introduction

Nanotechnology has an important role in our daily life ranging from tiny devices used for security purposes to most advanced nanoparticle-based treatments like drug delivery, medical imaging, preventive dentistry, etc. Despite its minute size, the nanoscale has enormous potential. It opens a new horizon for improvement in health care, be it in the field of diagnosis, prevention, or therapeutics.

For nearly one hundred years, dentistry has followed the same hygiene regimen to prevent oral disease. While fluoride treatments and sealants had a significant positive impact on oral health, there still exists a large unaddressed gap in the prevention of oral disease. Dental caries, which has a substantial disease burden worldwide, is the fourth most expensive condition to treat, according to the World Health Organization (WHO) [1]. According to a WHO research report from 2005, "adult dental caries is very widespread internationally, affecting around 100% of the population in the majority of nations" [2]. This extreme level of disease not only leads to tooth loss but also considerably place strain on the function and financial status of individuals. Here comes the role of prevention of oral diseases, before they take away our health, time, or wealth. Nanotechnology appears to be highly promising in this regard in that it is expected to bring positive impacts on dentistry, health care, and human life more profoundly than any recent developments as it is cost-effective and time-bound.

The following graph shows the year-wise number of publications on the role of nanoparticles in caries prevention (Figs. 1 and 2). The scope of future research in

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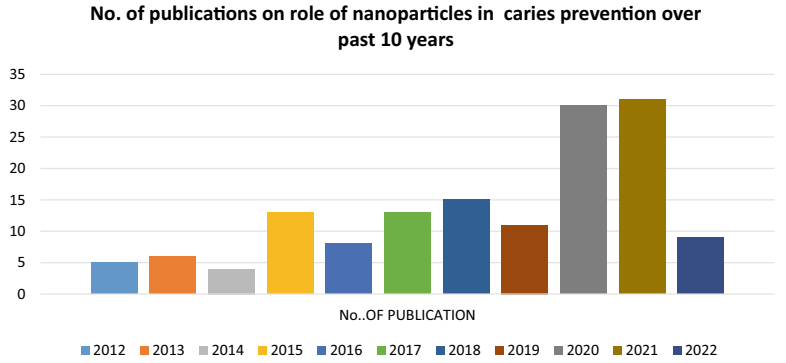

Fig. 1 Publications in the last decade regarding nanoparticles in dental disease prevention

this field is going to be tremendous, and nanotechnology is expected to transform the domain of caries prevention.

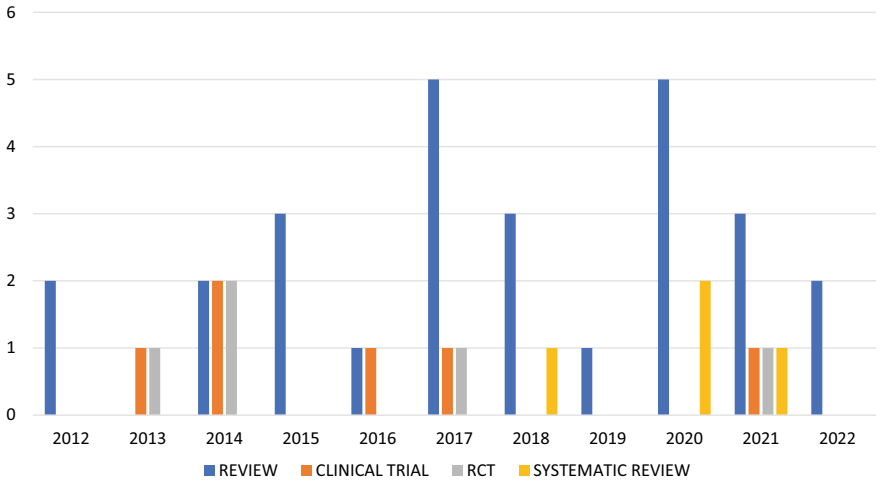

Fig. 2 Type of articles published during last 10 years

2 Dental Caries Prevention

2.1 How Do Caries Occur?

Dental caries is a term used to indicate a physiological breakdown in the biological tissues of teeth [3, 4]. A biofilm coats the oral environment, which is quickly re-established after brushing, or flossing removes it mechanically. The oral biofilm will renew, develop, and disperse after reformation unless it is interrupted again. During the development phase, these cariogenic biofilms sequester and retain oral acids. This decreases the pH in the oral cavity, and when the pH of the oral microenvironment drops below a critical threshold of 5.5 [4], cariogenic disease processes begin to emerge.

3 Limitation of Present Preventive Modalities

Preservatives and needless additional substances, such as alcohol, SLS, and parabens, are used in almost all modern dental products. These substances are not only hazardous to the oral environment but are also added to products to promote an acid–base model, enhancing fluoride absorption and extending the product's shelf life. Although fluoride has been demonstrated to be taken up in acidic environments, the exact quantity of acidity necessary has yet to be determined [4]. This acidic model has a number of flaws, but it does provide a low-pH environment, which cariogenic species favour. Furthermore, most dental products are unable to penetrate the biofilm's exopolysaccharide (EPS) structure, having little to no effect on the plaque fluid, which can lead to oral acid accumulation as the biofilm matures. Not only does the pH of the oral microenvironment have the ability to promote or inhibit remineralization, but it also induces changes in the types of bacteria present and there will be a shift towards increased cariogenic activity. In addition, the Point of Zero Charge (PZC) concept denotes that calcium exchange with hydroxyapatite (HA) is pH-dependant [4]. As the pH decreases, calcium attraction to HA is severely reduced, and instead favours hydronium ions (H+). This is especially true when the pH level dips below 5.5. Calcium's attraction to HA is only restated when there exists a stable, non-acidic environment. So all these findings suggest to revisit older modalities of prevention.

4 Role of Nanotecnology in Caries Prevention

With the development of engineered nanotechnology, dental biofim can now be penetrated deep while simultaneously stabilizing pH at 14. Silver nanoparticles are usually employed for this purpose. Silver, in its many forms, has been used for thousands

of years as a natural antimicrobial, antibacterial, antifungal, and antiviral agent. However, previous attempts to utilize silver compounds have been sullied by the lack of a delivery mechanism for use in the oral microenvironment. Silver, in its ionic form, is not effective, as the silver ions interact with various salts, enzymes, proteins, and saliva in the oral microenvironment. These interactions form by-products which cannot efficiently penetrate or alter the oral biofilm. Nanoparticles can efficiently overcome this problem. Nanoparticles, until recently, could not remain stable enough to be used in the oral microenvironment.

However, currently nanoparticles can be made using a capping agent which coats their surfaces and protects them from dissolving into ions [5]. It is this protection mechanism that allows nanoparticles to be effective over long periods of time while remaining stable at a small size. Nanoparticles can penetrate through biofilms and retain their properties before releasing ions, allowing them to be used with other combination agents and under multiple conditions. Nanoparticles to be effective in oral therapeutics must not only be alkaline but also be stable in the oral environment. However, this method is not challenge-free, as many properties of nanotechnology have yet to be elucidated; salt stability remains an obstacle for most nanotechnology applications, limiting their use.

Engineered nanoparticles can now be designed with a coating that matches the exopolysaccharide (EPS) bacterial coating of the biofilm. This provides a mechanism for selection while preventing the nanoparticles from being disrupted by other ion activity in the oral microenvironment. This not only provides a strong buffering capacity against acid attacks but also offers antibacterial and antimicrobial properties at a concentration of 1/80th that of Chlorhexidine (CHX) [6]. These particles can also be made completely non-cytotoxic to oral tissues, thanks to their superior coating and slow release of nanoparticles over time. This antibacterial, biofilm-penetrating, alkalizing agent stabilizes the PZC exchange complex around the tooth. Furthermore, it provides optimal calcium and phosphate delivery, subsequently stabilizing pH and thereby helping in prevention of dental caries as well as hindering the advancement of the condition.

4.1 Nanomaterials in Managing Oral Biofilms

The integral aspect of preventive dentistry is the prevention of the development of oral biofilm. To this effect the nanoparticles must typically be positively charged with particle sizes less than 130 nm in order to penetrate a negatively charged extracellular polymeric matrix of biofilm. Furthermore, the form of the particle is important because nanomaterials like graphene oxide have nano blades on their edges, and surface protrusions with nano-tipped spines can pierce bacterial cell membranes resulting in the release of intracellular components eventually leading to cell death.

Bacterial biofilms on the tooth's surface are responsible for dental caries, and complicated interactions between acid-producing bacteria and host factors are the modulating factor that controls caries formation. All solid substrates rapidly develop

a proteinaceous surface coating, known as a pellicle, upon exposure to oral fluids. The characteristics of the substrate are altered by this conditioning layer, which determines the surface charge and the type of chemical groups exposed at the surface. Dental plaque is a biofilm made of bacteria that colonizes the surface by attaching to the pellicle through adhesin–receptor interactions. Unique bacterial interactions (including quorum sensing and coaggregation) and heterogeneous bacterial populations define plaque maturation [6]. Each human host is a unique ecosystem of bacteria [6], and it is believed that the metabolic interactions between various bacterial species are crucial to the formation of the biofilm. Thus, particularly in the presence of dietary carbohydrates, the number of bacteria that cause caries, such as *streptococci and lactobacilli*, may develop. These bacterial species cause demineralization beneath the surface of the tooth by producing acids as by-products of the metabolism of fermentable carbohydrates.

Anti-adhesive surface coatings can be utilized in addition to traditional oral care to prevent the growth of dental biofilms since nanostructured surface topography and surface chemistry can both affect early bioadhesion [7, 8]. An illustration of a self-cleaning surface is the classic lotus effect in ultra-hydrophobic surfaces [9, 10]. However, due to surface wear and equilibration of the surface nano-topography by the pervasive pellicle layer, such nanostructured surfaces are not appropriate for usage in the oral cavity. Wear-resistant nanoparticles are being used in preventive dentistry to help avoid the pathogenic effects of persistent intraoral biofilm development over a longer period of time.

Nanomaterials for application in a range of oral healthcare products have recently been developed using biomimetic methods. For managing biofilms on the tooth surface, liquids and pastes containing hydroxyapatite particles are suitable, and those containing nanoparticles act as remineralizing agent in submicrometer-sized enamel lesions. However, research is currently being carried out in pursuit of non-technology solutions for the treatment of larger visible cavities.

Nanocomposite surface coatings have been created to modify the tooth surface in vivo [9]. Inorganic particles smaller than a nanometer are incorporated into a fluoropolymer matrix to create surface characteristics that are simple to clean [9]. Under the impact of physiological shearing forces in the mouth, these biocompatible surface coatings, also known as theta surfaces, can assist the detachment of adsorbed salivary proteins and adhering bacteria [10]. Patients with high caries risk may benefit from easy-to-clean coatings, such as those with xerostomia, a disorder that causes mouth dryness due to malfunctioning salivary glands. This also applies to people who are unable to practise good dental hygiene. Applications for dental sealants, coatings for restorations, dentures, or transmucosal use are all possibilities. The shear stresses from brushing teeth might make it easier to clean even tooth fissures that have been sealed with this substance.

Oral healthcare products containing bioinspired apatite nanoparticles, either alone or in conjunction with proteinaceous additives such as casein phosphopeptides, are yet another nano-enabled strategy for managing biofilms [11, 12]. Amorphous calcium phosphate (ACP) nanocomplexes with a diameter of 2.12 nm, stabilized by casein phosphopeptide (CPP), appear to be prominent in biomimetic approaches to

managing biofilms. CPP-ACP-treated germanium surfaces that are applied in the oral cavity for up to one week have been shown to significantly slow the formation of biofilms [11]. In vivo evidence suggests that CPP-ACP complexes reduce bacterial adherence by binding to the surfaces of bacterial cells, the core supporters of the intercellular plaque matrix, and to adsorbed macromolecules on the tooth surface.

It should be highlighted, nonetheless, that the study's clinical applicability is still restricted because germanium is not a biomineral. Other in vitro studies have demonstrated that non-aggregated and clustered hydroxyl apatite nanocrystalline particles, with an average size of 100 nm × 10 nm, may adhere to the surface of bacteria and interact with bacterial adhesins to prevent germs from adhering to the tooth surface. These size-specific effects of the apatite nanoparticles underscore the bioinspired approaches to biofilm control, which are believed to be more successful than conventional methods that employ toothpaste containing micrometer-sized hydroxyl apatite. The biomineral hydroxyl apatite (HA) has been used for years in preventive dentistry, but only nano-sized particles that are smaller than the size of a microorganism can interact effectively with bacteria.

The development of hybrid nanomaterials for the prolonged release of antibacterial medications and an improved affinity for enamel is a new advancement in the field of nanotechnology. For instance, the antibacterial mediator triclosan and the tooth-binding agents diphosphoserine and pyrophosphate are both included in the polymeric-based micelle system (Pluronic® P123). Another illustration is the interaction of silver fluoride nanoparticles with Streptococcus mutans due to the increased surface area of silver. Fluoride generates fluorapatite (FA), which requires a lower critical pH than hydroxyapatite (HA) to resist dissolution. Through the ionic gelation of tripolyphosphate nanoparticles, sodium fluoride is additionally loaded into chitosan, which also has inhibitory effects on S. mutans, to more efficiently distribute fluoride. Additionally, a hybrid nanoformulation with silver fluoride and chitosan that combines all the aforementioned benefits has been introduced.

5 Antibacterial Nanotherapy

Antimicrobial nanoparticles may prevent dental caries by preventing bacterial growth. Dental composites or dental adhesives have been infused with various nanoparticles (such as silver, zinc oxide, and polyethylenimine) to prevent bacterial development in a variety of ways. These processes include

- Bacterial cell membrane disruption.
- Inhibition of metabolism by disruption of active transport of sugars.
- Reactive oxygen species (ROS) production.
- Oral biofilm enzyme dysfunction by displacement of magnesium ions.
- Disruption of electron transport across the bacterial membrane.
- Inhibition of DNA replication.

In an in vitro model, nanoparticles were successful in decreasing the S. mutans and Lactobacillus acidophilus biofilms [13, 14]. Antibacterial nanocoating applied to tooth surfaces has been shown to be successful in eradicating bacteria, preventing bacterial adherence, and maintaining tooth integrity when exposed to biological fluids like saliva. Silver nanoparticles are important nanocoatings that are widely used for this purpose.

5.1 Antimicrobial Dental Nanomaterials for Caries Prevention

Nanoparticles are typically 1–100 nm in size. They might appear as nanorods, atomic clusters, spots, fibres, grains, films, or nanopores with a large surface area. They outperform conventional materials in terms of physicochemical qualities [1, 15]. Antimicrobial, antiviral, and antifungal properties of nanoparticles have been discovered. Furthermore, nanoparticles may improve the mechanical characteristics of dental materials, inhibit crack propagation, and improve fracture toughness. As a result, nanoparticle applications in dentistry have exploded in recent times. Metallic nanoparticles promote biomineralization by allowing demineralized (carious) tooth tissues to remineralize. Metallic nanoparticles can also overcome challenges in a range of oral conditions because of their ion balance in the oral environment. Researchers and physicians have studied many nanoformulations for caries prevention based on their potential advantages in diverse applications. There are two key ways that nanotechnology aids in the treatment of dental caries. The first strategy involves remineralization, which employs nanomaterials with the potential to release calcium and fluoride, namely calcium phosphate, calcium fluoride, hydroxyapatite, and fluorohydroxyapatite. The second strategy includes administering antibacterial nanoparticles such zinc oxide, silver, and quaternary ammonium polyethylene amine [16, 17].

6 Silver Nanoparticles

Silver nanoparticles are antibacterial agents with a broad range that can be utilized to prevent caries with no demonstrable microbial resistance. The particles' large surface area enables them to adhere to bacteria's outer cell membrane, disrupting the permeability and cell structure of the bacteria. As a result, silver nanoparticles may efficiently destroy bacterial cells at low concentrations [18, 19]. Silver nanoparticles have been used in caries control in vitro, and in clinical investigations. Various formulations of silver nanoparticles have been tested for their ability to inhibit caries-producing bacteria in research studies. Clinical isolate planktonic Streptococcus mutans and their mature biofilms are inhibited by silver nanoparticles

[20]. They also improve the microhardness of tooth tissues and have antibacterial properties [21]. The effects of silver nanoparticles integrated into dental materials were compared to silver nanoparticles alone. Remineralization was shown to be aided by silver nanoparticles in conventional sealants [22]. On the smooth enamel surface, the orthodontic bracket coated with silver nanoparticles exhibited efficiency in inhibiting S. mutans and reducing caries [23]. Silver nanoparticles have a comparable effect on caries prevention as other cariostatic drugs [24]. Silver nanoparticles put into a dental appliance made of poly methyl methacrylate or acrylic can suppress planktonic proliferation of cariogenic bacteria and biofilm formation while maintaining biocompatibility and mechanical qualities [25].

Silver-based nanomaterials may target multiple sites inside the cell at a relatively low concentration (0.5–1.0%) to stop bacterial development, which makes them efficient against biofilms [26–28]. Streptococcus mutans [29] was the subject of a comparative study to determine the antibacterial effects of chlorhexidine, silver, titanium dioxide, and silicon dioxide (SiO_2) nanoparticles (AgNPs, 60 nm; TiO_2 NPs, 23 nm; SiO_2 NPs, 14 nm). The results showed that $AgNO_3$ and AgNPs had the most effective bactericidal effects. In the silver nanoparticles' antibacterial action, proteoglycans found inside bacteria and on their cell membranes appear to function as locations where silver ions and AgNPs can bind. Silver ions can also bind with sulfuryl groups during the production of proteins, preventing the replication of bacterial DNA.

Silver nanoparticles provide multiple modes of action in order to prevent caries:

1. Penetrance of biofilms and disruption of biofilm attachments.
2. Prevention of bacterial adhesion to enamel.
3. Act as a delivery system for remineralizing agents.
4. Act as an alkaline neutralizing agent for oral acids.
5. Act as an agent to reduce acid release from oral bacteria.
6. Silver ion substitution into HA lattice (Remineralization).

When preparing AgNPs for use in dentistry, the following key factors must be taken into account:

(i) Nanoparticles bigger than 50 nm cannot penetrate dental biofilms due to an inverse association between size and efficacy in the diffusion of nanoparticles into the biofilm.
(ii) The presence of carboxyl and phosphoryl groups on the bacterial surface, which make the cell surface electronegative, may prevent negatively charged nanoparticles from diffusing through the biofilm.

Although silver nanoparticles have above-mentioned drawbacks, they are currently one of the most commonly utilized nanomaterials for caries prevention.

7 Gold Nanoparticles

Gold (Au) nanoparticles have antimicrobial activity against both bacteria and fungi. The antibacterial activity of gold nanoparticles (Au NPs) has been found to be enhanced when combined with tetracycline or ampicillin [30, 31]. Au NPs reduce ROS generation by lowering lipopolysaccharide-induced cytokine production, such as IL-1, IL-17, and TNF, and regulating the mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways [32]. In comparison to other nanoparticles, the concentration of Au NPs required to produce the intended effect is higher. Furthermore, they are said to have poor antibacterial action, making them less desirable for caries prevention than other nanoparticles.

8 Calcium Nanoparticles

The major inorganic component of teeth is hydroxyapatite (Ca5(PO4)3(OH)), which is made up of calcium and phosphate. Preventing caries requires a healthy balance of calcium and phosphorus [33]. In dental offices, insoluble calcium phosphates are difficult to use. Due to the intrinsic insolubility of calcium phosphates, soluble calcium and phosphate ions can only be employed at extremely low concentrations.

Furthermore, soluble calcium and phosphate ions do not mix well with dental plaque or form deposits on the tooth surface. As a result, in the remineralization process, the bioavailability of phosphate and calcium ions is always restricted. However, recent advances in nanotechnology have demonstrated the use of a variety of calcium nanoparticles in diverse applications. Calcium and phosphate ions can be recharged and released by amorphous calcium phosphate nanoparticles. As a result, nanoparticles of amorphous calcium phosphate can promote remineralization by releasing calcium and phosphate ions over a lengthy period of time. Because of their potential to remineralize lesions and thus supress dental caries, amorphous calcium phosphate nanoparticles can be added to orthodontic cement to prevent white spot lesions that develop during orthodontic treatment in certain patients [34]. In a biofilm model, an adhesive containing amorphous calcium phosphate nanoparticles can remineralize dentine lesions, form a strong bond interface, suppress secondary caries, and increase the life span of dental restorations. The remineralizing and antibacterial properties of amorphous calcium phosphate nanoparticles can be achieved by combining them with a variety of additional organic or inorganic agents. Various studies have employed quaternary ammonium methacrylate-amorphous calcium phosphate nanoparticles for remineralization, prevention of biofilm formation and lactic acid production, and increased dentine bond strength. The repair of the dentine–pulp complex and dentine production can be aided by a composite comprising quaternary ammonium methacrylate-amorphous calcium phosphate nanoparticles. According to one study, 2-methacryloxylethyl

dodecyl methyl ammonium bromide and amorphous calcium phosphate nanoparticles reduced demineralization and inhibited biofilm development while maintaining the shear bond strength of resin composites [35]. In an artificial caries scenario, a salivary slathering-protein-inspired polyamidoamine dendrimer combined with amorphous calcium phosphate nanoparticles could enhance enamel remineralization. An adhesive resin with triple agents of shells comprising triethylene glycol dimethacrylate, quaternary ammonium methacrylate, and amorphous calcium phosphate nanoparticles for the prevention of secondary caries was discovered to have antimicrobial and remineralizing properties. Other calcium-containing nanoparticles have recently been investigated; a dentifrice comprising nano-carbonated apatite and fluoride was shown to be an efficient remineralizing agent for preventing caries lesions in the early stages [36]. Fluoride may be combined with calcium and phosphate to generate fluorapatite (Ca5(PO4)3F), a more acid-resistant variant of hydroxyapatite. Fluoride can help to speed up the remineralization of carious lesions and prevent demineralization of enamel and dentin. Because of the high level of labile fluoride concentration in oral fluids, calcium fluoride nanoparticles were employed as anticaries agents. Calcium fluoride nanoparticles were shown to significantly decrease exopolysaccharide synthesis and limit biofilm formation in an in vitro investigation [37]. In addition, calcium fluoride nanoparticles can boost the remineralization effect. Therefore, calcium fluoride nanoparticles in nanocomposites provide great mechanical durability, strength, and continued fluoride release from the restoration.

9 Copper Nanoparticles

Copper nanoparticles can prevent root caries by inhibiting the development and colonization of *Streptococcus mutans* on root surfaces of teeth [38]. Silver nanoparticles are more expensive than copper oxide nanoparticles. With a large surface area and crystalline structure, they offer attractive physical qualities. Copper oxide nanoparticles are bactericidal particularly against cariogenic bacteria [39]. Copper oxide nanoparticles may be easily incorporated into polymers to create composites with distinct physiochemical characteristics. They can be included in dental adhesives to prevent early or carious white spot lesions because of the added antibacterial properties without compromising shear bond strength.

10 Titanium Nanoparticles

Because of their high biocompatibility, bioactivity, and antibacterial activity, titanium nanoparticles have potential applications in dentistry. Due to its potential to create microscopic holes in bacterial cell walls that enhance permeability and ultimately induce cell death, titanium nanoparticles can be used as an antibacterial treatment

against cariogenic bacteria and biofilm. Titanium nanoparticles also have a high degree of stability, photocatalytic activity, and reusability, making them a viable option for caries prevention.

Titanium dioxide nanoparticle has been extensively examined in the prevention of dental cavities. A promising restorative material for dental caries prevention is glass ionomer cement containing titanium nanoparticles. The addition of titanium nanoparticles to restorative glass ionomer cement increases microhardness, antibacterial activity, and flexural and compressive strength of the restoration without compromising enamel and dentine adhesion.

11 Magnesium Nanoparticles

Acid assault causes demineralization of hydroxyapatite in the caries process. As a result, alkaline nanoparticles may be a viable option for the prevention of caries. Magnesium is an alkaline metal that accounts for around 0.5% and 1% of the composition of enamel and dentin respectively. Through the release of magnesium ions, appropriate levels of serum magnesium might slow the progression of dental caries. The antibacterial and biofilm activity of magnesium oxide-nanoparticle-modified glass ionomer cement against cariogenic bacteria is substantial. So magnesium nanoparticles play a decisive role in caries prevention.

12 Zinc Oxide-Based Nanoparticles (ZnO NPS)

Dental cements have mostly consisted of zinc as a filler material. When zinc oxide is present as nanoparticles, the antibacterial activity of zinc ions is increased. Against S. mutans, Ag/ZnO nanocomposite exhibited improved antibacterial activity. The antibacterial process includes the direct destruction of cell structure and membrane function as well as the production of reactive oxygen species (ROS) to oxidize biomacromolecules.

Zinc oxide nanoparticles added to resin composites at a concentration of 2–5% can give antibacterial capabilities without affecting other qualities [40, 41]. Antimicrobial activity, hybrid layer integrity, and adhesive mechanical characteristics may all benefit from the inclusion of ZnO and Cu nanoparticles in universal adhesive systems. In the oral cavity, a Zn-containing mouthrinse has shown great substantivity and is potent against S. mutans. The sole drawback to using zinc ions in mouth rinses is that they have an unpleasant astringent taste.

The capacity of ZnONPs to interact with the cell membrane of several bacterial species may be the cause of their bactericidal activity and can be attributed to the following;

- Zn forms a strong bond with proteins and lipids that alters the osmotic balance and enhances membrane permeability [17, 42].
- ZnONPs can produce Zn2+ and ROS, which can also prevent the development of planktonic bacteria build up, increasing oxidative stress within the bacterial cell.

It is essential to draw attention to two crucial characteristics of ZnONPs' biological impacts as given below:

(i) These nanoparticles exhibit dose-dependent toxicity.
(ii) Compared to the antibacterial activity of the metal in its bulk condition, coating and functionalization of ZnONPs change zinc's antibacterial characteristics. Despite these encouraging effects, ZnONPs are significantly cytotoxic to human gingival fibroblasts. However, when these nanoparticles are incorporated in sodium-phosphorylated chitosan, which serves as a matrix for the creation of antimicrobial periodontal dressings [43], this toxicity is much decreased.

13 Titanium Dioxide-Based Nanoparticles (TIO_2NPs)

When subjected to near-UV and UVA light, TiO_2NPs undergo photocatalysis, generating ROS, mostly H_2O_2 and OH, which change the osmotic balance of bacteria. Additionally, TiO_2NPs have been shown to interfere with phosphorylation and result in oxidative cell death [44, 45]. TiO_2, even when unirradiated, may display antibacterial action, the mechanism of which is still unknown.

14 Chitosan Nanoparticles

N-acetyl-glucosamine and glucosamine residues randomly organized along a long polymer chain make up chitosan, a substance that has lately been proposed as a possible antibacterial agent for dental uses. Chitosan's composition, which includes deacetylated C2 amino groups that become protonated and positively charged at pH 6.5, may be the cause of its antibacterial properties. Thus, chitosan binds to bacterial cells leading to

- The outflow of ions and proteins from the microbial cell which in turn is due to increase in membrane permeability.
- Inhibition of mRNA transcription and changes in protein translation.

Chitosan binds to the lipoteichoic acid found in Gram-positive bacteria, changing the way that microbial membranes operate. Chitosan polycations compete with divalent cations in Gram-negative bacteria and engage in electrostatic interactions with the bacterial cell membrane. It is widely known that Mg_2+ or Ca2+ stabilize the bacterial outer membrane. The cell wall is disrupted when these ions are replaced with chitosan, and the activity of the degradative enzymes are also altered. Chitosan

can thus be used in the field of preventative nanodentistry because of its antibacterial properties.

15 Chlorhexidine

Chlorhexidine (CHX) has broad-spectrum antibacterial action and is a commonly used antiplaque agent [46, 47]. Two techniques are utilized to stop the rapid and uncontrolled release of free CHX from resin matrices: encapsulation and nanoparticulation. Due to the high rate of bioavailability and penetration of nano-encapsulated particles, the biological efficacy and cytotoxicity are increased [48]. Aggregatibacter actinomycetemcomitans, E. faecalis, Fusobacterium nucleatum, S. mutans, P. gingivalis, and S. sobrinus were among the bacteria against which the antibacterial ability of CHX nanoparticles was demonstrated in both planktonic and biofilm forms [49].

Nanocarriers such as spherical poly-lactic-co-glycolic acid, poly (ethylene glycol)-block-poly-(L-lactide), nano-silica wires, and spheres have all been studied for the sustained dispersion of CHX in the oral environment. In single or mixed cultures, C. albicans and S. mutans may not develop biofilms when exposed to the iron oxide magnetic nanoparticles (IONPs) and chitosan present in the CHX carrier nanosystem, as shown in a recent study [50]. CHX nanoparticles aid in creating a CHX-rich oral environment for a longer period of time and at a higher concentration than a conventional CHX digluconate solution. Furthermore, the treatment of oral conditions related to biofilms, such as dental caries, is aided by the hypothesized antibacterial effect of CHX nanoparticles.

16 Bioactive Glass

Due to higher Ca/P ratios and greater surface area, bioactive glass nanoparticles (BAG NP) outperformed ordinary BAG in terms of remineralization capacity thus delaying the progression of dental caries [50, 51]. When BAG NPs are exposed to an aqueous solution, they take on a mesoporous structure, allowing apatite to develop on the dentin surface. The precipitation of HA is triggered by an increase in pH. The mineralizing process is triggered by calcium and phosphate ions in the bioactive glass, along with minerals from saliva [52]. BAG NPs have been shown in vitro to increase dentin acid resistance by causing mineral formation on dentin surfaces [53, 54]. The new hydroxyapatite layer generated is comparable to enamel or dentin in appearance and abrasion resistance [55]. In comparison to sodium monofluorophosphate toothpaste, the fluoride-containing bioactive glass exhibited a higher ability for remineralization. The antibacterial benefit of bioactive glass nanoparticle toothpaste was also reported. S. mutans in biofilm can be inhibited by BAG NPs [56, 57]. By releasing alkaline ions that generate an increase in pH, BAG NPs can create an unfavourable environment for bacterial development. The addition of fluoride to

BAG increased the resilience of the material to acid degradation, allowing fluorapatite to develop on the tooth surface. Dentinal tubules were blocked and permeability was reduced as a result of the fluorapatite deposit on the dentin surface. Antibacterial activity against cariogenic bacteria, suppression of demineralization, and encouragement of remineralization are the major modes of action of nanoparticles of bioactive glass in caries control.

16.1 Reversing an Incipient Caries—Biomimetic Remineralization

Remineralization of early caries lesions has been described using a number of nanotechnological methods. In laboratory, animal, and human studies, CPP-ACP nanocomplexes have been demonstrated to support enamel remineralization and have anticariogenic efficacy. By forming amorphous nanocomplexes, the casein phosphopeptides stabilize calcium and phosphate ions. These complexes contain biologically usable calcium phosphate that can be used to remineralize carious lesions.

Recently, biomimetic therapy for early caries lesions has become more popular. The use of several forms of nano-sized particles of hydroxyapatite or calcium carbonate has received a lot of attention. Another approach is the employment of nanofillers as a component in resin restorations which induces the anticaries activity of restorations. Failure of dental restoration is primarily due to secondary caries and restoration fracture, reducing the lifespan of restorations. To prevent demineralization caused by caries at the resin composite–tooth interface, calcium and phosphate ion-releasing nanofillers have been developed, such as dicalcium phosphate anhydrous (112 nm in size) and amorphous calcium phosphate (116 nm in size). When the pH of the resin composite is reduced in vitro, these additives allow the resin composite to release calcium and phosphate, giving caries-inhibiting capabilities. Nanocomposites comprising 40% amorphous calcium carbonate nanoparticles have been found to rapidly neutralize a lactic acid solution with a pH of 4.0 by increasing the pH to 5.69 within 10 min.

Restoration fracture and secondary caries may be reduced using nano-CaF2-containing composites with strong flexural strength and long-term fluoride release. CaF2 and dicalcium nanocomposites have recently been discovered.

Novel nanocomposites containing antibacterial compounds such as CHX (10%) and quaternary ammonium dimethacrylate (7%) alone or in conjunction with silver nanoparticles (0.028%), as well as calcium and phosphate ion-releasing nanofillers, have recently been developed. Biofilm colony-forming unit counts, metabolic activity, and lactic acid generation of S. mutans biofilms were all reduced when these antibacterial components were incorporated into nanocomposites [58–60].

16.2 Calcium Carbonate Nanoparticles

Calcium carbonate nanoparticles effectively prevent the onset of caries. Calcium carbonate (CC) nanoparticles may be retained well on oral surfaces due to their colloidal particle size and capability for delivering calcium ions. They serve as a delivery system for the gradual, continuous release of calcium ions in high concentrations into the oral fluids around them (saliva and dental plaque). Additionally, CC nanoparticles may raise the pH of the surrounding fluid. Accordingly, when added to an experimental tooth dentifrice, CC nanoparticles were successful in remineralizing developing enamel defects. Compared to its macro counterpart, nanoscale calcium fluoride (CaF2), which serves as a labile reservoir for fluoride (F), has been shown to be extremely soluble and reactive with dicalcium phosphate dihydrate. Due to CaF2's high solubility, its interaction with dicalcium phosphate dihydrate may consume a significant quantity of CaF2, which allows a significant amount of F to be integrated into the stable reaction product (apatite). As a result, a mouthwash containing CaF2 nanoparticles demonstrated more F deposition (2.20.3 g/cm^2) than a rinse made of sodium fluoride (NaF) (0.310.06 g/cm^2). By raising the F-content in oral fluids and hence promoting tooth remineralization, the CaF2 rinse has the potential to be employed as an anticaries agent.

16.3 Hydroxyapatite Nanoparticles

Other biomimetic methods for remineralizing early enamel erosions are based on nanoscale hydroxyl apatite particles [15]. Due to their comparable form, crystallinity, and chemical composition (Ca10(PO4)6(OH)2) to enamel nanocrystals, hydroxyapatite (HA) nanoparticles can be used to replace lost enamel nanocrystals. Because they enhance the surface area for binding and permit stacking of the nanocrystallites, HA nanoparticles can be used as a filler to fix minor depressions on enamel. For instance, it has been noted that HA, which has a size of 20 nm, may effectively occupy space within acidic erosion-induced nanodefects. The newly formed biomimetic mineral covering can then be created using the deposited and adsorbed HA nanoparticles. Adapting the size of the apatite particles to the scale of the sub-micrometer- and nano-sized diameter by erosive demineralization of the natural apatite crystallites can considerably increase healing at the enamel surface [27]. The 20 nm size of hydroxyl apatite matches the dimensions of the nanodefects produced at the enamel surface during acidic erosion quite well. Under in vitro settings, these particles tightly adhere to the etched enamel surface and, interestingly, prevent further erosive demineralization. Therefore, the use of properly sized nano-apatite particles might simultaneously help to treat and prevent enamel lesions caused by early erosive lesions.

While the collagen network is unaffected in the early stages of caries attack, acids produced by bacterial metabolism induce mineral loss from the hard tissue. This organic framework is remineralized using HA nanoparticles (HA NPs), which can

function as a transporter for lost ions or as direct replacements for missing minerals. By replenishing calcium and phosphate ions in areas where minerals have been dissolved, HA NPs have been utilized in dental care products like dentifrices and mouthwash to encourage enamel remineralization and restore enamel integrity [61]. In situ research with toothpaste containing HA NPs showed that the nanoparticles may enter dental porosities and create a protective coating on the tooth's surface [62].

HA NPs, which are found in toothpaste and provide a biomimetic covering that matches the biological hydroxyapatite of enamel in appearance and structure, help in enamel regeneration. The new layer of apatite demonstrated resistance to toothbrushing because of chemical linkages between the artificial and natural enamel crystals. On artificially produced incipient caries-like lesions, nano-HA paste showed a protective layer with globular deposits in contrast to fluoride varnish and casein phosphopeptide–amorphous calcium phosphate (CPP-ACP). With HA NPs, including pit and crack sealants, a remineralized region was seen at the sealant–enamel interface [63]. Additionally, they revealed improved ion release and a higher degree of conversion [64]. Dental composites containing HA NPs improved enamel remineralization at a potentially cariogenic pH of 4 [65].

17 Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP)

CPP-ACP nanocomplexes inhibited demineralization and increased remineralization of enamel in vitro and in vivo investigations by localizing at the tooth's surface, buffering phosphate and calcium-free ion activities, and maintaining super-saturated state. They bind plaque and tooth surfaces to generate a calcium and phosphate reservoir [66]. CPP-ACP NPs with *L. rhamnosus* (probiotic strain) were shown to have efficient remineralizing and antibacterial properties in toothpaste [67]. To remineralize early dental caries and white spot lesions, CPP-ACP and fluoride were indicated. When compared to fluoride, CPP-ACP demonstrated a somewhat poorer capacity for remineralization of early enamel caries. When additional caries preventative therapies like dental sealants and resin penetration are available, CPP-ACP nanocomplexes cannot be employed as a substitute for fluoride.

18 Nanocomposites for Caries Prevention

Several Ca2+ and PO4 3-releasing dental nanocomposites were established for their remineralizing effect since Ca2+ and PO4 3-are necessary for remineralization; as a result, they aid in avoiding recurring (secondary) decay around or under restorations. As Ca2+ and PO4 3-releasing fillers, various kinds of nano calcium phosphates, such as dicalcium phosphate anhydrous, tetra calcium phosphate, monocalcium phosphate

monohydrate, and carbonate hydroxyapatite, were employed. Degradability and the volume percentage of the CaP form affect the release of Ca and PO4. Since these nanoparticles are combined with additional fillers, like whiskers fused with nano-sized silica, they might still be employed for high-stress bearing applications. When the pH is lowered from neutral to a cariogenic level, the Ca2+ and PO4 3-can also be released on demand, and the level of their release rapidly increases with an acidic pH. Since nano calcium phosphate fillers, such as carbonate hydroxyapatite, have the potential to chemically bond to damaged enamel and dentin and produce a protective layer, they can stop the effects of acid or bacterial attack.

19 Enamel Biomimetic Synthesis—Caries Lesions Repaired with Enamel-Like Nanomaterials

Enamel is unable to repair due to its non-regenerative nature following the demineralization of the surface and the subsequent cavitation. Artificial enamel production using biomimetic techniques could restore superficial damage to enamel and increase the longevity of teeth [68]. The production of hierarchically ordered apatite crystals has been studied in vitro to replicate the formation of hierarchically structured apatite crystals. Using acellular nanotechnological methods, enamel-like nano- and microstructures were created [69].

In order to create structures that resemble the hierarchical nanostructure of tooth enamel, mixtures of nano-sized mineral particles, nanocrystal pastes, or calcium phosphate ion solutions with different biological additives or surfactants were used. Surfactants used as reverse micelles or micro-emulsions for nanoscale structure formation and self-assembly are one of the most extensively utilized nanotechnology approaches [70]. By altering hydroxyapatite nanorods with surfactants, this method mimics the natural biomineralization process that occurs during the development of enamel. This allows the nanorods to self-assemble into an enamel prism-like structure. The technique of self-organization generated by amelogenin is a promising way to obtain the arrangement of apatite nanoparticles in complex-oriented enamel-like materials. Amelogenin is a significant extracellular matrix protein involved in the formation of real tooth enamel, and it has been used to create biomimetic enamel-like apatite layers. Amelogenin aids in the formation and structuring of apatite crystals [71]. The natural enamel protein amelogenin has been utilized to modulate calcium and phosphate crystallization in vitro, resulting in the formation of nano-sized rod-like apatite crystals. Remineralization of the etched enamel surface is achieved with this approach, which involves the creation of a mineral layer comprising needle-like fluoride hydroxyapatite crystals with diameters of 35 nm.

Currently, a biomimetic method has been employed to regulate the crystallization of HAP under biophysical circumstances at 37 °C using single crystalline hydroxyapatite micro-ribbons instead of amelogenin templates. As a result, the fluoride concentration had an impact on the morphologies and orientations of the crystals

that had formed. From disorganized clusters of nanoflakes to clusters of nanoneedles that are almost precisely aligned along the c-axis of the substrates, the hydroxyapatite crystals undergo a significant metamorphosis. All these are well recognized methods for biomimetic enamel synthesis.

20 Caries Vaccine

As a fresh approach to preventing dental caries, several attempts are now being made to create an effective anticaries vaccine. It was discovered that using a DNA vaccine to induce cellular and humoral immune responses was an efficient, secure, stable, and affordable immunogenic method. Many potential anticaries DNA vaccines have undergone or are undergoing animal or human investigations. The majority of anticaries vaccines limit bacterial mass build-up by either inhibiting the glucosyltransferase enzyme or blocking the surface protein antigen (PAc). The pathogenic components that cause S. mutans to adhere to tooth surfaces include the PAc and glucosyltransferases. The immunogenicity of anticaries DNA vaccine has been improved by the use of customized delivery vehicles, such as anionic liposomes in chitosan/DNA nanoparticle complexes. In order to permit the release of the vaccine in a pH-dependent way, the surface charge of the delivery vehicle may also be pH-dependent.

Various types of anticaries vaccines are

1. Passive immunization.
2. Active immunization.
3. Recombinant and DNA vaccines.
4. Subunit vaccines.
5. Synthetic vaccine.

Using self-assembling nanoparticles that connect the glucan-binding region of the Streptococcus mutans glucosyltransferase (GLU) to the N-terminal domain of ferritin, new developments in nanotechnology have aided in the development of anticaries vaccine and improved its immunogenicity. Recombinant DNA vaccines benefit from the use of nanotechnology in the production of anticaries vaccines. Trimethyl chitosan nanoparticles, particularly pVAX1-wapA/trimethyl chitosan vaccination, induced a stronger immunological response in animal studies than naked pVAX1-wapA, according to Li et al. [72]. These findings highlight the potential of nanotechnology in the development of anticaries vaccines.

21 Conclusion and Future Challenges

Despite the advances in nanotechnology, routine clinical application of this science for caries therapy is not feasible at present. Although the Ca2+ and PO4 3-releasing

nanocomposites can sustain stresses, it is challenging to predict and manage their mechanical behaviour in comparable clinical settings. Not much research has been done on the ion release profile in limited environments. It is necessary to strike a balance between the release of ions and the unpredictability of the caries development pace. Designing nanomaterials must carefully take into account a number of important factors, including the ideal particle size of nanofillers needed for remineralization, the pattern in which released ions are precipitated, and the orientation of precipitated nanocrystals with respect to collagen fibrils. All in vitro research up to this point has suggested that nanotechnology may effective in hindering the progression of early dentinal caries in its outer but not deeper layers. However, controlling deep and substantial lesions necessitates a full comprehension of the caries advancement procedure. Additionally, adding nanofillers to dental composites may help to prevent recurring caries surrounding restorations. Regardless of the progress with anticaries vaccinations, their use in human beings has not been investigated yet. As a result, they are not available for routine use currently. Limiting variables can include diverse oral flora, high salivary flow, challenging antigen delivery, enzymatic vaccine breakdown, and poor internalization.

It is clear that nanotechnology has been successfully applied to dentistry for a while, especially in the creation of restorative materials. The technological difficulties of manufacturing these materials to make use of the nanoscale features have been addressed by researchers. The prevention and management of oral biofilms, in particular, hold great promise among other areas. Although making up just around 20% of the bulk of the biofilm, practically all therapies for treating biofilms have concentrated on destroying the bacteria that are present in the biofilm. The remaining portion is a polysaccharide matrix, and it may be wise to think about attacking this matrix rather than the bacteria as it keeps the microbial populations together. Utilizing nanotechnology for dental treatments opens up a lot of possibilities since the complexity of the requirements can be kept to a minimum, making it more attainable. The price of the device or therapy being produced with nanomaterials is an issue that should be taken into account. Since they will be too expensive to synthesize and hence too expensive for customers to procure, the economic part of it needs to be further researched in order to make it more affordable for clinical use. Cost is a crucial element that is sometimes overlooked in the development of novel medicines and gadgets but is ultimately an important barrier that will determine whether iteventually succeeds or not. Nevertheless, the discipline of dentistry is expected to experience significant advancements, thanks to nanotechnology, and this fact augurs well for both patients and dentists in future.

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Chapter 4
Nanocomposites and Other Restorative Materials

Yanni Tan and Jianfeng Lyu

1 Introduction

Restorative dental materials are those materials that can be used to restore dental tissue lost caused by trauma, tumor, or caries. Some restorative materials that are composed of two or more different types of materials and contain nanoparticles are called nanocomposites, such as nano glass ionomers and resin-based composites. This chapter introduces the clinically commonly used nanocomposites and other restorative materials from the perspective of material science, including glass ionomers, resin-based composites, and inorganic-based cement materials such as calcium silicate-based cement, calcium phosphate cement, and calcium sulfate-based cement.

2 Glass Ionomer Cements (GICs)

Glass ionomer cements (GICs) have been commonly used in filling and repairing dental cavities due to their ability to release fluoride, adhesion ability with tooth tissue, and translucency. GICs are made from polymeric water-soluble acid solutions and silicon aluminum fluoride glass powder with the addition of phosphate ($AlPO_4$). The polymers used in GICs include polyacrylic acid, acrylic/maleic or acrylic/itaconic copolymer, or acrylic/2-methylene butanedioic copolymer [1]. Some clinical GICs products mix the polymeric acid powder with glass powders, then blend it with water, which can increase the content of polymeric acid in the cement without affecting the viscosity of the liquid. The advantage of these materials is that they have

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the ability to form chemical bonds with enamel and dentin. With time, GICs have been shown to form an ion-enriched interfacial zone with dentin, which is probably the reason that GICs exhibit the high durability of the adhesive bonds [2]. However, GICs have many disadvantages, such as poor handling properties, high solubility at early setting stage, and insufficient mechanical properties for restoring molar [3].

In order to improve their poor mechanical properties, various modifiers have been added to the cement, such as bioactive apatite, ZnO, Zn, SrO_2, fibers, SiO_2, and so on [4]. In addition, nano-additives such as apatite-based nanoparticles, glass nanofibers, ceramic nanoparticles, and carbon nanotubes have been used to improve the performance of GICs [5]. It was reported that GICs containing hydroxyapatite and fluorapatite nanoparticles had improved mechanical properties [6, 7]. Adding hydroxyapatite-silica nanoparticles into commercial GICs can improve their hardness, compressive strength, flexural strength, and shear bond strength [8], as well as prevent microleakage both at occlusal margins and gingival margins [9]. Nano-apatite containing GICs exhibit enhanced bonding with the tooth surface, which may be because of the strong ionic linking formed between the apatite nanoparticles in the cement and Ca^{2+} in the tooth, or because of the great surface area of nano-sized particles, which makes the crystals infiltrating into demineralized dentine and enamel pores [10].

Other efforts have been made to improve the antimicrobial activity and the ability to prevent enamel demineralization. Chlorhexidine and nano-sized sodium trimetaphosphate (n-TMP) were incorporated into GICs and the results showed that GICs containing 1.25% chlorhexidine and 14% n-TMP exhibited higher antimicrobial/antibiofilm activity and enamel anti-demineralization, rarely decreasing the mechanical properties [11]. Silver nanoparticles were also used to improve the antimicrobial activity of GICs [12, 13]. Although the influence on mechanical properties was insignificant, it has significant color change and introduces pores within the cements [14].

Other additives are also applied in order to improve more functions or properties. For example, cellulose nanocrystals were incorporated into GICs and significantly improved the mechanical properties and increased the fluorine-release of all GICs [15]. Bioactive glass nanoparticles can improve cell viability other than compressive strength [16]. Fluorapatite nanoparticles were used to increase the fluorine release ability [17].

However, other than the in vitro and in vivo investigations, it is necessary to do more clinical trials for warranting the clinical application of these modified materials.

3 Resin-Based Composites

Resin matrix composites are the most applied materials clinically used for repairing dental caries because of their advances in operability, mechanical properties, and biocompatibility. Resin matrix composites mainly consist of polymerizable resin matrix, silanized inorganic fillers, and an initiator system. The resin

matrices used in commercial resin-based composites are: Bis-GMA (bisphenol-A glycidyldimethacrylate), TEGDMA (triethylene glycol methacrylate), UDMA (urethane dimethacrylate), PEGDMA (polyethylene glycol dimethacrylate), Bis-EMA (6): (ethoxylatedbisphenol-A dimethacrylate(bisphenol-A: ethylene glycol = 1: 6)) [18]. Inorganic fillers as the reinforcement phase can significantly improve the mechanical strength, wear resistance, elastic modulus, color difference, no X-ray resistance, polishing performance and surface smooth and other properties of dental resin [19–22]. Nanoparticles are widely used as fillers, which are also called nanofillers, such as ZnO [23], Ag [24], TiO_2 [25], SiO_2 [20], CaF [26, 27], clay nanoparticles, hydroxyapatite nanorods [28], etc. The composition, shape, content, and size of nanofillers are important factors that affect the properties of resin-based composites [19, 29, 30]. Modification of nanofillers is one of the common methods for improving the properties or functions of restorative materials. For example, TiO_2 nanoparticles can be introduced to the mixed filler to adjust the color of dental resin composite, making the color more beautiful [31]. Inorganic compounds containing fluoride have been used to treat and prevent dental carries, as fluoride ions can inhibit microbial growth and metabolism, reduce demineralization and promote remineralization of enamel and dentin [3, 27]. One study showed that the nanocomposite containing 20 wt.% CaF_2 nanoparticles prepared by two-step "co-precipitation and spray-drying" method exhibited better mechanical properties and excellent long-term F-release ability than other group samples [27].

The antibacterial performance of resin-based composites has been the study topic in recent years because bacterial microleakage has been considered to be the most significant risk in restorative dentistry. One of the important methods to improve the antibacterial performance of the materials is to incorporate nanofillers that exhibit antibacterial activity, such as Ag nanoparticles and ZnO nanoparticles. In the meantime, the addition of the antibacterial nano agents should not affect the mechanical properties and the color stability. Ardestani et al. [19] studied the effect of silica silica-blow-spun nanofibers containing Ag nanoparticles on an industrial composite low-viscosity filled resin. When 0.5 wt.% silanized nanofiber was added, the material had a stronger inhibitory effect on *Streptococcus mutans*, with sufficient roughness, bending strength, and hardness. Cao et al. [24] developed a resin-based composite containing core–shell AgBr/cationic polymer nanocomposite (AgBr/BHPVP). The nanocomposite has long-lasting antibacterial activity and the dual effect of contact sterilization by cationic polymers and release sterilization by sustained releasing Ag^+ ions. ZnO nanoparticles have been used as antimicrobial fillers due to their broad-spectrum antibacterial activity, long-term environmental stability, biocompatibility, and non-toxicity [23]. Yang et al. [32] prepared dental resin composites with regular-shaped SiO_2–ZnO nanoparticle clusters (CCs) made by the spray-drying method. This complex filler can enhance antibacterial activity while maintaining mechanical and aesthetic properties.

Other than the antibacterial activity, mineralization or remineralization is another function that has been focused on by researchers [33]. Calcium phosphate compounds including hydroxyapatite nanofillers are often used to improve the remineralizing capabilities of dental resin composites as they have a similar chemical composition

with mammal's teeth and bone, and exhibit good biocompatibility and bioactivity. Calcium phosphate nanofillers can release high levels of calcium (Ca) and phosphate (P) ions. These ions can re-mineralize and promote the formation of a mineralized layer on the surface of the material, which can inhibit the occurrence of secondary caries and strengthen the tooth structure. Amorphous calcium phosphate nanoparticles were incorporated into resins for caries inhibition [34]. Hockin H. K. Xu's group has been studying the nanocomposites with calcium phosphate and published several papers [35–37]. Recently, they incorporated 20% (mass fraction) amorphous calcium phosphate into a low-shrinkage-stress resin matrix with antibacterial property and developed a dental resin nanocomposite with remineralization and antibacterial properties [38]. Liu et al. [39] prepared a photocurable bioactive dental resin composite by combining the silanized hydroxyapatite whisker grafted by poly (double GMA) with silanized silica nanoparticles. The material has good apatite forming ability and good mechanical properties.

In addition, modification of the organic resin matrix of a dental nanocomposite is also very important to make the combination properties satisfy the clinical expectations inside the harsh oral environment conditions including reducing the polymerization shrinkage related strain and stress, introduction of new monomers with new structure, antibacterial ability or lower viscosities [33].

4 Other Restorative Materials

4.1 Tricalcium Silicate Cement

Mineral trioxide aggregate (MTA), which is commonly used in root canal filing, consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate, and bismuth oxide and served as radiopacifier [40]. Although MTA has achieved great success in clinical dentistry, including root canal filing and pulp capping [41], it still has some drawbacks, such as long setting time, low biocompatibility, poor anti-washout performance, discoloring, and so on [42]. Therefore, as the main component of MTA, tricalcium silicate (Ca_3SiO_5, C_3S) is expected to substitute MTA, because of its self-setting property and good bioactivity [43].

C_3S has a variety of crystal types, including triclinic (T1, T2, or T3), monoclinic (M1, M2, or M3) at room temperature and rhombohedral (R) when heated [44]. In the crystal structure, C_3S is composed of calcium ions, silicon-oxygen tetrahedrons, and solo oxygen anions, with a complex crystal structure [45]. While dicalcium silicate don't exist oxygen anions that is easier to combine with H^+ in water. Due to independent oxygen anions, C_3S represents more intense hydration and shorter curing time compared to dicalcium silicate. The hydration is a process where the C_3S phase reacts with water to produce calcium hydroxide ($Ca(OH)_2$) and calcium-silicate-hydrates (C-S–H) nanocrystalline gel surrounding C_3S [46, 47]. The process of C_3S hydration can be scribed as Eq. 1 and the microstructure after hydration is

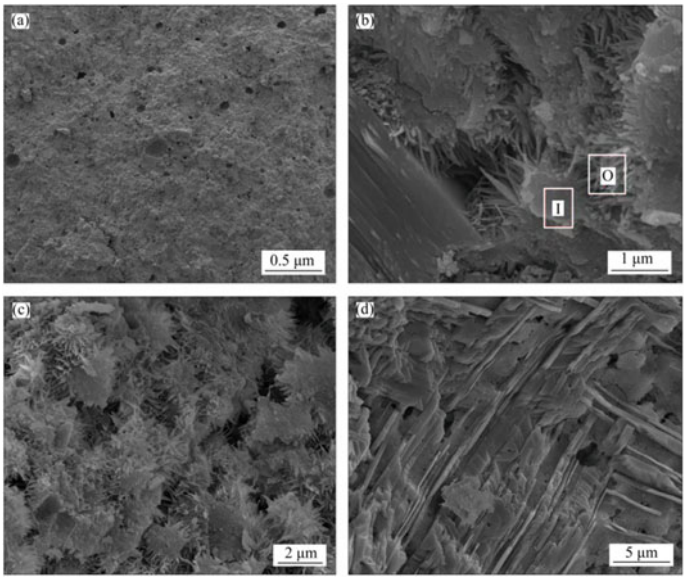

Fig. 1 SEM images of the product of C_3S hydration (**a**, **b**) (I: Inner product, O: outer product), fibrillar C–S–H (**c**), lamellar portlandite (**d**) [51]

shown in Fig. 1. With the process of hydration, C_3S pastes set and become harder, due to the C-S–H excellent mechanical properties to the cement. So, for sufficient hydration, it demands that the C_3S powders have a high specific surface area.

$$Ca_3SiO_5 + 5.2H_2O \rightarrow 1.2Ca(OH)_2 + (CaO)_{1.8}SiO_2(H_2O)_{4.0} \quad (1)$$

The synthesis methods of C_3S powder mainly include solid phase synthesis method [48], sol–gel method [49], two-step precipitation method [50], and Pechini method [51]. The sol–gel method is the most common method for preparing C_3S powder used in the laboratory with $Ca(NO_3)_2$ and TEOS as raw materials. There is also a novel and facile preparation method with CaC_2O_4 and TEOS as raw materials [52]. As a wet-chemical route, this method maintains the advantage of powder size and avoids the by-product of oxynitride which has a negative influence on the environment. Pechini method is simple, efficient, and widely used [53, 54]. The technique includes mixing nitrate solutions, complexity of cations with citric acid, esterification of citric acid complexes with ethylene glycol, and heating of concentrated solutions under agitation [55]. The final powders possess high porosity, high surface energy, and high free energy [51]. However, NO_2 gas which is harmful to humans and the environment is formed during the Pechini process because of using $Ca(NO_3)_2$ as the raw material. Thus, it is necessary to eliminate harmful by-product.

C_3S is a promising material for dental treatment. C_3S promotes the proliferation and differentiation of root tip stem cells and the generation of dentin. In the oral therapy field, it can be used as root canal filling, apical deformation induction, pulp capping agent, repair root perforation, treatment of dentin allergy, and promote dentin remineralization [56]. C_3S have excellent biocompatibility and can induce HA formation in simulated oral solutions. At the same time, the highly alkaline $Ca(OH)_2$, produced by hydration, endowed C_3S with good bacteriostatic performance. Compared with MTA, the curing time of pure C_3S was about 3 h, less than that of MTA, and there was no discoloration of teeth [57]. As a restorative cement, C_3S also could repair the broken mandible as the bone filler padding in gaps. C_3S, as a bioactive ceramic, promotes the proliferation of bone marrow mesenchymal stem cells, which then differentiate into osteoblasts repairing defective bone tissue through matrix mineralization [49, 58, 59].

There are lots of studies about enhancing the compressive strength, decreasing the setting time, achieving anti-washout property, and obtaining radiation impenetrability of C_3S cement for better application in dental therapy. Ion doping is an effective way to modify the property of C_3S and its cement, such as Zn [60, 61], Sr [62], Fe [63], La [64], and so on. Lin et al. [60] synthesized Zn-doped C_3S by sol–gel method and found that Zn-doping could inhibit the formation of free CaO, while prolonging the setting time. Eltohamy et al. [61] found that Zn-doping improved compressive strength and antibacterial ability, and also significantly increased the proliferation of rat bone marrow stromal cells and alkaline phosphatase (ALP) activity. Except for ion doping, calcium chloride ($CaCl_2$) [65], magnesium phosphate [49, 66], disodium hydrogen phosphate [67], γ-polyglutamic acid [68], and vaterite [69] have been incorporated into C_3S to reduce the setting time or enhance the bioactivity.

In addition, radiopacifiers need to be mixed with C_3S to get applicable radiopacity. Bismuth oxide (Bi_2O_3) is the traditional radiopacifier in MTA. However, Bi_2O_3 has potential cytotoxicity [70] and also is the main reason for tooth discoloration [71]. Other materials such as zirconium oxide (ZrO_2), calcium tungstate ($CaWO_4$), niobium oxide (Nb_2O_5) or ytterbium trifluoride (YbF_3) have been used to replace Bi_2O_3 [72, 73]. Roberta et al. [74] compared the effect of micro ZrO_2, $CaWO_4$, Nb_2O_5, and Bi_2O_3 on the premixed cement, and found that C_3S with micro ZrO_2 has the shortest setting time and higher flowability, indicating that micro ZrO_2 is the most suitable to replace Bi_2O_3. C_3S combined with radiopacifiers and other ingredients to form a premixed bone cement has a practical application. Queiroz et al. [75] evaluated novel reparative materials composed of pure C_3S, ZrO_2, and biosilicate. Biosilicate, C_3S, and ZrO_2 show lower solubility and higher radiopacity than Biodentine (a commercial root canal filler) while keeping similar biocompatibility and bioactivity. Wu et al. [76] developed a premixed C_3S/sodium phosphate dibasic (SPD)/ZrO_2 cement with polyethylene glycol as the liquid phase. It was found that the premixed cement possesses anti-washout property and good sealing property due to SPD's positive effect on hydration.

However, in order to promote the C_3S cement with new formulations for clinical application, systematic studies are necessary, including setting time, mechanical properties, anti-wash property, X-ray radiopacity, in vivo cytotoxicity, antibacterial

activity, biocompatibility, and bioactivity, as well as clinical trial. In addition, the cost is another factor that needs to be considered.

4.2 Calcium Phosphate Cement

Calcium phosphates gained attention in the field of dental repair in the 1990s beginning with Brown and Chow of the Paffenbarger Dental Institute. Calcium phosphate cement includes tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$), dicalcium phosphate dihydrate (DCPD; $CaHPO_4 \cdot 2H_2O$), tricalcium phosphate (TCP, $Ca_3(PO_4)_2$), monocalcium phosphate monohydrate (MCPM; $Ca(H_2PO_4) \cdot H_2O$), hydroxyapatite (HA, $Ca_{10}\ (PO_4)_6(OH)_2$), and so on [77].

Among the different calcium phosphates, TCP has been a focus of great attention regarding its use as raw material for bone repair and periodontal treatments [78]. TCP has three types of crystal structure: α-TCP (high temperature), β-TCP (low temperature), and α'-TCP, corresponding to monoclinic, rhombohedral, and hexagonal symmetry respectively [79]. The α'-TCP phase can't exist at room temperature, so for the bone cement application, only α-TCP and β-TCP are considered. The structure of α-TCP is less densely packed than β-TCP, as the theoretical density is 2.866 g/cm^{-3} to α-TCP and 3.066 g/cm^{-3} to β-TCP. Thus, the loose structure causes faster dissolution and degradation in a physiological environment. α-TCP can react with water to form a calcium-deficient apatite in accordance with Eq. (2), and β-TCP's hydration follows Eq. (3), which needs acid condition. Due to this hydration process, TCP cement absorbs water surrounding the powder and generates calcium-deficient apatite which connected solid phase. Thus, during hydration, the cement becomes hardened.

$$\alpha - Ca_3(PO4)_2 + H_2O \rightarrow Ca_9(PO_4)_5HPO_4OH \tag{2}$$

$$\beta - Ca_3(PO4)_2 + H_3PO_4 \rightarrow 6H_2O + 3CaHPO_4 \cdot 2H_2O \tag{3}$$

The preparation of α-TCP needs precursor's long time calcine in high temperature (>1200 °C) and rapid cooling to room temperature, while the β-TCP can synthesized at a lower temperature. In addition, the precursor with a molar ratio Ca/P ~ 1.5, could be calcium-deficient hydroxyapatite, CDHA; amorphous calcium phosphate, ACP; or β-TCP [80–82]. Therefore, it is a little difficult to synthesize pure α-TCP, because of the transformation of α-TCP to β-TCP and the existence of other calcium phosphates. Sinusaite et al. [83, 84] synthesize the pure α-TCP at a low temperature (750 °C) using the as-prepared precipitates (precursor) washed with enough acetonitrile, ethanol or isopropanol. It was found that the synthesized α-TCP possesses the nano size, which means a large specific surface area, beneficial for cement hydration. However, this method is not eco-friendly, because it introduced a lot of organic chemicals.

Despite TCP cements have positive biocompatibility and bioactivity, there are still several challenges for dentistry and orthopedics application. Such as, TCP cement has low compressive strength, no antibacterial property, and poor anti-washout performance. Thus, many researchers modify TCP cement with other biomaterials [85–87], or dope other ions in TCP structure [84, 88–90]. Shi et al. [85] introduced Ca-binding agents (citric acid and sodium alginate) into α-TCP cement to improve the physicochemical properties utilizing their synergistic effects. The formation of SA-CA gel networks significantly accelerated the setting, improved the anti-washout ability, and enhanced the mechanical strength of TCP cement. Di Filippo et al. [91] developed an antimicrobial and radiopaque calcium phosphate bone cement by adding gentamicin sulphate-loaded solid lipid microparticles and barium sulfate. Mn^{2+} could be doped into α-TCP crystal structure so that it could slow the intense hydration, meanwhile, it won't decrease the biocompatibility [84, 92]. Yuan et al. [93]found that appropriate Sr+ doping would increase the setting time and compressive strength of α-TCP thanks to the effect on the size of the powder.

About β-TCP, there are also a lot of researches on enhancing the biological properties. β-TCP cement has a rapid self-setting time of approximately 1 min making its utilization difficult in dentistry therapy [94, 95]. On account of the complexation, appropriate citric acid concentration could be an inhibitor to prolong the setting time, sacrificing little mechanical strength [96]. Lee et al. [97] mixed chitosan-alginate complex solution with TCP/MCPM powder to form a better injectable bone cement with good washout resistance. Kim et al. [98] found the injectability of the β-TCP cement paste was improved mainly due to the mechano-chemical modification, and the compressive strength was enhanced by the addition of ZrO_2 after ball-milling. Arahira et al. [99] prepared mixtures of α-TCP and β-TCP with various compositions that were prepared through the α-β phase transition of α-TCP powder at 1000 °C for various periods. It's noted that with the increasing content of β-TCP, diametral tensile strength decreased and setting time prolonged, but the cell proliferation and ALP activity increased. Ag+, Zn^{2+}, and Cu^{2+} could improve the antibacterial property of TCP cement [100]. Compared with C_3S, TCP has better biocompatibility and shorter setting time, thus they could be an additive into C_3S cement, but their low compressive strength inhabits their use in dentistry applications.

4.3 Calcium Sulphate Cement

Calcium sulphate (CS) cement has been used as orthopedic materials due to its safety in handling and storage and its cheap price [101]. The main three types of CS are fully hydrated $CaSO_4 \cdot 2H_2O$ (gypsum), partly hydrated $CaSO_4 \cdot 0.5H_2O$ (hemihydrate CS, HCS), and anhydrous $CaSO_4$. The anhydrous CS and HCS could absorb the water to form $CaSO_4 \cdot 2H_2O$, which follows as the Eqs. (4) and (5) [102]. With this hydration, the paste sets and hardens. Same as TCP and C_3S cement, hardened CS cement is not a compact solid, but a loose porous structure comprising interlocking crystals in the form of plates and needles [103]. This porous structure empowers

CS cement degradability which can eventually eliminate the implanted cement bad effort with the formation of new bone.

$$CaSO_4 \cdot 0.5H_2O + 1.5H_2O = CaSO_4 \cdot 2H_2O + \text{heat} \quad (4)$$

$$CaSO_4 + 2H_2O = CaSO_4 \cdot 2H_2O + \text{heat} \quad (5)$$

Compared to anhydrous CS, HCS, commonly known as plaster of Paris, is extensively used in medical industries due to its stability. HCS consists of two structural forms: α- and β-forms according to the different preparation methods. Synthesis of beta hemihydrate is achieved by heating gypsum to between 45 and 200 °C under vacuum or dry air [104]. Gypsum must be heated in an acidic or salt solution, or above 97.2 °C in water under pressure to obtain α-hemihydrate CS. Also, α-HCS could be synthesized by liquid phase reaction. Kong et al. [105] successfully controlled the morphology of α-HCS using reverse microemulsions of water, n-hexanol, cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (SDS). With the increasing of SDS/CTAB or CTAB/H_2O, the c-axis length decreased, and morphology transformed from whisker, column, rod, plate, and eventually nanogranules. The different morphology might have an influence on the hydration process of α-HCS. Chen et al. [106] found that the particle sizes of α-CSH rods were significantly affected by the type of modifier and the reaction temperature when hydrothermally prepared by gypsum. With modifier ($MgCl_2$, sodium citrate, and sodium dodecyl benzene sulfonate) content increasing, it would be easier to generate short and thin rods. The small size of HSC is beneficial for hydration and increasing the solution rates in simulated body fluid. Nakagawa et al. [107] successfully controlled the size of HCS rod by adding the different content of Ag nanoparticles using the micelle-mediated phase separation phenomenon.

Considering the poor performance of fast setting and low compressive strength (about 15 MPa), the solely utilizing of HCS is generally inadvisable. HCS cement could be combined with various calcium phosphate salts [108–110] or some biocompatible organic materials [104, 111]. Cai et al. [112] found that the introduction of micro-nano calcium phosphate not only affected the morphology of CS cement crystals, but also produced calcium phosphate particles with smaller diameters to fill the gap between the CSC crystals, which reduces porosity, improves mechanical properties, and delays the degradation of CSC. Du et al. [113] designed and prepared an injectable abalone shell/calcium sulfate bone cement. The abalone shell was coated evenly on the HCS surface, thus prolonging the injectable time and improving the solidification and mechanical properties of HCS. Chen et al. [114] developed a calcium citrate/Vitamin D3-loaded calcium sulfate composite cement with higher mechanical strength (28.87 MPa), better injectability, and appropriate setting time (23.7 min). The loaded Vitamin D3 can promote calcium and citrate deposition at defect sites participating in bone formation. To enhance the compressive strength, $BiFeO_3$ was introduced into HCS cement by ball-milling [115]. The compressive strength of CSH doped with $BiFeO_3$ increased by 3 times compared to pure-CSH.

The bioactive glass was also combined with HCS to prepare a composite cement to improve biocompatibility for better bone regeneration application [116]. In addition, HCS is always a favorable and degradable main component in other bone cement. Ji et al. [117, 118] developed a tricalcium silicate/sodium alginate/calcium sulfate hemihydrate (45/45/10 wt%) composite bone cement. After adding HSC, the final setting time could be reduced from 68 to 21 min. A biodegradable C_3S/gluconodelta-lactone/ HSC composite cement developed by Ding et al. [119] had a short setting time (less than 15 min) and high preliminary mechanical property (5.27 MPa in the first hour).

5 Conclusion

Because of the natural characteristic of human bone and teeth, that is, they consist of an inorganic calcium phosphate compound, mainly hydroxyapatite, most nanocomposites and other restorative materials are based on calcic chemicals or contain calcic nanofillers, or use calcic compounds as a modifier. Glass ionomers and resin-based nanocomposites contain nanofillers such as hydroxyapatite nanoparticles and CaF. Inorganic-based cement materials are based on calcium silicate, calcium phosphate or calcium sulfate. In order to improve the mechanical properties, biological properties or other functions, additives or modifiers are introduced such as ZnO, SrO_2, SiO_2, Ag, TiO_2 nanoparticles, etc. Another effective way is ion-doping with functional ions like Zn^{2+}, Ag^{+}, F^{2-}, Mn^{2+}, etc. In order to promote those new formulation materials for clinical application and meet the clinical requirements, systematic studies are necessary, including physicochemical properties, mechanical properties, and biological properties, as well as clinical trials for warranting the clinical application of these materials. In addition, product costs are another important factor that needs to be considered.

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Chapter 5
Sustained Drug Delivery—The Nano Advantage

Ali Nematollahzadeh, Farrokhfar Valizadeh Harzand, and Zahra Vaseghi

1 Introduction

Materials in the range of 1–100 nm are known as nanomaterials. Owing to the high surface area, nanomaterials show special properties [1]. Nanomaterials have numerous applications in medicine leading to many advances in this field, of which dentistry, oral, and maxillofacial surgery are no exception. This chapter provides a detailed outline of nanoparticles and their usage as dental sustained drug delivery systems (DDS) and their advantages. Advanced nanomaterials play important role in the integration of nanoscience and biotechnology. Therefore, they can be extensively applied in the diagnosis and improvement of diseases. It is expected that nanomaterials will cause a great change in orthodontics, prosthetics, periodontics, surgery or restorative dentistry, and medical nanodontics as a new field, having a valuable place in improving the quality of life [2]. Nanotechnology which provides the basis for improving the treatment, prevention, and protection of teeth and mouth against diseases, has significantly paved the way [3]. Nanomaterials have shown promising applications in medical science for a variety of applications such as dental fillings and sealants for luster, strength, and durability. In addition, nanoparticles with antimicrobial activity used in restorative composites not only prevent tooth decay, but also have a significant effect on improving the health of the oral environment. On the other hand, the feasibility of designing targeted nanomaterials-based drug delivery systems in the treatment of diseases is one of the benefits of nanomaterials usage in medical dentistry [4].

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2 Nanomaterials in Sustained Drug Delivery

In the traditional method of treating diseases, drugs are usually taken orally or by injection, which causes the drugs to be distributed throughout the human body. Such conditions cause damage to the cells and tissues that are healthy and will have side effects and even serious problems for patients [5]. Targeted drug delivery is known to be a good solution to this problem because, in addition to treating the intended tissues, both the dosage of the drug taken by the patient and its side effects are significantly decreased in this system. This method has been reported to be very effective and efficient, especially for cancer therapy and diseases related to the nervous system [6]. Combination methods are common for therapeutic applications.

Sustained release systems enable the delivery of medicine in a controlled manner that results in drug delivery for a prolonged time. This viewpoint of drug delivery is principally applicable to drugs that are quickly processed and removed from the body shortly after their entrance. Sustained release is a distinctive method in which the concentration of a drug is kept at a regular level in the target tissue by regulating the rate of drug release. While in the immediate release, a rapid onset of drug action results in a high plasma concentration quickly and even passes over the tolerable maximum plasma concentration. In the zero-order drug release, the drug release rate is constant over the entire release duration. It provides the best control of drug concentration in the plasma and has the benefits of improving patient adherence to medical advice and reducing the number of drug intake by the patient. Although several techniques can be used to achieve zero-order release, most of them are complicated, costly, laborious, and difficult to manufacture. Therefore, instead of zero-order release the sustained release approach that overcomes most of the above-mentioned difficulties can be an appropriate drug delivery system. The different release approaches are shown in Fig. 1.

Due to the mentioned advantages of nanotechnology, nanomaterials and nanostructures can also be helpful for dental applications. The nano-drug delivery system by combining an antimicrobial agent can be applied directly to the periodontal pocket as a controlled drug delivery system [7]. Achieving the necessary effectiveness in the treatment of periodontal patients with controlled drug delivery systems relies on the qualification of the drug delivery system to release the antimicrobial agent at the appropriate concentration and to store the drug for a proper time. An ideal drug delivery system for periodontitis therapy includes a biodegradable and adhesive drug delivery system that delivers the drug in a restrained /stable way during a couple of weeks [8]. Magnetic nanoparticles coated with aminosilane show good antifungal and antibacterial activity against microbial biofilm as an antifouling agent. These nanoparticles are effective in treating local infections caused by oral microflora. In addition, a tetracycline delivery system based on a hydroxyapatite nanocarrier was used to eliminate infection and regenerate bone, and the results of this study showed that nanoparticles loaded with tetracycline were biocompatible and can serve as osteoconductive bone substitutes [9].

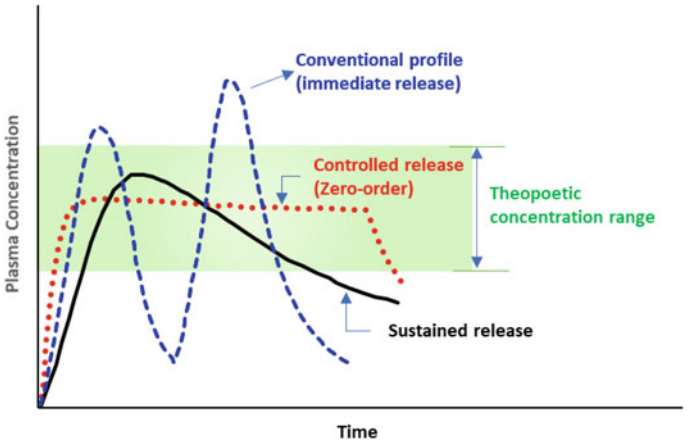

Fig. 1 Schematic representation of plasma concentrations of a conventional immediate-release dosage form sustained-release dosage form, and zero-order controlled release

In addition to the antimicrobial effect, calcium phosphate nanoparticles loaded with chlorhexidine also have a mineralizing effect. To improve bioadhesion, calcium phosphate nanoparticles were coated with carboxymethylcellulose, which prevented the growth of *Escherichia coli* and *Lactobacillus casei* by adhering to enamel and dentin to prevent dental caries. Injection systems can be a good option for the easy and fast release of nanoparticles in periodontal pockets. Polydopamine nanoparticles can be used as an injectable antioxidant defense platform in the removal of reactive oxygen species (ROS) to treat periodontal disease caused by oxidative stress. The results showed that this platform, in addition to significant ROS removal, showed high biodegradability and low systemic toxicity [9].

Depending on the type of prescription, numerical predictions provide satisfactory approximations of the drug dose and its intended release characteristics, geometry, dimensions, composition, and preparation steps for drug forms. Real phenomena such as mass transfer, dissolution, and drug diffusion in drug delivery can be described by a real mechanical mathematical model based on equations. Most of the governing equations are partial differential equations, and to solve them, we need initial and boundary conditions in the drug release environment. If the membrane is not swollen and complete, sync conditions are provided during the release time and drug permeability remains constant; the first-order release synthesis (regardless of system geometry) for the drug is as follows (as stated in Fick's law) [10]:

$$\frac{dM_t}{dt} = \frac{ADKc_t}{l} = \frac{ADK}{l}\frac{M_0 - M_t}{V}$$

where

M_t Absolute cumulative amount of drug released at time t;
c_t Concentration of the drug in the release medium at time t;

M_0 Initial amount of drug within the dosage form;
V Volume of the drug reservoir;
A Total surface area of the device;
l Thickness of the membrane;
K Partition coefficient of the drug between the membrane and the reservoir;
D Diffusion coefficient of the drug within the membrane.

2.1 Zero-Order Model

Dissolution of the drug from drug systems that release the drug slowly but do not disaggregate is shown as follows [11]:

$$Q_0 - Q_t = K_0 t$$

where

Q_t the amount of drug dissolved in time t;
Q_0 the initial amount of drug in the solution;
K_0 zero-order release constant expressed in units of concentration/time.

This equation can describe the drug dissolution of different drug types, such as some percutaneous systems, poorly soluble drug-coated matrix tablets, osmotic setups, and so on.

2.2 First-Order Model

When drug release follows first-order kinetics, the following equation can describe it [11]:

$$\frac{dC}{dt} = -Kc$$

which is also expressed as follows:

$$\log C = \log C_0 - Kt/2.303$$

where

K first-order rate constant expressed in units of time^{-1};
C_0 initial drug concentration;
K first-order rate constant;
t time.

This equation is mostly utilized to describe the dissolution of drugs in pharmaceutical structures such as water-soluble drugs in permeable matrices.

2.3 Higuchi Model

Mathematical descriptions for integrated dispersions also become extremely complicated. For the simplest thin-film geometry with minimal edge effects, the second temporal root correlation between the quantity of drug released from a thin lotion film with a large amount of drug was expressed by Takro Higuchi [10]

$$\frac{M_t}{A} = \sqrt{D(2C_0 - C_s)C_s t}$$

where

M_t the cumulative absolute amount of drug released at time t;
A surface area of the film exposed to the release medium;
D drug diffusivity in the carrier material;
C_0 initial drug concentration of the drug in the carrier material;
C_s solubility of the drug in the carrier material.

2.4 Korsmeyer-Peppas Model

Korsmeyer-Peppas model [11] can be used to describe the drug release from the polymer network as follows:

$$M_t/M_\infty = Kt^n$$

where

$M_t M_\infty$ a fraction of drug released at time t;
K release rate constant;
n release exponent.

In this equation, the mechanism of drug release is specified by the value of n.

For cylindrical tablets, different values of n correspond to the following mechanisms:

$0.45 \leq n$ Fickian diffusion mechanism,
$0.45 < n < 0.89$ non-Fickian transport,
$n = 0.89$ Case II (relaxational) transport,
$n > 0.89$ super case II transport [11].

3 Nanomaterials in Dental Medicine

The nanoparticles used in targeted drug delivery systems are mainly colloidal particles with a diameter of less than 1 μm. In general, the nanoparticles used in this field are divided into three categories: (1) Nanoparticles that are the essential part of the drug constitution, (2) Nanospheres in which the particle matrix is loaded, dissolved, or dispersed with the active drug, and (3) Nanocapsules in which the active drug is dissolved or dispersed in a hydrophilic or lipophilic media. Due to the benefits of degradable and biocompatible materials mentioned above, nanoparticles used as drug carriers are mainly made of gelatin and albumin as natural polymers, or polylactides and polyalkyl cyanoacrylates as synthetic polymers [12]. The loaded drug is released through diffusion, destruction, or erosion. Due to the high surface area, nanoparticles have a high carrying capacity and allow the integration of hydrophilic and hydrophobic drugs and diversify the routes of administration [13].

Natural polymers such as chitosan and gelatin and synthetic polymers including poly (lactide) (PLA), poly (glycolide) (PGA), and polycaprolactone (PCL) are available for the design of drug nanocarriers [14]. Biodegradable polymers have stable structural properties and controlled drug release. Polymer nanoparticles have shown significant efficiency in delivering antigens, peptides, and non-peptide drugs such as insulin and anti-cancer drugs to the disease site.

In the field of dentistry, mainly toothpaste, mouthwashes, and pills play the role of drug carriers with an uncontrolled rate of drug release [12]. A controlled drug delivery system can have numerous benefits in dental medicine and periodontal disease treatment. A controlled drug delivery system with long-term release can be used at the desired location, such as the periodontal pockets [12].

Destruction of microbial biofilms is one of the main concerns of oral health. The susceptibility of oral bacteria to antibiotics and drugs increases with the growth and maturation of the bacteria. Bacterial species including *Streptococcus mutans* and *Lactobacilli* cause an acidic environment in the mouth leading to a considerable reduction in the efficacy of antibiotics. New nano-based drug delivery methods offer platforms to address these problems that help to prevent and treat infectious oral diseases [15]. Fungal infections of the mouth, such as candidiasis, can cause cancer if left untreated. Drugs such as amphotericin B are useful in treating such diseases, but their use may cause kidney toxicity. In contrast, Amphotericin B lipid nanostructures consisting of amphotericin B lipid complex, in addition to preventing candidiasis, have shown very low renal toxicity [16].

Fluoride ions interfere with bacterial growth metabolism, preventing the growth of bacteria that lead to acidification of the oral environment and tooth decay. Due to the high volume-to-surface ratio, nanoparticles allow the loading of large amounts of fluoride ions and provide controlled treatment with the sustained release of the ions. Sodium fluoride/chitosan particles have been reported to be useful in the continuous release of fluoride ions [15]. Calcium fluoride/chitosan and chitosan/fluoride nanoparticles can release fluoride ions in an acidic medium and accelerate calcified

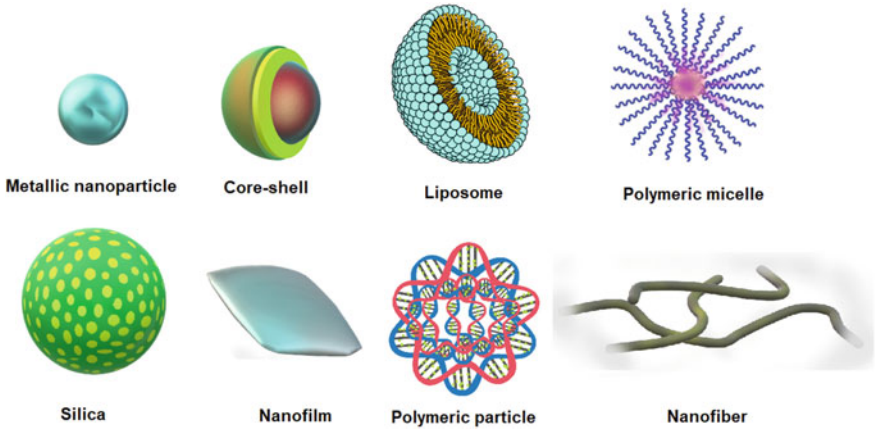

Fig. 2 Representation of nanoscopic drug delivery systems used in dentistry

tissue remineralization. Fluoride-based nanoparticles are also used in mouthwashes to prevent tooth decay [17].

Strontium (Sr^{2+}) arouses the differentiation of mesenchymal stem cells and prevents bone resorption by suppressing the activity of osteoclast cells. Ranilite strontium (SrRn) is known as an anti-osteoporosis drug [15]. In a study that used a coating agent in the pulp to induce SrRn mineralization of tooth pulp, the results showed that SrRn increased the proliferation of dental papilla cells. Collagen/SrRn composite gels have also been used as drug delivery systems to treat topical periodontitis. The results of studies show that the presence of Sr^{2+} increases bone binding and sustained drug release [15]. Figure 2 shows the most common targeted drug delivery systems in dental applications [15].

Coating implants with nanoparticles creates significant bone conduction because of the high surface area of nanoparticles. Chitosan nanoparticles have been used to deliver bone morphogenetic proteins and ciprofloxacin as coatings on titanium implants. Due to its high biocompatibility, chitosan particles reduce drug delivery problems in tissues, and in addition to covering the surface of titanium, which causes uniform bone regeneration, it is also used as a drug carrier in cancer therapy. In addition to chitosan nanoparticles, hydroxyapatite nanoparticles and silica nanoparticles are used as coatings on titanium and drug carriers for targeted drug delivery applications [6].

Porous calcium phosphate (CaP) granules were developed with biodegradable PLGA nanoparticles containing dexamethasone for bone regeneration. The results showed a controlled release of dexamethasone within a month. The use of carbon nanotube (CNT) in gene therapy and pharmacology in genetic applications has also been proven. A group of researchers used a single wall carbon nanotube (SWNT) with the anticancer drug cisplatin to treat squamous cell carcinoma, known as one of the most typical oral cancers. The advantage of using CNTs is their capability to conduct the composite and help with bone repair and nerve stimulation [6].

Periodontal disease caused by microorganisms results in a lack of soft tissue adhesion and alveolar bone resorption, leading to envelope formation or gingival resorption, which consequently results in periodontal tissue destruction [18]. In addition, periodontal disease does not end with oral injuries alone; Instead, it accesses the systemic circulation through the envelope epithelium and stimulates the inflammatory immune response of the host, subsequently leading to systemic problems which affect the cardiovascular system and diabetes [19]. Owing to the promising talent of pathogens to invade the gums, non-surgical methods and techniques of bone flap surgery cannot eliminate periodontal microorganisms. Choosing a suitable antimicrobial agent and a useful method of drug intake, as well as dosage modes, has a positive effect on the overall clinical outcome of treatment. Systemic antimicrobial treatment is only efficient when taken in sufficient doses to reach the intended concentration in the envelope area [20]. Most systemic antimicrobial agents withstand microbial resistance. On the other hand, lack of access to the infected area, achievement of sufficient concentration, and weak penetration into the tissue can lead to treatment failure. An ideal local drug delivery system (LDDS) should ideally offer a controlled drug release attitude, preserve the drug concentration for a long time, be eco-friendly, and be biocompatible without causing any tissue irritation. LDDS is used in a number of structures including fibers, gels, tapes, films, microparticles, and nanoparticles [19]. Figure 3 shows a schematic of a micro/nano-drug delivery system [19].

Metallic and polymeric nanoparticles, quantum dots, nanocomposites/nanogels, liposomes, and nanofibers have been used in periodontics for numerous clinical practices. Nanofiber-based scaffolds with electrospinning, emulsion method, mixing, and surface modification are used to combine different therapeutic agents in dentistry

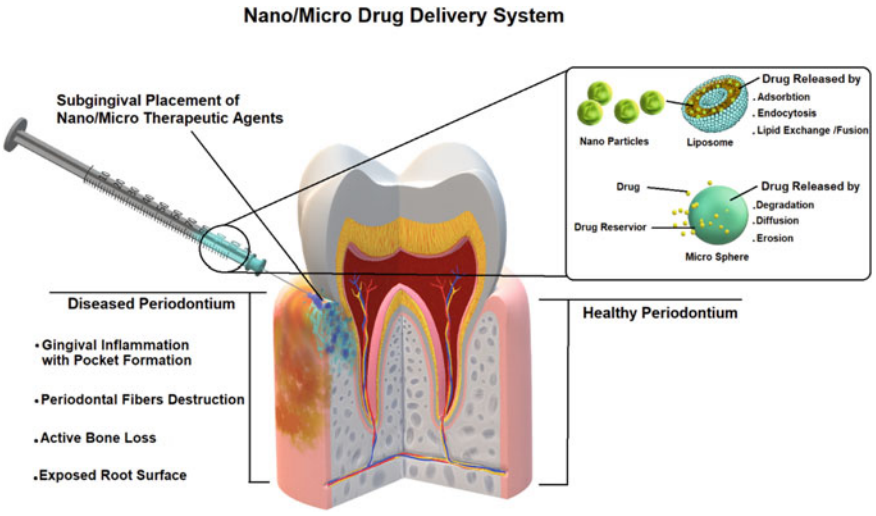

Fig. 3 Nano/micro dental drug delivery system

[21]. Factors such as fiber diameter, drug release rate, polymer degradation or erosion rate, drug dissolution rate, and drug release rate from nanofibers are determined. Physicochemical changes occur in electrospun smart nanofibers when exposed to factors including pH, temperature, light, electric field, and magnetism so that the release rate is optimized with these factors. For example, methylene blue encapsulated PLGA nanoparticles are capable of targeting special oral pathogens leading to the release of reactive oxygen species through photodynamic treatment. Killing microorganisms utilizing reactive oxygen species is recognized as an efficient curing strategy in periodontics [19].

Studies show that much progress is still to be made on nanotechnology and its subdisciplines. Undoubtedly, it is almost impossible to study all nanoparticles and their applications in dentistry. However, in recent decades, nanotechnology has proven many applications and methods in medicine and pharmaceuticals. The applications of some nanoparticles and nanostructures in oral drug delivery are summarized in Table 1.

Some remarkable superiorities of these nanocarriers are their biocompatibility, eco-friendliness, facile production method, and efficient drug loading and delivery. Also, the nanoscale drug delivery systems can improve the drug delivery system's properties including hydrophobic drug solubility, drugs stability, bioavailability and absorption, diffusivity with low mass transfer resistance, targeting a specific

Table 1 Some applications of nanoparticles in oral drug delivery

Discipline	Nanoparticles/Nanostructures	Applications	References
Oral and maxillofacial surgery	HA NPs, Au NPs, carbon nanotubes, electrospun polylactic co-glycolic acid nanofiber, SWNT blended with nanocomposites, cerium oxide nanoparticles, copper/carbon hybrid nanoparticles, chitosan-gold nanoparticles, chitosan-poly(ε-caprolactone) nanofibers, composite chitosan/hydroxyapatite nanofibers	Anti-cancer drug delivery, early detection of oral cancer, used as scaffolds for new bone formation, Hard and soft tissue engineering, predictable and efficient bone engineering, trabecular bone pattern, enhance vascularization of bone grafts, bone regeneration, wound healing	[22]
Oral medicine	Silica NPs, ZrO_2NPs, HA NPs, TiO_2 NPs, porous calcium phosphate granules, PLGA nanoparticles, cerium oxide nanoparticles	Antibacterial therapy, drug or gene carriers, targeting agents for the treatment of cancer, NPs against Multi-Drug Resistance (MDR) cancer cells, localized delivery of drugs, improved solubility of CNTs and biocompatibility	[22]

tissue/cells, reduction of the dosing intervals, reduced systemic toxicity, and tuned release rate of drugs.

3.1 Composites Dental Nanomaterials as Drug Carries

Oral diseases negatively affect the well-being and quality of life of the facilitators besides their negative effects on health. Bacterial and systemic inflammatory vulnerabilities created due to poor oral health can lead to adverse results such as uncontrolled diabetes, cardiovascular disease, and respiratory disease [23]. More than one-third of all age groups throughout the world have experienced tooth decay. Metal and alloy composites such as titanium, cobalt, chromium, and gold are used to repair decayed teeth due to their toughness and high resistance to abrasion and breakage [24]. Despite the high wear resistance of these composites, low biocompatibility and the adverse effects of ions released over time have become a matter of concern. Resin-based dental composites, amalgams, glass ionomers, and resin-modified glass ionomers are among the most widely used composites [25]. These composites are used in dental operations as a restorative material, cavity sealant and fissure, cavity liner, veneer, crown, root sealer, and orthodontic appliances. These materials are divided into two phases. The first phase is based on the organic resin matrix; while the second phase is mainly based on mineral/organic filler [26]. The organic resin matrix phase is mainly composed of a mixture of multifunctional monomers and light-sensitive primers; whereas micro/nanofillers that are mainly used as reinforcements are involved in the inorganic/organic filler phase. Resin composites are directly and indirectly used to repair and replace the lost tooth structure or as a direct filling material. Despite many advances in the performance of composites in recent years, their useful life and longevity as a restorer, the annual failure rate of restorative composites is reported to be 3–11%, so that the approximate life of these materials is about 10 years [27]. Secondary decay and fracture of the composite mass are recognized as the principal proof of the failure of composites [25]. Since the last few years, the creation of permanent interfaces and intermediate phases between the matrix and the amplifier has become an important issue. The chemical reactions between the matrix and the amplifier, pairing agents, and chemical modification of the component surfaces have contributed to some extent to the bonding of the matrix to the encapsulated amplifiers. Polymer matrices and inorganic filling particles are commonly used as reinforcements for direct restoration of teeth and their beauty. Glass ionomers are used for tooth restorations, fissure sealants, lotting agents, liners, and bases, and as root sealants. Materials such as hydroxyapatite, silica, zirconia, graphene, and silver nanoparticles (Ag NPs) are added to better the mechanical characteristics including hardness, compressive strength, and flexibility of glass ionomers for dental applications [28]. On the other hand, it has been reported that the combination of alumina/zirconia and hydroxyapatite with glass ionomer increases antibacterial activity and biocompatibility. Nanomaterials are effective in the properties of restorative composites [29].

3.2 Carbon-Based Nanomaterials

Carbon nanotubes as prominent carbon-based materials are known for their flexibility, strength, and biocompatibility [30]. Studies have shown that carbon nanotubes have mechanical properties in addition to antimicrobial characteristics [31]. This feature paves the way for the use of tubes as coating materials for dental implants and bone tissue engineering scaffolds. Furthermore, carbon nanotubes can be used to produce dental coatings with outstanding features such as bioactivity as a delivery system for agents with antimicrobial characteristics and the feasibility of tissue regeneration [28].

In recent years, ceramic materials such as glass matrix ceramics, polycrystalline ceramics, and resin matrix ceramics have been used instead of traditional amalgams and fillers [28]. Accumulation of oral biofilms on implant surfaces is the most prevalent cause of dental implant breakdown. Graphene is another nanomaterial composed of a thin layer of carbon atoms with a hexagonal honeycomb lattice making this material the thinnest known substance in the world. This exceptional feature has granted fantastic properties to graphene, such as good electricity and heat conduction, high mechanical strength, and very low mass [32]. Graphene, as a carbon-based nanomaterial, is used to treat various bacterial biofilms, which are a major cause of tooth decay and periodontal disease [33]. Graphene has got other applications in dentistry including its incorporation in implants, membranes, resins and cement, teeth whitening, detection of dental bacteria, and tissue engineering [34]. Graphene/zinc oxide nanocomposites have been reported to be effective in the treatment of *Streptococcus mutans* biofilms [35].

3.3 Hydroxyapatite (HAP)

About 97% of tooth enamel is mainly composed of Hydroxyapatite ($C_{10}(PO_4)_6(OH)_2$). Hydroxyapatite nanoparticles (HAP-NP) are used in dentistry as a compound similar to a tooth and bone structure due to their high biocompatibility in physiological processes. By combining Hydroxyapatite particles with CNTs, a useful composite can be obtained to close the orifice and prevent the nerves from being directly exposed to external stimuli. High biological activity and high reactivity of HAP nanoparticles, which arises from their large surface area, results in their quick adhesion onto the dentin, followed by enamel apatite resulting in its repair. Hydroxyapatite, as an artificial enamel, covers small cavities caused by erosion and decay, thereby preventing further decay. Studies have shown that hydroxyapatite can attach to bone and does not cause any inflammatory reactions or toxic effects. This material provides better and immediate integration of the titanium implant with the teeth and surrounding tissues by forming a crystal with the mineral components of the tooth [35]. The use of Hydroxyapatite nanorods for the strength

of dentin restorations has been reported in the literature. Hydroxyapatite nanorods can be used as a suitable substitute for silicate restorative materials [36].

3.4 Metal Oxide Nanoparticles

Impressive promotions in nanotechnology resulted in the development of new nanomaterials like metal oxide nanoparticles for numerous dental plans such as reinforcement of the filler, attacking oral pathogens, transferring calcium phosphate compounds to the teeth, supplying sufficient contrast during imaging, and improving the capacity of bonding interface for mineral compounds [37]. Titanium oxide is a cost-effective, non-toxic substance that can be widely used in dentistry. The incorporation of titanium oxide results in a considerable reduction in the number of bacterial colonies which subsequently prevents the formation of biofilms on the teeth. On the other hand, the use of this nanoparticle due to its unique photocatalytic properties in resin-based implants increases the polymerization synthesis and photocatalytic activities of the implants [38]. Zinc oxide nanoparticles (ZnO-Np) are among the nanomaterials that have been studied as mineral fillers in restorative materials. Zinc oxide nanoparticles can activate the growth of minerals through their bioactivity and negatively affect the growth of bacteria. Zirconium dioxide (ZrO_2) is used in dental implants because of its tooth-like color, antibacterial properties, and low toxicity. By merging zirconia with other nanomaterials such as alumina, its mechanical properties and toughness are improved. The resulting composite material can be used as a suitable coating to reduce the formation of biofilm on teeth [35].

ZrO_2 releases fewer ions and is more biocompatible than titanium implants. ZrO_2/HAP composites increase the interaction between bone and implant and are suitable for bone repair. The use of zirconium and similar ceramic materials such as zirconium and aluminum oxides compensates for the lack of mechanical strength of HAp composites and adds to the antibacterial and biocompatibility properties of the composite [39]. Since surface morphology and roughness have a great impact on the interaction between bone and implants, it is recognized as one of the most vital properties of a dental implant. Similar bone integrity is a significant advantage over traditional implants. Nano ZrO_2 has become a promising option over titanium enamels due to its chemical stability, high biocompatibility, high fracture toughness, and flexural strength. Zinc carbonate HA nanoparticles have also been shown to be effective preventative and control agents for biofilm formation.

In addition to the above, metal oxide nanoparticles including copper oxide (CuO), iron oxide (Fe_2O_3), cerium oxide (CeO_2), and tantalum oxide (Ta_2O_5) Have been reported useful in reducing the formation of multispecies biofilms and improving the mechanical properties of restorative composites [40]. Table 2 gives useful information regarding various metal oxide nanoparticles which have been used in different aspects of dentistry.

Engineered nanoparticles, in the form of nanofilms, nanomembranes, nanoscaffolds, and composites, have attracted much attention in diagnostic imaging,

Table 2 Application of metal oxide nanoparticles in various disciplines of dentistry

Discipline	Nanoparticles	Applications	References
Prosthodontics	ZrO_2 NPs, TiO_2 NPs, CuO, Ta_2O_5, CeO_2, Fe_2O_3, Ag NPs, CNT reinforced ceramics, Graphene/zinc oxide nanocomposites	Biofilm management on the tooth surface, antibacterial and bacteriostatic properties, better fracture toughness and surface finish, improved flexural strength and fracture toughness, dental aesthetics	[28]
Preventive dentistry	ZnO NPs, HA NPs, Zinc carbonate HA NPs, carbon nanotubes, ZrO_2	Low surface tension allows nanoparticles to get to the smallest fissures and dental ducts, used directly before dental fillings, dental caries inhibiting properties, tooth remineralization, intense desire to absorb and neutralize the oral microflora, improves the surface properties of restorative materials	[38]
Periodontics	Nanospheres and nanocapsules, HA NPs, nanocrystalline HA	Bio-membranes for correction of osseous defects, osteoconduction and osteoinduction, Selective differentiation and growth of epithelial cells during periodontal surgery	[28]
Orthodontics	Alumina NPs, ZnO NPs	Coating of metal nanoparticles on brackets and arc wires reduces surface irregularities and reduces friction, nanoparticle modified orthodontic bonding materials, NPs coating act as a solid lubricant film to ease the sliding of orthodontics wire over the bracket	[18, 22]
Restorative dentistry	Nanofilled resin-modified glass ionomer cement, single walled nanotube (SWNT), bioactive glass nanoparticles, Ag NP, ZnO NPs, HA NPs	Nano particle-filled restorative composite resins, nanoflled bonding agents, nanoflled glass ionomer cement, treatment of dental hypersensitivity, improved esthetics, strength, and durability of composite resin, wear resistance	[14, 18, 20]
Dental implant	Au NP, ZnO NPs, TiO_2 NPs	Surface modifications of dental implants	[7, 18, 21]

cancer treatment, and targeted drug delivery [41]. Extensive studies have been performed on the regeneration of bone, cartilage, or dental tissue using nanomaterials, with promising results for the medical sciences. Natural or synthetic organic nanoparticles have shown many advantages in dentistry, implantology, periodontics, endodontics, and wound healing [42]. High colloidal stability, better dispersion, and improved surface reactivity make these materials a new solution in treatment, prevention, and tissue repair.

3.5 Nanozymes

Nanozymes are nanomaterials that display enzyme-like behaviors. Nanozymes are recognized as an important way through which nanomaterials and biological systems are connected [43]. In recent years, these materials have motivated numerous researchers owing to their useful characteristics such as ease of synthesis, high stability, low cost, and adjustable catalytic activity. Nanozymes can be promising alternatives to natural enzymes in a variety of applications. In enzyme-induced cellular metabolism, oxygen undergoes single electron reductions, leading to the formation of reactive oxygen species (ROS) such as OH ·, OOH ·, $O_2{\cdot}^{2-}$, and H_2O_2 radicals. ROS with low dosage plays an important role in signal transduction, cell proliferation, and the body's defense against pathogen invasion. On the other hand, when ROS levels increase unusually, redox homeostasis is destroyed and the structure is seriously damaged which consequently leads to the function of macromolecules in the cell with oxidative stress. Oxidative stress may result in diseases including chronic inflammation leading to diabetes, cancer, cardiovascular, neurological, and pulmonary disorders. Enzyme systems such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) protect cells against the harmful effects of ROS by modulating intracellular ROS levels. Nanozymes can modulate ROS levels in cells due to their enzyme mimicry activity. Nanozymes convert superoxide to H_2O_2 and subsequently to O_2 and H_2O, thereby reducing intracellular ROS levels [44]. Studies have shown that several natural enzymes, including proteolytic enzymes and amylase, can be used to treat tooth decay and mouth ulcers, owing to their antibacterial, inflammatory, and immune-boosting activities. However, natural enzymes have poor stability in environments with high temperatures and extreme pH, and other disadvantages such as time-consuming separation and purification, high production costs, and difficult storage for long periods limit their use [45]. In addition to the intrinsic properties of nanomaterials such as photothermal and fluorescence effects, nanozymes have properties such as antibacterial, anti-inflammatory, and antioxidant activities. Furthermore, the use of nanozymes in tumor treatment, tissue regeneration, and disease diagnosis has been reported [46].

Nanozymes are embedded in the dental plaque biofilm structure, and under acidic pH, convert H_2O_2 to free radicals, break down extracellular polymeric materials, and kill bacteria [47, 48]. Nanozymes provide bioavailability by staying in the plate

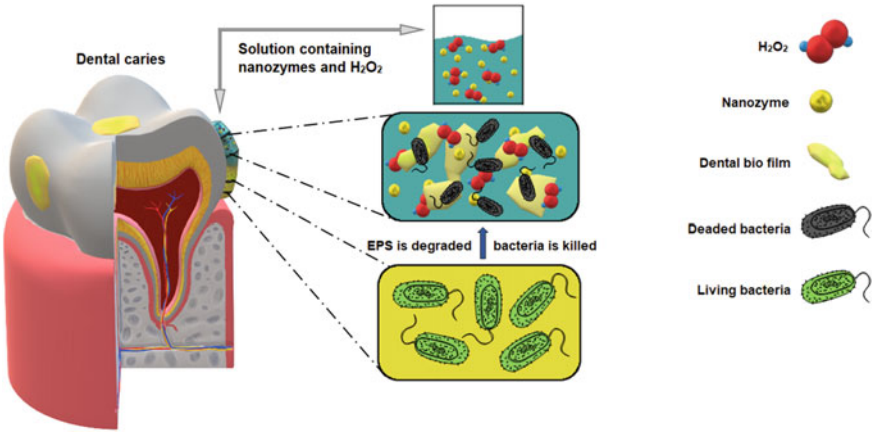

Fig. 4 Schematic of mechanisms of nanozymes in the elimination of oral bacteria

biofilm and maintaining activity. In addition, they are stable in physiological environments and can be activated by changing the pH caused by the activity of pathogenic bacteria. The mechanisms of nanozymes in the elimination of oral bacteria are shown in Fig. 4. Fe_3O_4 nanoparticles are effective in H_2O_2 catalysis by peroxidase-like activity [46]. Nanoparticles such as iron oxide, cerium dioxide, and copper/carbon hybrid nanoparticles are known as nanozymes [49].

4 Nanomaterials in Oral and Maxillofacial Surgery

Oral and maxillofacial diseases deal with the influence of physical, chemical, and biological factors as well as systemic malfunctions on the soft and hard tissues of the craniofacial, face, and dental arches. The application of nanomaterials has helped to cure oral and maxillofacial diseases by providing significant improvements in oral and maxillofacial healthcare [50]. Titanium is the most widely used implant material for cranial and maxillofacial applications due to its biocompatibility and high strength [51]. The mechanical properties of pure titanium are very similar to bone in terms of Young's modulus. But as a bioinert substance, it prevents tissue integrity. To promote bone integrity and prevent periprosthetic infection, many efforts are made to create surface structures suitable for implantation. Many studies have reported that nanometer-controlled titanium surfaces can be fabricated by a variety of surface modification techniques, including mechanical, chemical, and physical methods, or a combination of them [52]. Mechanical techniques, such as grinding, polishing, machining, or blasting, are used to obtain specific surface topographies to improve the adhesion of biologically active molecules and accelerate biomineralization. The plasma polymerization technique is used to create a surface. Bioactive materials can be used on titanium substrates for better stabilization of the bioactive molecules such

as DNA, heparin, and glucose oxidase [53]. NH_2 and COOH-based plasma polymers are used intermittently for bacterial adhesion and to prevent biofilm by coating the layers with a suitable antibacterial agent such as silver nanoparticles (AgNPs) with good chemical reactivity. To improve abrasion resistance, corrosion resistance, and blood compatibility, advanced physical techniques are achieved within 1–10 nm of the modified surface layer. Chemical and electrochemical treatments are more cost-effective than physical methods. Chemical methods include acidic or alkaline treatments, hydrogen peroxide purification, chemical vapor deposition, and electrochemical techniques. Specific surface topographies are created through these techniques to improve corrosion resistance, improve biocompatibility, bioactivity, or bone conduction, and create different nanostructures in terms of nanotubes, nanopores with cavity morphology, or nanotubes with different diameters. Different sizes are obtained from 15 to 300 nm in diameter and different lengths by adjusting the anodizing conditions [53]. In addition to supporting the attachment of target tissue cells, implant surfaces are designed to prevent bacterial adhesions. Biofilms consist of clumps of sticky microorganisms with several high-density species that adhere to the surface of the implant, affect the function of the implants, and may cause local inflammation [53]. Nanoparticles are extensively used worldwide as efficient antibacterial agents, and today, modification of titanium surface using antibacterial properties of metal nanoparticles is a common approach in clinical treatments. Nanoparticles with antimicrobial activities which are used to coat titanium surfaces include silver, zinc, gold, selenium, copper, fluorine, and calcium nanoparticles. TiO_2 nanotubes and silver nanoparticles are used to improve titanium levels and antibacterial applications. On the other hand, selenium nanoparticles have been found to be useful in killing cancer cells and treating bone cancer [53].

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Chapter 6
Nanotechnology for Pain-Free Dentistry

Hamid Reza Vanaei, Sofiane Khelladi, and Abbas Tcharkhtchi

1 Introduction

The problem of dentistry always engaged with its pain. This issue comes from the inappropriate medical services, and the fact that the painful incidents during the dental procedures act as a barrier for further operations. Nowadays and with the development of the technology, it has been argued that pain-free dentistry is a substitutional approach to reduce the pain resulting from the dentistry and its related operations. Using this approach, surgical and numerous dentistry operations are free of pain and increased the tendency of performing different dentistry operations.

To address the advantages of pain-free dental operations, the most important one could be mentioned as the relationship between the patient and dentist that facilitate the requirements for doing dentistry operations. On the other hand, it means that the less the pain in dentistry operations, the more the healthy situations.

However, the concept of pain-free dentistry has been developed nowadays and turns into the incorporation of nanotechnology and pain-free dentistry. The expression "nanotechnology" has first utilized in 1959, and was the implementation of materials and devices in the scale of nano. Devices such as nanorobots or nanomachines, and materials with the scale of less than 100 nm, have been widely used from the time of this technology's development. In general, there are four generations of nanotechnology that are summarized as follows: (1) Active nanostructures as 3D transistors, actuators, biodevices; (2) Passive nanostructures as ceramics, polymers,

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composites, aerosols; (3) Nanosystems; and (4) Molecular nanosystems in atomic designs. Accordingly and based on the existence of these definitions, the application of nanotechnology in dentistry termed as "nanodentistry" could be classified as follows: inducing anesthesia, hypersensitivity cure, tooth repair, salivary diagnostics, nanoceramic technology, and the most important ones: nanodentistry as a bottom-up approach and nanodentistry as a top-down approach.

This chapter focuses on the incorporation of nanotechnology and dental medicine. We concentrate on literature that presented the role of nanotechnology in dental medicine and the associated nanobiomaterials as well as the pain-free dentistry.

2 Role of Nanotechnology in Dental Medicine

The emergence of the application of nanotechnology in dental medicine has made this approach a promising technique. In fact, a high level of oral health could be obtained by employing nanomaterials and implementing the potential treatments in dentistry to be able to facilitate the common treatments that already exist in the industry [1].

The main key factor of employing nanotechnology in biomedical subjects, particularly the dental medicine, relates to the possibility of construction of the nanostructures with a nanoscale precision. One can note the advantages of the nanotechnology in dentistry such as disease diagnosis, restorative dentistry, rehabilitative dentistry, or even regenerative dentistry. However, based on the application and the existence of the technology in dental medicine, there are two approaches in general: Top-down and bottom-up. The top-down approach normally works on the implementation of the existing materials to be applied in nanoscale and enhance their quality. Whereas, the bottom-up approach refers to the creation of smart devices which is capable to force the fabricated structures to be assembled in a nanoscale to be able to apply them in macroscale fabricated structures (Fig. 1) [2].

By developing the application of nanotechnology in the area of dental medicine, different nanomaterials have been included to be applied in this regard. By applying nanomaterials, oral health could be obtained as a result of using dental nanorobots. They are capable of operating a program in different operations and thus monitor them remotely to perform a reconstruction of the desired parts [3]. Accordingly, the dental medicine received much attention using the knowledge of tissue engineering and also the stem cell that are capable of performing cartilage reformation, bone augmentation, and integration of dental implants. Using tissue engineering, nanoscale fibers could be a promising part in the bone tissue engineering in order to improve the mechanical strength of the desired parts. By using Titanium implants as the main bio-nano-based dental implant, it has been observed that the reaction to the biological environment could be increased by increasing the surface area of the fabricated part. Also, efforts have been taken into account to produce artificial structures to be able to apply them as the artificial tooth. Employing dental composites, researchers have added fillers to the resin polymer to improve and augment the mechanical strength of the fabricated

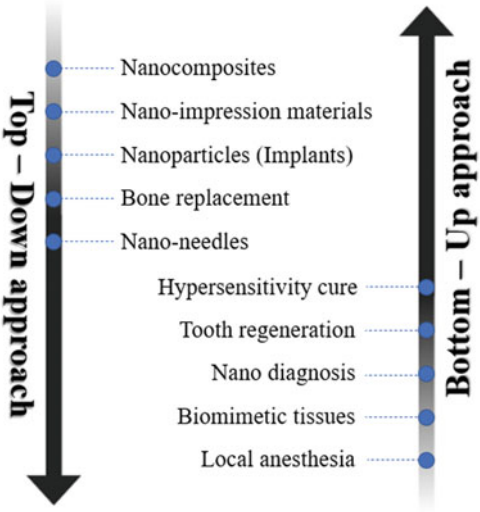

Fig. 1 Schematic representation of the application of nanotechnology in dentistry

structures. However, further research indicated that nanoparticles should be replaced with other nanoscales additives in order to produce more smooth surfaces that are suitable for the artificial teeth [4]. Worth mentioning to say that the in-vitro studies as well as the physicochemical and mechanical characterizations give us the possibility of obtaining the high-strength composite parts to be applied in dental medicine.

Given the above-mentioned explanation through the application of artificial composite teeth in dental medicine and also the importance of surface engineering, one can note the importance of surface engineering in dental medicine and consequently the importance of the application of nanotechnology in dental medicine termed as "nanodentistry". From the very beginning, several applications of nanotechnology have been addressed and due to the fact that almost all of them are applicable in dental medicine, nanodentistry has received a sustainable attraction through dental nanorobots and tissue engineering [5].

3 Nanobiomaterials in Dentistry

3.1 An Overview on Applicable Nanoscale Constructs in Biomedical

Following the explanation on the different approaches on the application of nanomaterials, there are several types of nanomaterials that are widely being used such as nanoparticles, nanospheres, nanofibers, nanopores, and nanoshells.

Nowadays, nanoparticles play an important role and they are mostly applicable in resin-based composites and consequently in the dental composites. Several works have focused on various characteristics of the nanoparticles to improve their application in this area [6]. Nanospheres have particular application for repairing natural tooth. Nanorods are also using in some materials such as Hydroxyapatite-based composites based on their bioactivity and they are capable of providing artificial models [7]. The role of nanofibers in biomedical application is inevitable and strengthening composite materials is one of the most important parts in dental composites. The main advantage of nanofibers refers to their improved physicochemical and mechanical properties. In comparison with the presented nanoscale materials, nanopores are one of the cheapest options for fabrication purposes particularly for observation of the shape of the DNA molecules for cancer treatments [8]. Furthermore, to observe and absorb the infrared lights and apply them as a source of heat for destroying the cancer cells, nanoshells are widely being used in recent years. Accordingly and based on the nature of their application, nanoshells have several applications in dental medicine including the prevention of photodegradation of teeth or preparing the photonic crystals.

3.2 An Overview on Nanomaterials in Dental Medicine

The key-point for researchers and manufacturers is the fabrication of high quality and good surface roughness for dentistry objectives such as implants. However, the quality of tissue-implant interface is an important issue in employing the nanoscale materials in dental medicine. Also, the implant surface roughness is the most important factor in design of them which is an important issue in consideration of various characteristics that are important in dental medicine. Accordingly, the use of different types of nanoscale materials and their application in dentistry has been briefly presented in Table 1.

Table 1 Different types of nanoscale materials in dentistry [9–15]

Nanomaterial	Application
Single-walled carbon nanotubes	Improvement of the mechanical properties of the resin-based composite
HA nanocrystals	Dental implant coating
Diamond nanoparticles	Coating orthopedic implants in bone regeneration
Yttrium-stabilized zirconia nanoparticles	Coating for biomedical implants
Calcium carbonate nanoparticles	Remineralization of enamel lesions
Spherical silica nanoparticles	Dentine hypersensitivity
Glass nanoparticles	Enhancement of mineral content of dentine

4 Nanotechnology and Pain-Free Dentistry Methods

4.1 Nanotechnology in Dental Diseases

4.1.1 Tissue Engineering

With the promising progress in the implementation of tissue engineering in dentistry, nanotechnology and its application in tissue engineering have a remarkable development of scaffold materials. The objective of this approach relates to the enhancement of the mechanical properties of the applied materials. As the nanoscale surface of the material performs different physicochemical characteristics, almost all bioactive molecules tend to interact with desired materials and thus it is more convenient to evaluate the behavior of the proposed structures. So, nanotechnology-based tissue engineering is capable of fabrication of the structures that are applicable in dental medicine.

To mention these approaches, there are several examples such as the implementation of collagen fibers in tissues. The structure of the collagen molecules gives us the opportunity of operating them in bone tissues comprising collagen fibers [16]. In fact, the development of nanoscale fiber-based scaffolds goes to the regeneration of dental tissues (e.g., dentin-pulp complex). Based on the large surface area relative to volume, several surface-related characteristics govern the cellular behavior of the fabricated structure. This is related to the fact that dentin and pulp are broadly used in nanoscale fibers scaffolds that can be fabricated using different techniques such as phase-separation or electrospinning. Compared with other methods, electrospinning is the most powerful method for producing the nanoscale fibers. Recently, there are several works that have been working on the nanoscale fiber-based scaffolds including bioactive glasses and biopolymers. The most common outcomes of them have shown that the characteristics of the fabricated scaffolds, in particularly the mechanical strength, have improved with better biological behavior [17]. In parallel to these explanations, it is worthy to mention that nanoscale fiber-based matrix has been developed to highlight the hypersensitivity of the dentin [18].

4.1.2 Root Canal Disinfection/Sterilization

To sterilize root canals, several efforts have been taken into account to apply chitosan or Zinc oxide in dental treatment approaches. The fact is that the antibacterial performance could be improved in the treated dentin, and it is required to perform some surface treatments through the root canal dentin using phosphorylated chitosan that will help to preserve the inhibitory effect on the root canal dentin. Accordingly and with reference to the several possibilities, another option is the use of metal oxide containing nanoparticles that shows the same antibacterial behavior. It has been found that these nanoparticles are one of those adherents that are effective and leave significant effect on the root canal dentin.

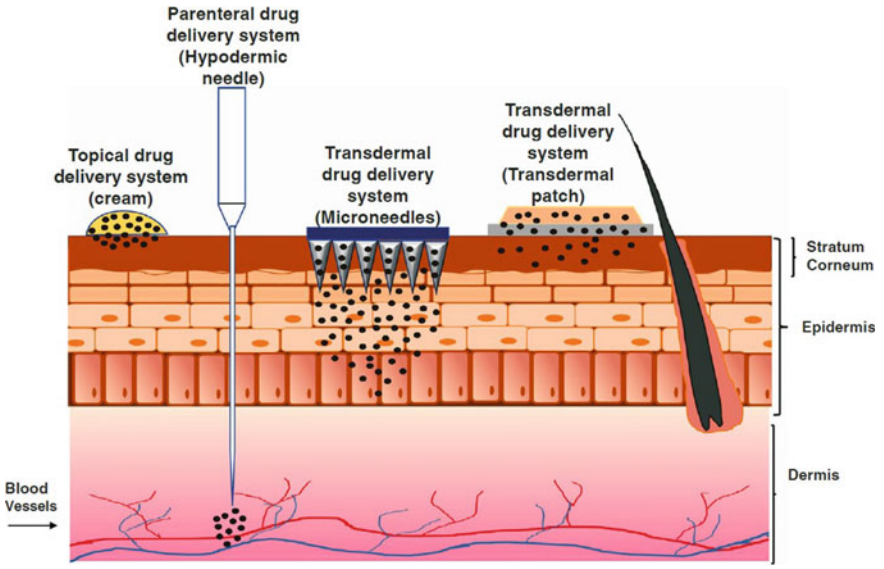

Fig. 2 Schematic representation of comparison of topical, parenteral, and transdermal drug delivery systems utilized in the management of pain (adapted from [19])

4.2 Pain-Free Dentistry

Pain management is one of the most important issues in medical aspects, and it is one of the main concerns of treating the pain in order to be able to reduce it. Accordingly, the fast development of personalized medicine gives the opportunity of implementing various issues and techniques through the pain management. Nanotechnology is one of the most applicable approaches in medicine that has been promisingly progressed in recent years. As shown in Fig. 2, it is capable of providing drug modality to control pain without any complications.

Numerous efforts have been taken into account to develop proper means of treatment to detect and evaluate the dental diseases. Presumably, there are researchers that are broadly trying to develop these tools, whereas, there are still missing spots in their recognition and development of these tools to meet the minimum requirements. The mentioned issue also exists for the parts combined with the nanotechnology and the employment of nanoscale materials. Incorporation of nanotechnology and dentistry is increasingly becoming a subject of broad and current interest in the industry and medical science, and it is required to pay a strong attention in treatment of dental diseases using nanotechnology.

One of the most important issues that exists in dental treatments is the pain problem that creates a barrier between patients and the tendency to perform their dental treatments. It has been remarkably announced that the advances in dental medicine

Table 2 Common applicable lasers in dentistry

Laser type	Delivery system	Application
Argon	Continuous wave	Tooth bleaching
CO_2	Continuous wave	Treatment of oral ulcerative lesions
Diode	Continuous wave	Surgical wounds
Nd: YAG	pulse	Root canal therapy
Helium–Neon	Optical fiber	Frenectomy and gingivectomy
HO: YAG	Pulse	Treatment of oral lesions

have enabled dentists to perform pain-free dental treatments and some of the most important pain-free ways in dentistry are briefly explained as below.

4.2.1 Lasers

Nowadays, laser treatments play an important role in dentistry and they are normally utilized for soft tissue treatments. Prior to this step and innovations in the industry of dental medicine, the use of conventional methods keeps people away from doing the dental surgeries, whereas, laser treatments perform a pain-free surgery without bleeding. The mechanism of this operation is based on the transformation of the light from the laser to the target using the fiber-optic cables. In this approach, there are several types of lasers working on either the dental treatments or soft tissue treatments. The main feature is the difference in lasers' wavelength that distinguishes them different from each other. So, it depends on the dental work that let the dentist to choose the type of the laser use in the operation treatments. A brief explanation of the different types of lasers further with their application and delivery system is summarized in Table 2 [20].

4.2.2 Nitrous Oxide

Also known as laughing gas, it is a mild relaxing element and is applied for managing and reducing the pain in dentistry. To apply during the surgery or dental treatment, a colorless nitrous oxide and oxygen are mixed and within minutes of breathing, it will have its effect. One can refer to the name "laughing gas" that comes from the fact that the gas makes some operation to reduce the sensitivity of the nervous system. To mention the benefits of utilizing the laughing gas by dentists, we can mention its safety and also the sedation that gives to the patient during the operation. However, there might be some siding effects such as headaches, sleepiness, or excessive sweating in the case of high level of nitrous oxide. To avoid these impacts, it is recommended to breathe for about 5 min with excessive oxygen, which helps cleaning the lungs from the remained gas and thus facilitates the awareness of the patient. With reference

to the given explanations, the advantages of utilizing the nitrous oxide comprise as the possibility of titration, maintaining the reflex integrity, rapid onset of action, and no need to have any escort. Also, the limitations of applying this oxide mainly refer to the expensive equipment and the fact that during the treatment/surgery, patient should breathe through his/her nose [21].

4.2.3 Implant Dentistry

Dental implants have been widely used in the past decades. Titanium-based implants are the main type that are utilizing in dentistry. Based on several studies that have been performed on the characteristics of these implants, it was found that the mechanical properties of them are not high enough and the limitation of their strength is still a limiting point. Furthermore, based on the significant progresses in the fabrication of nanoparticles, the application of nanotechnology in dentistry and particularly the dental implants have been raised to fabricate high-strength dental implants. Efforts have been taken into account in modifying the external surface in the nanoscale, and it has been observed that the chemical reactivity altered the surface biomolecular interactions. Indeed, the importance of the application of the dental implants relates to the interface of tissue-implant. So, modifying their external surface is inevitable, and it is required to perform a good surface roughness. In the case of the Ti implants, it is required to improve the interaction of the external surface of the implant with the surrounding environments by adding a ceramic layer and avoid the corrode reactions. To do this, nanoscale ceramics were produced to be applied on Ti implants. They mainly include the calcium phosphate compounds or hydroxyapatite (HA) that are categorized as the most important coating materials in this application. Although HA coating makes the external surface of the implant tough enough to prevent the degradation and corrosion of the dental, the particles' size and morphology play a crucial role on their interface adhesion [22].

5 Conclusion

Human life has been deeply influenced by the rapid progress in technology, and it is particularly impacting several aspects such as the health care. It is capable of changing health care in several ways such as proposing novel techniques for disease prevention, establishment of nanorobots, or drug delivery. In recent years, nanotechnology has shown significant impact in dental medicine to cover several features in dentistry and its related operations/surgeries. The impact of nanotechnology on dental medicine has created massive changes on improving the traditional facilities by fabrication of several types of nanodevices such as nanorods, nanotubes, or nanofibers. Furthermore, nanorobots are capable of controlling microscopic level in different features of dental cares. Accordingly, pain management is one of the main concerns in the field of dentistry and by development of the nanotechnology in performing pain-free

dentistry, researchers and industry are working hard for development of pain-free dentistry techniques including the nanotechnologies.

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Chapter 7
Nanorobotics in Dentistry

Rampalli Viswa Chandra

1 Introduction

Nanorobots are measured on the nanometer scale, which are basically theoretical microscopic devices. They are also referred to as nanites or nanomachines, and have a diameter of 0.5–3 μm and components that are between 1 and 100 nm in size [1–12]. In humans, these nanodevices are used primarily for protection or treatment against pathogens. At cellular and molecular levels, these nanorobots can respond to certain programs, that allow to perform accurate procedures [10–12]. They can be operated by clinicians using acoustic signals and also electromagnetic waves. The first nanorobot was designed with high navigational accuracy to crawl or dip through human tissues [13]. Due to their small size, nanorobots are rarely seen by the naked eye. Nanorobotic theory suggests that "nanorobots are microscopic in size and it would probably be necessary for very large numbers of them to work together to perform microscopic and macroscopic tasks" [14].

Nanorobotics is primarily being applied for minimally invasive surgery with excellent control over precision instruments, which are proved to be wonderful tools for surgeons. Surgeons use joystick handles instead of instruments, to control robotic arms containing microinstruments and perform micromovements in cellular level surgery. Nanorobots should ideally integrate diagnostic precision, surgeon's motor skills, and sensations with haptics and reality [15].

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2 History

The first person to study nanorobots was Robert Frietas, which were made in resemblance to red blood cells and named them as respirocytes [16]. In the late 1990s, the term nanorobot was mostly used by the robotic circle. Eric K Drexler and Robert A Freitas were two pioneers in the field of nanorobotics. The concept of molecular machinery and manufacturing was explained by Drexler while the concept of medical nanorobotics was described by Freitas. Science fictions, TV series, and books have become the source for nanorobotics to reach large audience. In 1966, Issac Asimov published a book 'Fantastic voyage' which described a miniscule submarine able to travel through the human blood stream while Michael Crichton in his book 'Prey' described an intelligent nanorobot that threatens humans. In the nonfictional context, the word nanorobot is the correct technical term for serious engineering studies [17].

3 Mode of Action of Nanorobots

For nanorobots, carbon which is the main element used is in the form of diamond nanocomposite because it is chemically inert. For special purposes, light elements such as nitrogen and oxygen can be used. The passive diamond coating of nanorobots externally will provide a flawless, smooth surface and evokes less immune reaction from the body. Metabolization of glucose, oxygen, and externally supplied acoustic energy will be the powerhouses of nanorobots [17–19].

Computers capable of performing around 1000 computations per second are used to control nanorobots [18]. Broadcast-type of acoustic signaling establishes communication with the device. In the body, a navigational system is installed that provides high precise accuracy to all flowing nanorobots and also maintains track of the other devices in the body. The surface antigens present on cell surface help nanorobots to distinguish between various cells [17–20].

Nanorobots manufacturing involves actuators, control, sensors, and power communications. It requires interfacial signaling across spatial scales, between organic and inorganic as well as biotic/abiotic single office visit. In dentistry, nanorobots can be used to covalently bond with diamondized enamel. Continuous oral health maintenance with the help of destroying caries-causing bacteria is achieved by mechanical "dentifrobots" and the nanorobots even repair blemishes on the teeth where decay has set in [18].

In real time, dental nanorobots might use specific motility mechanisms to acquire energy, penetrate human tissue with navigational precision, and manipulate their surroundings. Nanorobot functions could be controlled by a nanocomputer that executes pre-programmed instructions in response to local sensor stimuli. Also, strategic orders can be issued directly by the clinician via acoustic signals to nanorobots. Dental outlook for nanorobots identified by previous researchers (Goene

et al. 2007; Freitas 1999) has included to build nanoparticles by combining atomic elements and the creation of nanoscale objects [19].

4 Dental Nanorobot

The dental nanorobot should have a nanocomputer which will execute planned missions, receive and process signals, communicate with other nanocomputers, it responds to external control and to various monitoring devices. Researchers have designed various shapes for medical nanorobots, based on the place of action and biomimetism. Freitas suggested that intravascular structure of nanorobot should have a sphere shape, resembling leucocytes and red blood cells. Medical nanorobot should be designed in accordance with a flagellated bacteria for controlled movement through the blood vessels [19–21] (Fig. 1).

As dental nanorobots need to be quick in fulfilling their tasks, they should have a spider-like body. Diamondoid structures are used for manufacturing dental nanorobots. Diamondoid molecules are diamond-like structures with circular saturated hydrocarbons. Exceptional atomic structure is the unique property of diamondoids. They have properties like thermal and chemical stability, self-assembly, more resistant, but are lighter than steel [20, 21].

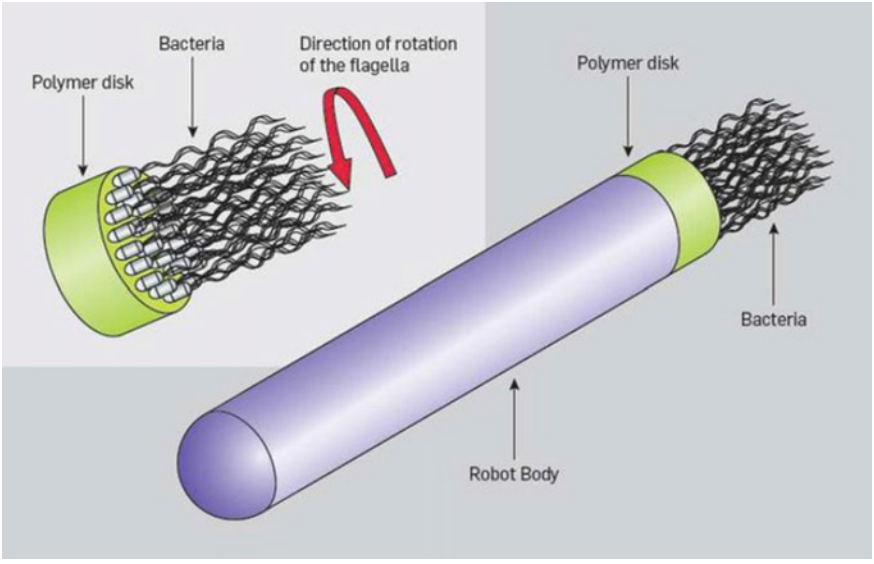


Fig. 1 Magnetic micro/nanorobots contain two components—fabrication of micro/nanorobots and incorporation of magnetic components

5 Types of Hypothetically Used Nanorobotic Systems [11, 22, 23]

(a) Microbivores—Primary function of microbivores is to destroy pathological microorganisms found in the human bloodstream. In phagocytic defences, microbivores are 1000 times faster-acting than antibiotics and are able to extend the therapeutic therapy of the clinician to the wide range of potential microbial threats, such as local infections. They are also used for making artificial mechanical cell walls.

(b) Respirocytes—These are considered to be artificial blood cells which deliver large volumes of oxygen than natural red blood cells, i.e., up to 236 times of oxygen to the tissues per unit volume. Medically respirocytes have various applications which include transfusable substitution of blood; perinatal, neonatal, and lung disorders; partial treatment for anemia; enhancement of cardiovascular/neurovascular procedures; prevention of asphyxia; artificial breathing; and a variety of sports, battlefield, veterinary, and other uses.

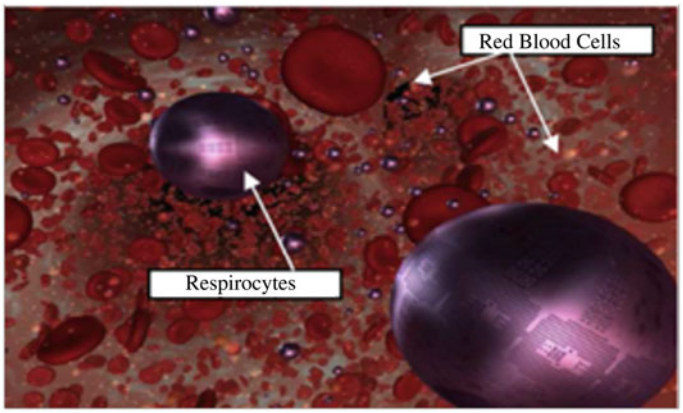
(c) Clottocytes—These are artificial mechanical platelets. Even in moderately large wounds, clottocyte may allow complete haemostasis in as little as ~1 s. Considered to natural system, this response time is 100–1000 times faster.

(d) Pharmacytes—These nanorobotic devices are manufactured for pharmaceutical drug delivery. Within the human body, these pharmacytes will be self-powered, with digitally precise transport that is controlled by computer nanorobotic devices, and specific cells can also be targeted by pharmacytes.

(e) Chromallocytes—Chromallocytes were aimed to use in chromosome replacement therapy and gene delivery. This hypothetical mobile cell repair nanorobot is capable of limited vascular surface, travels into the capillary bed of the targeted tissue or organ, and then causes extravasation, histonatation, cytopenetration, and complete chromatin replacement (Fig. 2).

6 Applications of Nanorobots in Dentistry

Research in nanodentistry covers a wide range of areas including diagnosis of diseases (nanodiagnosis), prevention of dental caries (nanoprevention), and dental treatments (nanotreatment). Nanorobots can be used for inducing analgesia, desensitization of tooth, realign tissue, straighten irregular teeth, and to increase durability of natural teeth. Furthermore, nanorobots are used in preventive, restorative, and curative dentistry. Their applications are as follows (Fig. 3).



Respirocytes

Fig. 2 Respirocytes

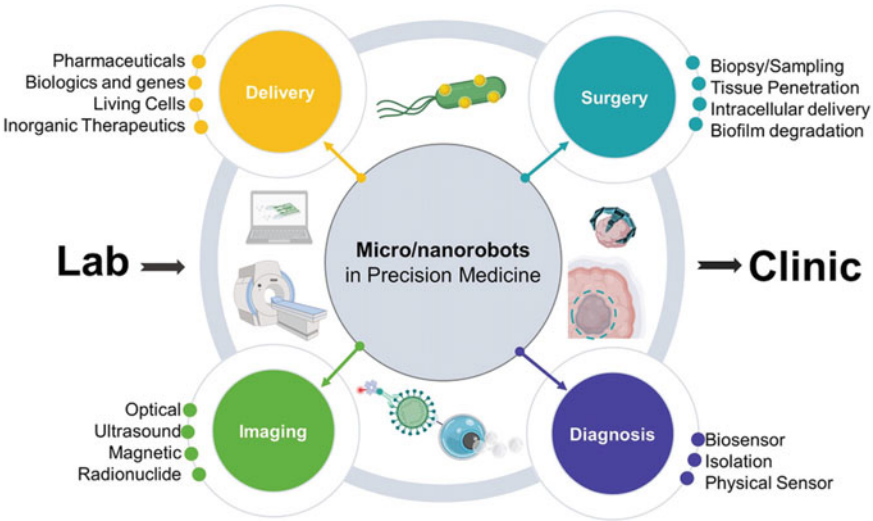


Fig. 3 Applications of nanorobots

7 Nanodiagnosis

Potential applications of nanorobotics devices include early detection of diseases such as cancer at the cellular and intercellular levels, pharmacokinetics, and detection of biomarkers and pathogens. Life-threatening oral diseases such as oral and pharyngeal cancer have been listed as the most common type of cancer in many countries, affecting thousands of people annually.

Identifying cancer-related cell changes using polymeric nanoparticles and, subsequently, detecting cancer at an early stage using minimally invasive techniques greatly improves prognosis. Applications of nanodiagnosis include sensor systems such as 2 25 nanoelectro mechanical systems (NEMSs), oral fluid nanosensor test (OFNASET), and optical nanobiosensors [11].

7.1 NEMs

Nanoelectro mechanical systems (NEMs) transform the biochemical signal and cantilever array sensor into sensitive technology suitable for the detection of microorganisms and their DNA at the molecular level. This technology is useful in diagnosing oral tumors and diabetes mellitus diseases, and also in detecting bacteria, viruses, and fungi [23].

7.2 OFNASET

Oral fluid nanosensor tests (OFNASET) are micro-electromechanical systems consisting of protein and RNA biomarkers. The system is based on connecting target RNA molecules or proteins to the surface of the sensor efficiently and utilizes microfluid technology, defined as the automation of analytical laboratory procedures onto a single device or "chip." A combination of salivary mRNA biomarkers like Oz/ten-m homologODZ, IL-1b, IL-8, and salivary transkriptome-SAT and salivary biomarkers such as IL-8 and thioredoxin has been shown to have precise sensitivity and specificity for the diagnosing of oral cancer [11] (Fig. 4).

7.3 Optical Nanobiosensors

These use the principles of optics for the transduction of biochemical interactions into suitable output signals. The fiber-optic tool allows non-invasive analysis of intercellular protein such as cytochrome C, which is important for cellular level energy production and involved in programmed death of cell or apoptosis [23].

Digital Dental Imaging: With the advent nanotechnology, there is an advancement in even digital imaging. Decreased radiation dose with high-quality imaging can be obtained using nanophosphor scintillators in digital radiographs.

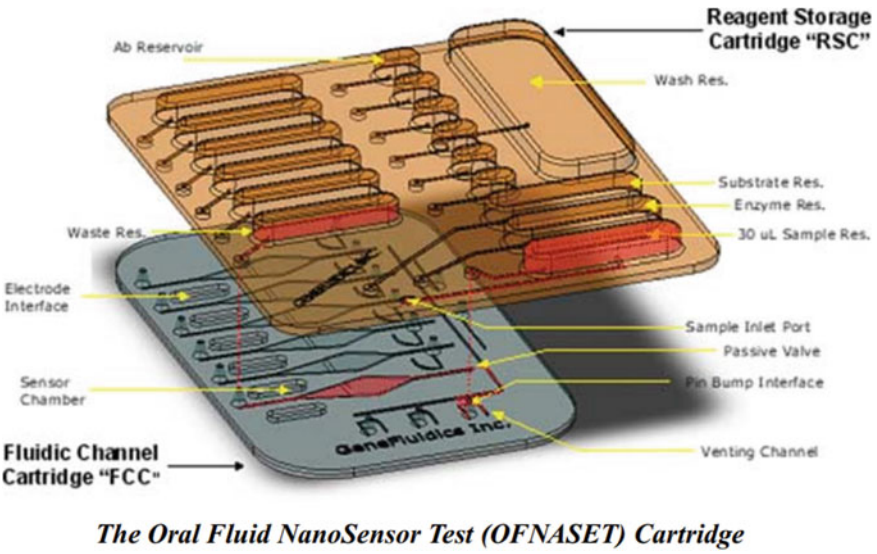


Fig. 4 OFFSET—Oral fluid nanosensor test device

8 Nanoprevention

Remineralization represents a major advancement in the clinical management of lost enamel surfaces, and the introduction of nanoparticles in fissure sealants, fluorides, and toothpastes can assist in the prevention of dental caries.

8.1 Sealant

Braun et al. demonstrated that, relative to conventional sealant materials, fissure sealants containing nanofillers exerted minimal effect on laser fluorescence values during caries detection. Therefore, nanomaterials may be used to assess caries progress underneath sealants and to initiate appropriate treatment in a timely manner. However, further research on the properties of these materials and the long-term reliability of these treatment strategies is necessary [11].

8.2 Nanosilver Fluorides

Although silver diamine fluoride application is a conservative, effective, and non-invasive treatment option for the prevention of dental caries, it often causes staining

of the teeth through deposition of silver particles on the dentin layer and formation of a silver phosphate layer, resulting in aesthetic deterioration.

This has led to the development of fluorides containing silver nanoparticles that produce decreased staining as the particles are smaller and therefore do not undergo oxidation. Smaller silver particles also have the added benefits of increased surface contact with cariogenic cells.

One such new formulation, known as nanosilver fluoride (NSF), contains silver nanoparticles, chitosan, and fluoride, and exhibits antimicrobial properties against Lactobacilli and Streptococcus mutans. Laboratory and clinical studies have shown that NSF is safe to use in humans, is economical, and can be used to arrest active dentin caries in children [11].

8.3 Nanorobotic Dentifrices (Dentifrobots)

Nanorobotic dentifrices can cover all subgingival surfaces, when delivered either by mouthwash or toothpaste, where it metabolizes trapped organic matter into harmless and vapors. Well-designed dentifrobots can identify and destroy pathogenic microorganisms that exist in the plaque. When swallowed, these invisibly small dentifrobots which are purely mechanical devices safely deactivate themselves [2].

Nanohydroxyapatite toothpastes deliver a nanocrystalline form of hydroxyapatite particles at a size 20–50 nm which is conducive for natural repair. Previous studies have reported that nanohydroxyapatite toothpastes inhibited caries development and exhibited higher remineralizing potential compared to those containing amine fluorides, suggesting that these were effective alternatives to fluoride toothpastes [11] (Fig. 5).

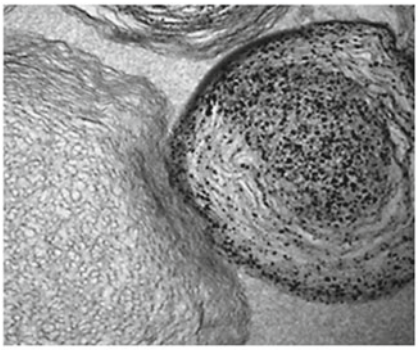


Fig. 5 Multilayer liposome with nano-HA particles

9 Nanotreatment

There is significant growth in the field of science exploring incorporation of nanotechnology in dental treatments, resulting in vast improvements in the materials used for oral therapy.

9.1 Nanoanesthesia

- Millions of active analgesic micronsized dental nanorobot "particles" in colloidal will be instilled by the dentist on the patient's gingival.
- The nanorobots then reach dentin by migrating into the gingival sulcus and at the CEJ they pass through the basement membrane or loose tissue.
- They enter the dentinal tubules up to 1–4 μm depth, once reaching the dentin and by a combination of chemical gradient, temperature difference, and navigation under nanocomputer control they move towards the pulp.
- Within 100 s, the moment of nanorobots from surface of tooth to the pulp chamber occurs.
- Once installed in the pulp chamber, they exert their control over neuronal impulse, analgesic nanorobots shut down all sensitivity in any particular tooth requiring treatment.
- As the dental professional presses the handheld control, the selected tooth is immediately anaesthetized.
- The dentist controls the nanorobots to revive all the sensations and will exit from the tooth after the procedure is completed [2, 24].
- Analgesia by nanorobotics offer patient comfort with reduced anxiety, no needles, greater selectivity, control the effect of the analgesic, also reverses the action with avoidance of complications [25].

9.2 Hypersensitivity Cure

Dentin hypersensitivity is another field of dental nanorobots. Higher surface density of up to eight times is seen in natural hypersensitive teeth dentinal tubules and diameter is also twice as large than nonsensitive natural teeth.

- Dental nanorobots, using natural biomaterials, could selectively and precisely occlude specific tubules within minutes.
- The nanorobots enter dentinal tubular holes that are 1–4 μm in diameter once they reach the dentin, and travel toward the pulp, guided by a combination of chemical gradients, temperature differences.

- There are various pathways by which nanorobots travel from dentin to pulp. Because of significant tubular branching patterns, dentin tubular density may present as a challenging factor for navigation.
- In approximately 100 s, the nanorobots can complete their journey into the pulp chamber. This is with the assumption that the total path of length of about 10 mm from the surface of tooth to the pulp and average speed of about100 μm/s [2].

9.3 Nanocomposites

- Cavity Preparation and Restoration—Multiple nanorobots that are invisible to the naked eye are used for cavity preparation and even restoration of teeth. Maximum conservation of sound tooth structure is achieved by limiting the preparation of cavity to the decayed enamel and dentin [8].
- In dentistry, microfillers and microcore materials in composites have been used for a long time. Even if filler particle size cannot be reduced below 100 nm, nanocomposite filler particles are small enough to be produced at the cellular level.
- These nanoparticles increase the compressive strength of the material.
- To improve polishability and esthetics, particles of minute size, such as zirconium dioxide, are also used. However, brittleness and cracking or fracturing after curing are the disadvantages of using these zirconia particles.

To overcome this issue, hybrid composites and composites containing a wider range of filler particles were used. Due to nanoparticle clumping they are weak, despite displaying strength and esthetics. This problem can be addressed by surface coating process during the filler particle manufacturing procedure, thus reducing weak spots and providing equal strength throughout the fill of the core.

A smoother, creamier consistency with improved flow characteristics is seen with the uniform amalgamation of filler nanoparticles. Once the material reaches its hardened state, these characteristics add up to the dentin-like curability and also polishability of the material [13].

9.4 Nanocomposite Denture Teeth

Although porcelain dentures exhibit high wear resistance, they are fragile, poorly connected to the denture base, and difficult to polish. Conversely, resin dentures are easy to manipulate but are highly susceptible to abrasion.

Nanocomposite dentures contain uniformly dispersed nanosized filler particles and polymethylmethacrylate, which deliver superior surface structure, polishability, surface hardness, and wear resistance [11]. According to literature, acrylic teeth and

microfill composite teeth are less durable than nanocomposite artificial teeth and have a lower resistance to abrasion.

Nanotechnology has enhanced the characteristics of various kinds of fiber materials. Polymer-reinforced nanofibers with diameter of nanometer size have a greater surface area and permit an easier addition of surface modalities compared to polymer reinforced microfibers.

9.5 Nanolight-Curing Glass Ionomers

The combination of nanotechnology and fluoraluminosilicate technology was found to greatly improve the wear resistance, esthetics, and polishability of glass ionomer materials. Clinical indications of this material include core build-ups; class I, III, and V restorations; sandwich technique; and restorative treatment of primary teeth [11].

9.6 Impression Materials

- The addition of nanofillers in vinyl polysiloxanes was seen to improve the hyrophilic properties, flow, and accuracy of siloxane impression materials when compared with traditional vinylpolysiloxanes.
- Therefore, nanofillers may be integrated into conventional vinyl polysiloxanes in order to improve their properties and produce fewer voids, better model casting, and advanced accuracy.
- Nanotech elite H–D plus (Zhermack Italy) is an example of such an impression material that is commercially available. This material has several advantages including high tear resistance, increased fluidity, resistance to distortion, hydrophilic properties, and snap set and heat resistance, thus reducing errors caused by micromovements [10].

9.7 Orthodontic Nanorobots

- In contrast to the current techniques which require weeks or months to proceed to completion, orthodontic nanorobots could directly influence the periodontal tissues which include gingival tissue, periodontal ligament, cementum, and alveolar bone structures, allowing fast painless tooth alignment, rotations, and vertical repositioning within minutes to hours [25].
- A novel stainless steel wire with an electroless nickel phosphorous filled with fullerene-like inorganic nanoparticles of tungsten disulfide (IF-WS2) nanotechnology could provide a new way to minimizing friction during tooth movement.

Studies evaluated the noxious effects of fullerene-like nanoparticles and found them to be biocompatible.

- Advantages of these wires include corrosion resistance, smooth surface finishing, high strength, and good deformability [26].

9.8 Nanosolution (Nanoadhesives)

Nanosolutions are used as bonding agents as they are dispersible nanoparticles. This helps in an uniform and perfectly mixed adhesive consistently [27]. The advantages include high dentinal bond strength, stress absorption, high shelf life, good marginal seal, no additional step of acid-etching and will also have fluoride release.

9.9 Nanoencapsulation

- Tissue targeted release systems have been introduced by South West Research Institute (SWRI). These nanocapsules are available in varied forms like vaccines, local antibiotics, with less complications.
- In 2003, Osaka University in Japan made possible the targeted delivery of dedicated nanoparticles and this might be developed to focus even on oral tissues.
- Literature suggested the use of triclosan in reducing inflammation. So, an effort can be made to introduce local drug delivery of triclosan encapsulated in nanocapsules for the treatment of periodontal diseases [28].

Several novel products were manufactured by SWRI;

- Protecting outfit and mask, by incorporating antipathogenic nano-emulsions and nanoparticles.
- Medical appendage for immediate cure: with various types of wound dressings.
- Bone targeting nanocarriers: These materials are encapsulated with calcium phosphate which integrate with natural bone easily.

9.10 Tissue Engineering and Dentistry

- Bone augmentation, treatment of orofacial fractures, temporomandibular joint, pulp repair, periodontal ligament regeneration, and implant osseointegration are various applications of tissue engineering in dentistry.
- In implantology, tissue engineering helps in early loading of implant. This enables placement of implant that is biologically much stable than the conventional implants and thus reducing prolonged period of waiting.

- With the use of nanocrystalline hydroxyapatite, bone grafts with better properties can be introduced. Studies have suggested that nanocrystalline hydroxyapatite helps in stimulation and proliferation of cells for periodontal regeneration.

Currently, for tissue engineering purpose, various researchers are trying to manufacture cytocompatible biological nanomaterial scaffolds with encapsulating cells such as stem cells, chondrocytes, and osteoblast [29].

9.11 Nanoneedles

In the manufacture of suture needles, nanosized stainless steel crystals can be used, which can make oral and periodontal cellular surgery possible in the near future.

Commercially available examples include RK 91, Sandvik Bioline, and AB Sandvik Sweden needles [11].

9.12 Bone Replacement Materials

- Bone grafts closely reflect the chemical composition and structure of natural bone. The adhesion and growth of osteoblasts on nanophase hydroxyapatites were found to be significantly higher than those on conventional hydroxyapatite.
- Similar to other synthetics, nanophase hydroxyapatite materials reduce the risk of immune rejection and stimulate better cell growth.
- One such nanoproduct is made up of two bonelike components: calcium-containing nanocrystals with the size of natural bones, and collagen to mimic the soft tissues around the natural bones.
- Another nanorobotic product is made up of a mixture of nanocrystalline calcium sulfate particles and calcium sulfate hemihydrate powder, and promotes bone regeneration over a longer period of time [25].
- It can be used to repair bone defects caused by facial injuries and periodontal surgery. Nanocrystals consist of loose microstructure and nanopores situated between the crystals. The surface modification of the pores can be done by the addition of silica molecules as they adsorb protein. Bone defects can be effectively treated by using these hydroxyapatite nanoparticles [13].

9.13 Tooth Reconstruction

- Nanodentistry plays an important role in natural tooth repair through genetic engineering, tissue engineering, and regeneration, as well as production and installation of a whole new tooth in vitro.

- The use of nanorobotic technology to manufacture and install new teeth with the same mineral and cellular components as the original tooth structure would greatly improve the whole treatment plan [30].

9.14 *Dental Implants: Structure, Chemistry, and Biocompatibility*

- The success of osseointegration is determined by factors such as surface contact area and surface topography. Nevertheless, bone bonding and stability also play an important role. By using nanotechnology, bone growth and increased predictability can be effectively accelerated in implants.
- For effective osteoblast formation, nanoparticles of hydroxyapatite and calcium phosphate can be deposited to create a complex implant surface.
- These new implants enhance the integration of nano-coatings resembling biological materials to the tissues and are more acceptable [31].

Surface alteration at nanoscale level on titanium endosseous implant surface can alter cellular and molecular response that can benefit implant osseointegration.

- At present three nanostructured implant coatings in use are diamond; which provide hardness, toughness, low friction; and possess increased osteoblast adhesion proliferation with mineralization, and graded metalloceramics with the ability to limit adhesion problems.
- Nanoscale surface structuring optimizes cell colonization, surface chemistry, and attempts to control and optimize the chemical surface properties of an implant material.
- Wettability due to the observation that cell adhesion and subsequent activity are generally better on hydrophilic surfaces. Nanoscale processes allow structuring and chemistry modification which would play a role in increasing wettability.
- HA nanoparticles used in repairing osseous defects are Ostim ® HA, VITOSS ® HA + TCP, NanOssTM HA.

9.15 *Bionic Mandible*

Similar to normal mandible in function and sensation, the bionic mandible is helpful to reconstruct the entire mandible. Using nanotech-enabled robotic myoelectric prosthetic limb, the first bionic arm was constructed by Todd Kuiken and his team. Just like this bionic mandible reconstruction is also possible near future [32].

9.16 Dentition Renaturalization

Cosmetic dentistry can be revolutionized by this technique. Initially, old amalgams restorations may be removed and the teeth remodeled with natural materials. This is followed by complete coronal renaturalization procedures in which all previous procedures may be undone and all the teeth remanufactured to become identical to natural teeth [33].

10 Nanodentistry—An Interdisciplinary Approach

Nanorobotics is a strong promising tool in revolutionizing oral health care and esthetics. Enormous changes in medicine and dentistry as nanodentistry is emerging as an interdisciplinary field that is undergoing fast development. And the applications of nanodentistry is seen in various fields of dentistry.

10.1 Nanotechnology in Oral Medicine and Radiology

- Nanomedicine, atomic force microscopy, and scanning tunnel microscopy are major breakthroughs that pave way to many advancements. Resolution in the order of fractions of a nanometer is demonstrated by these technologies and also creates topographical three-dimensional images.
- It helps in diagnosis of inflamed tissues and tumor that is rapid, early, sensitive, and economically feasible, which poses major challenges in regular oral medicine and radiology [34].

10.2 Nanotechnology in Periodontics

For clear diagnosis of periodontal diseases, Quantum dots (QDs) coupled with immunofluorescence can be used that precisely label specific periodontal pathogens. To enhance healing of inflamed tissues, lead-free and cadmium-free quantum dots are used in periodontal therapy [35].

10.3 Nanotechnology in Endodontics

Endodontic sealers incorporated with bioceramic-based nanoparticles have helped obturation by gaining access to difficult irregular dentinal surface. The use of Quaternary ammonium polyethyleneimine (QPEI) nanoparticles also provides better antibacterial properties [34].

10.4 Nanotechnology in Oral and Maxillofacial Surgery

Devices like nano-LIPUS (low-intensity pulsed ultrasound) are used for stimulating fibroblast growth factors with enhancement of wound healing, allowing bone growth into titanium-coated implants, and also in distraction osteogenesis.

10.5 Nanotechnology in Maxillofacial Prosthodontics

Effective antimicrobial activity can be seen by the incorporation of silver nanoparticles in prosthodontic materials such as silicone and polymethylmethacrylate. Incorporation of titanium dioxide and cerium dioxide nanoparticles showed an impact on mechanical properties such as strength and color stability.

10.6 Nanotechnology in Orthodontics

In orthodontics, nanocomposites and nanoionomers (consisting of nanoagglomerated particles and nanoclusters (made up of fluoroaluminosilicate glass and zirconia) improve chemical adhesion, improve bond strength, mechanical properties.

Apart from these applications, there are many more researches being carried out to introduce newer applications in the field of nanorobotics. However, there are certain limitations in this field and the challenges faced have been discussed below.

11 Limitations of Nanorobotics

(1) Engineering Challenges:

- Mass production technique feasibility.
- Simultaneously manipulating and coordinating activities of large numbers of nanorobots.

(2) Biological challenges:

- Developing biofriendly nanomaterial without any adverse effects on human body.

(3) Social challenges Ethics:

- Acceptance from public
- Regulation of human safety.
- Challenges faced by Nanorobotics.

Engineering Challenges:

- Even though the field of nanorobotics is fundamentally different from that of the macrorobots due to the differences in scale and material, there are many similarities in design and control techniques that eventually could be projected and applied.
- It has become possible to attempt the creation of nanorobotic devices and interface them with the macroworld for control, due to the modern scientific capabilities.
- There are countless such machines which exist in nature, and there is an opportunity to build more of them by mimicking nature.

Biological Challenges:

- To ensure compatibility with all intricate of human body, it is essential to develop bio-friendly nanomaterial.
- Smaller particles can interact with other living systems increases because they can easily cross the skin, lung, and in some cases the blood/brain barriers. These nanoparticles can be toxic.
- Further biochemical reactions like the creation of free radicals that damage cells can occur once they are in the body.
- The body has built defence for natural particles it encounters, but the danger of nanoparticles is that it is entirely new to the defense system and is known to be toxic.

Social Challenges:

- Employes manufacturing these nanoparticle products are at the highest risk.
- The National Institute for Occupational Safety and Health (NIOSH) reports that over two million Americans are exposed to high levels of nanoparticles and published safety guidelines to follow in nanoindustry [36].

Nanorobots are going to play an essential role in the future in the medical field. Nanorobotic technology promises huge advances in extending healthy lifespan as they are efficient in eliminating every infected cell. Life is going to be easier and simple with the advent of nanorobots. Nanotechnology is foreseen to change health care in a fundamental way. It forms the basis for early disease diagnosis and prevention. It will be useful in therapeutic customized to the individual patient and can be used in drug delivery and gene therapy.

12 Conclusion

Enormous changes will be seen in the fields of medicine and dentistry with nanotechnology. However, as with all advantages, it may also pose a risk for misuse. Nanorobots which remained only in fictions in earlier era are now becoming a new reality. Dentistry has become less stressful to the dental surgeon with the various applications of nanotechnology in dentistry. In the future, nanodentistry becomes

the best method to maintain oral health. More success in the field of nanotechnology can be achieved with further research.

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Chapter 8
Nanobiotechnology in Regenerative Dental Medicine

Henry A. Adeola

1 Introduction to Dental Nanotechnology

The emergence of the dynamic field of nanotechnology has ushered in impactful and exciting progress over the last century in various fields of biomedical sciences [1–5], as well as other fields such as electronics [6–8], aerospace [9], catalysis [10–12], automobile [13], food processing [14], pharmaceutical [15] and cosmetic [16] sciences. The field of dental sciences which involves the management of oral diseases and other conditions that affect the mouth, teeth, gum and hard tissues of the maxillofacial complex [17–21] also benefits immensely from a range of applications of nanobiotechnology (NB) to improve oral health conditions, and hence this chapter is dedicated to discussing the notable current and potential application of NB in the field of regenerative dental medicine.

1.1 Basic Nanotechnology Principles

Nanotechnology harnesses the phenomenal atomic and molecular behaviour of materials at a nanoscale size (1–100 nm) to provide solutions to a vast array of scientific applications [22, 23]. The burgeoning knowledge of variation in structural design, shape microstructure, size and surface charge of nanomaterials holds great promise for improving the medical application [24], and avoiding the toxic side effects of nanoparticles. The concepts behind nanotechnology were first espoused by the 1959 seminal lecture of renowned material physicist, Richard Feynman, entitled "*There's Plenty of Room at the Bottom*" at the American Physical Society [24–26], and there

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has been an exponential increase in its application in recent times. Although nanomaterials are commonly classified as carbon-based, organic-based, inorganic-based and composite-based, there are several other classification systems that are based on chemical composition, shape, origin, dimensionality and crystallinity, as described elsewhere [27]. Common nanomaterials such as nanofibers, nanoplates, nanotubes, quantum dots (QDs), nanowires have played crucial roles in the biomedical sciences [28–30]. Production of nanoparticles is generally engineered by anthropogenic activities or as naturally available biological species. Such natural nanomaterials are independently present in the earth spheres [23], while engineered nanomaterials result from grinding particles mechanically, smoke, engines exhaust, etc. Although there is manifold benefit in the production of nanoparticles using various sources, a major pitfall in the artificial synthesis of nanoparticle is the uncertainty around its environmental safety and molecular behaviour [14, 31–34]. The safety, stability, security and effectiveness pitfalls have been potentially addressed by the environmentally-friendly and cost-effective green synthesis, which uses plants and microbes for synthesis [35].

1.2 Biological Regeneration and Nanomedicine

As opposed to tissue repair, which focuses on adaptive mechanisms to restore functionality to tissue after injury, albeit with the formation of scar tissue; tissue regeneration involves the restoration of tissue to its original morphology, population and function [36]. The molecular and cellular underpinning mechanisms for these pathophysiological homeostatic processes are yet to be fully elucidated [37]. Under a normal physiological circumstance, the functional restorative body mechanisms are highly efficient; however, several host and environmental factors may militate against this optimal tissue recovery and may lead to formation of scar tissue or wound dehiscence, following injury [36, 37]. Heterogeneity and diversity has been observed, in the constitutive regenerative capacity of various organisms in the field of tissue repair and regeneration [38]. Many organisms are capable of complete regeneration following injury, while others are not. It also appears that the degree of regeneration and scar tissue formation within the same species also differ, depending on the age. In fetal skin, several studies have described the ability to perfectly regenerate damaged tissues [38, 39]. This phenomenon has also been described in murine experiments [40]. Among other possible molecular mechanisms, the absence of transforming growth factor beta (TGF-β) has been identified as a key factor in the scarless regeneration of tissues [41, 42]. Regenerative medicine aims to use a multidimensional approach to leverage current understanding of the repair and regeneration mechanisms, to develop biomaterial or cell/tissue composites that are biomimetic and biologically compatible for tissue regeneration [43–45]. This aim is achieved in three keyways, which includes the use of cellular systems (e.g. transplantation with stem cells), hybrid/complex systems involving cells and biomaterials, and the exclusive use of biomimetic biomaterials [43]. The interest of these three distinct approaches of regenerative medicine has been furthered with the use of inorganic and organic

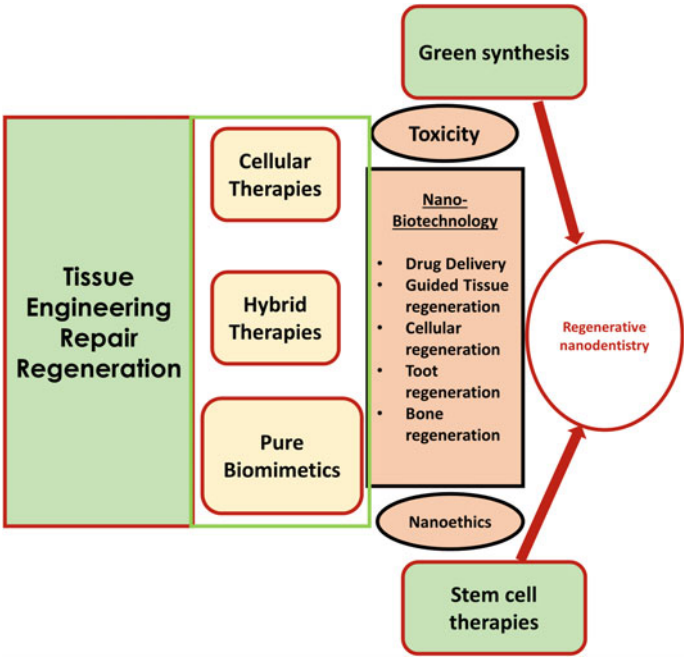

Fig. 1 Prospects of translation of regenerative nanobiotechnology using nanoparticles for regenerative nanodentistry. Potential obstacles may be overcome by the use of green synthesis and establishment of good nanoethical regulations to prevent toxicity

nanoparticles and has been well-discussed elsewhere [46–48] (see Fig. 1). Dental tissues like the rest of human tissues are made up of molecules, and the application of nanotechnology to molecular science will foster remarkable progress in regenerative nano-dental science problem management, leveraging molecular knowledge to improve and maintain "molecular-scale" dental health [49]. The application of nanotechnology has also revolutionized the field of regenerative dental medicine using these three key tissue engineering and regeneration for graft design, development of scaffolds, and restoration of dental and maxillofacial tissues, as described hereafter.

1.3 Regeneration and Nanotechnology in Dental Medicine

The emerging field of nanodentistry explores the application of nanotechnology to the management of dental conditions [27, 50, 51]. Beyond all normal body connective tissues (such as bone, nerves, blood vessels, fatty tissues and other soft tissues), the mouth, jaws and maxillofacial complex also contains specialized epithelial mucosal tissues (gum, floor of mouth; as well as buccal, lingual and palatal mucosa), glandular

tissues (salivary glands), fibrous tissues (periodontium), muscular tissues (tongue muscles, palatal muscles, as well as muscles of facial expression, deglutition and mastication) and other dental hard tissues such as enamel, dentine and cementum [52–56]. A good understanding of the biochemistry and interconnectedness of these various tissues is an important basis for the effective application of nanotechnology in the field of regenerative dental medicine. The burgeoning application of nano-dental approaches in the field of regenerative dentistry presents a matchless opportunity for tissue restoration beyond the confines of the possibilities of orthodox maxillofacial tissue reconstruction and restorative dentistry.

2 Nanobiomaterials Used in Dental Medicine and Maxillofacial Pathology

As previously introduced in Sect. 1.2, biologically compatible nanobiomaterials (organic/inorganic) development for regenerative dental medicine, leverages three key approaches of either using cellular, hybrid or pure biomimetic nanomaterials [43]. Over the years, there have been continued efforts to improve biocompatibility and reduce the toxicity of nanoparticles [3, 57–60], using functionalized biodegradable nanomaterials in tissue engineering [61]. Organic nanoparticle assembly has been improved for small molecule delivery. For instance, hydrophobic and hydrophilic blocks of copolymer micelles (sized 10–100 nm) within non-ionic water-soluble shell attenuates the uptake capability of the innate phagocytic mechanism on the nanobiomaterials, thereby increase the biological function, stability and bioavailability of these materials [62]. A wide range of nanomaterials have been used in regenerative dentistry for targeted imaging and small molecule delivery. This includes near-infrared-absorbing carbon (e.g. carbon nanotube or graphene), metals (Ag, Au, Pt, Ag, or Pd), quantum dots (QD)-based nanostructures (such as CdSe or CdTe), upconversion composite nanoparticles (such as NaGdF4:Yb:Er) and magnetic iron oxides [27, 63]. The scope of nanotechnology in regenerative dental medicine involves the use of both synthetic and natural nanobiomaterials for tissue engineering/rejuvenation, leading to the maintenance of good oral health. These highly beneficial nanobiomaterials may be employed to augment the natural properties of dental materials and treatments. The use of nanobiomaterials in the field of dental medicine (Table 1) ameliorates many of the canonical problems associated with dental treatments such as pain, disfigurement and malocclusion; as well as supports improved aesthetics, self-confidence and beauty [64–66].

Table 1 Nanobiomaterials used in the field of regenerative dental medicine

Regenerative nanobiomaterials delivery system	Use/mechanism of action	Type of regenerative material	Ref#
Copolymer micelles	Small molecule delivery	hybrid	[62]
Near infrared-absorbing carbon	Small molecule delivery	inorganic	[27, 63]
Upconversion composite nanoparticles	Targeted imaging	Hybrid	[27, 63]
Quantum dots	Targeted imaging	Hybrid	[27, 63]
Magnetic iron oxides	Targeted imaging	Inorganic	[27, 63]
Collagenase	Improves bone-to-tooth tissue remodelling	Enzyme	[65]
Nanostructure surface coating	Optimize the mechanical properties of dental materials and bone substitutes	Organic	[73]
Magnetic nanoparticles conjugated with nonviral, viral or polymeric platforms	Gene delivery	Hybrid	[79]
Nanofiller Engineered nanomaterials	Nanocoating for implants, antibacterial agents	Hybrid	[80]
NanoGIC	Calcium fluoroaluminosilicate glass powder with an aqueous homo-/copolymer of acrylic acid used as liners, fissure sealant, luting agents and as an adhesive in clinical paediatric and restorative dentistry	Hybrid	[97, 98]
Nanocomposite	Regeneration of bone and dental tissues	Hybrid (metallic-, polymeric- or ceramic-based)	[112, 113]

2.1 Nanobiomaterials for Dental Regenerative Drug Delivery

There has been an increased public appetite for cellular transplantation, as well as genetic and medical therapies that seeks to replace and rejuvenate diseased or damaged cells, tissues or organs. A controlled release with targeted delivery plays a pivotal role in precision/personalized medicine [67]. As the development of nanobiomaterials expands, the eligibility criteria for engineered tissue and cell therapeutics has evolved under the regenerative medicine advanced therapy (RMAT) categories, as established in Section 3033 of the 21st Century Cures Act [68]. The RMAT designation by the Food and Drug Administration (FDA) provides fast-track opportunities for affordable, and effective regenerative materials (and products) to be

introduced into mainstream management of patients [68, 69], using cellular therapies, biologics, biomimetic prosthetics and tissue bioengineering. Similar regulations have been adopted by the European Union (EU) to grant hospital exemption to Advanced Therapy Medicinal Products (ATMP) which are employed for treatment of conditions to which conventional therapeutic approaches have proven ineffective [70–72]. Using nanoparticles as delivery vehicles has the potential to reduce toxicity and improve therapeutic efficacy of current drug regimens, novel therapeutics and repurposing of previously undevelopable bioactive (but suboptimal) pharmacological agents [73]. Obstacles related to solubility, bioavailability, pharmacokinetics and potency of new molecular entities can now be overcome with the use of nanobiomaterials [73, 74]. Various nanoparticles have been used for regenerative drug delivery in the field of dental medicine. For instance, dental therapies have been delivered noninvasively using nanosized liposomal vesicles [65]. Enzymes, such a collagenase, have been nanoparticle-delivered for bone-to-tooth tissue remodelling in implantology and periodontological settings, significantly improving the tolerance and outcome of dental surgical procedures [65]. Increased *DMP-1* gene expression has been observed *in-vitro*, following laser photobiomodulations of inflamed dental pulp stem cells [75]. This might potentially have relevance in pulp tissue regeneration following injury. Cell therapies that mimics the intricate in-vivo milieu are now possible with deeper understanding of the role of fibroblasts and other skin and mucosal cells in the highly orchestrated wound healing process [76]. Nanobiomaterials have been employed to optimize the mechanical properties of dental materials and bone substitutes, and they have also been used for nanostructure surface coating of dental prosthesis and implants [73]. Inorganic nanoparticle surfaces have been functionalized to act as drug sensitiser for oral squamous cell carcinoma [4, 77]. Furthermore, the salivary flow barrier to topical therapies in the oral cavity have been overcome by the synthesis of novel nanoencapsulant/nanocarrier-aided drug delivery systems [78]. Not least, genetic materials have been directly transferred to cells and tissues using magnetic nanoparticles conjugated with nonviral, viral or polymeric platforms, for gene delivery [79]. There is a great prospect for the benefits of nanoparticle drug delivery system in dental medicine, albeit pharmacokinetics, encapsulation, coordination chemistry, cleavability (REDOX or enzymatic) and electrostatics are important factors to be considered in targeted covalent/non-covalent ligand conjugation of nanobiomaterials [67].

2.2 Tooth Structure Enhancement with Engineered Nanomaterials

The unique physical and histological architecture of the oral cavity, as well as the biochemical and microbiological composition of saliva, oral biofilms and dental hard tissue, requires demands adaptation of the conventional nanomedical approaches to

specifically developed engineered nanomaterials (ENM) that are practical, beneficial, bioavailable and useable in the oral cavity environment [80]. Although the compact crystalline structure of the dental enamel may prevent ENM penetration, the arrangement of the dentinal tubules is advantageous to the penetration of ENMs for restorative and regenerative dentistry purposes [80]. Nanofiller ENMs have been used to structurally fortify restorative materials, while ENMs have also been used as nanocoating for implants, personal oral care (e.g. dentifrices) and antibacterial agents in dentistry [80]. Even though literature evidence suggests that oral toxicity is low, ENMs crossing the gastrointestinal tract have been identified as responsible for systemic disruptions and gastrointestinal pathologies [27]. Optimization of the surface nano-topography of metallic dental implant with surface modifications, biochemical anodization and creation of nanoscale adhesive topography has significantly improved tissue engineering and osteointegration in maxillofacial and implantology practices [81, 82]. Despite the evolving benefits of ENMs, a cloud of uncertainty still persists about its safety and potential adverse effects [83].

2.3 Emerging Application of Nanobiomaterials in Regenerative Oral and Maxillofacial Pathology

In maxillofacial pathology, nanomaterials have been used for tooth repair, tooth renaturalization/realignments, tooth sensitivity treatment, covalently bonded diamondized enamel, as carriers of local anaesthetic agents (nanoanesthesia) and as nanorobotic dentifrices [84–86]. Nanobiomaterials have also lent themselves for use in reengineering of periodontal tissues, bone and graft regeneration/augmentation, orthodontic nanorobots, pulp repair and stem cell therapy, orofacial fractures, nanofill restorative dental materials, as well as temporomandibular joint regeneration [27, 87, 88]. Mobile nanorobots have been used to intercept pulpal nerve impulses by transporting active anesthetic agents to the pulp through dentinal tubules. This approach has been used to reduce complications in the management of periodontal diseases [89, 90]. Nanocrystalline hydroxyapatite, nanoassemblers and programmable, 0.5–3 μm diameter diamond nanorobots are also used in restorative dentistry [64]. Luminescent near-infrared luminescent quantum dots and superparamagnetic iron oxide nanoparticles (SPIONS) have been used to target specific cancer antigens in conjunction with enhanced magnetic resonance imaging [27, 91]; precision biosensors and nanochips are currently explored for oral cancer biomarker detection [84, 92]. Specific applications of nanobiomaterials to regeneration of dental and maxillofacial structures and tissues are further discusses hereafter.

2.4 Hybrid Nanomaterials in Regenerative Dentistry

Various dental tissues are required to work harmoniously for the tooth to be structurally and functionally sound. The durability (and integrity) of restorative materials aimed at replacing dental structures such as the dentine, enamel, pulp and cementum depends on a sound knowledge of the biochemistry and pathophysiology of these tissues. Restorative materials are therefore expected to be aesthetically pleasing, bear occlusal forces during mastication (and other parafunctional forces), and retain their stability in the moist environment of the mouth [93]. Tooth loss is a common dental problem among paediatric and adult age group, which leads to masticatory problems, pain and aesthetic embarrassment [94, 95]. Untreated carious lesions may lead to pulpal inflammation and tooth tissue damage; while periodontal diseases may lead to tooth loss [93]. This presents a challenge that requires a "close-to-normal" replacement of dental tissues or teeth. Although the hierarchical and mechanical structure of the enamel (which has a very high inorganic content) has not been biologically regenerated, this tissue can be remineralized and fortified with ions. Research efforts have been made previously to regenerate the completely functional tooth, albeit the generated features were not perfect to withstand the normal pH changes and masticatory function of the oral cavity [93, 96]. In the effort to improve restorative and regenerative materials in dentistry, nanobiomaterials have been incorporated into restorative materials to improve durability, aesthetics and function.

2.4.1 Nanomaterials and Glass Ionomer Cements (GIC) in Regenerative Dentistry

The main composition of glass ionomer cements (GICs) which has been widely used as liners, fissure sealant, luting agents and as an adhesive in clinical paediatric and restorative dentistry is calcium fluoroaluminosilicate glass powder with an aqueous homo-/copolymer of acrylic acid [97, 98]. With a closely similar coefficient of thermal expansion to the tooth, and other highly desirable physicochemical properties [98–101], GIC have played a key role in restorative dentistry for decades. However, some of the limitations to the use of GIC have emerged to be poor fracture toughness, delayed setting time, poor mechanical properties and high moisture sensitivity [99, 102]. Efforts aimed and improving the mechanical and physical characteristics of GIC without compromising its biochemical properties include the incorporation of nanomaterials. For example, titanium oxide (TiO_2) nanotubes have been used to improve the performance of GIC in a study [98]. This study concluded that incorporation of TiO_2 nanobiomaterials into GIC resulted in positive influence on extracellular matrix (ECM) and cell morphology, superior microhardness as well as fluoride release. Sliver nanoparticles have been used by Magalhaeces et al. (2012), to improve the antimicrobial properties of GICs against *Streptococcus mutans* [103], thus improving the management and restoration of primary and secondary enamel surface caries [104]. Using an in-vitro approach, Siqueira et al. (2015) demonstrated

the safety of silver nanoparticles (NAg)-containing GIC [105]. A recent review covering the years "2000–2018" showed that modification of GICs with nanofillers significantly improves physicochemical properties such as surface energy, particle distribution and surface area [104]. Research is ongoing on various nanotechnological approaches that have been used to improve the application of GIC in regenerative dentistry [106, 107].

2.4.2 Nanocomposites in Dental Regeneration

Biologically active nanocomposites have also been used for tissue regeneration and repair. Due to multiple nanoparticulate biological interfaces, this material is highly sought after for regenerative biomedical applications [108]. Nanocomposites can be a hybrid of inorganic and/or organic biocompounds that are metallic-, polymeric- or ceramic-based [108]. This confers a wide range of properties on nanocomposites for regeneration of bone and dental tissues. Biocompatible, wear-resistant and easy-to-clean nanocomposite coatings have been successfully used to manage formation of biofilm on tooth [85, 109]. Graphene/zinc oxide nanocomposite (GZNC) has been shown to possess therapeutic potential against the development of *Streptococcus mutans* biofilms [110]. Bonding agents for orthodontic brackets have also been developed using nanocomposites [111]. Also, biodegradable multifunctional nanocomposites have been developed for the application of drugs and bioactive compound for promotion of dental tissue regeneration [112]. Nanocomposites have also been used in the emerging field of Hydrogel-based regenerative endodontic procedures. Bekhouche et al. (2020) developed a nanocomposite that incorporates fibrin hydrogel with clindamycin-loaded Poly (D,L) Lactic Acid, for effective control of infection during root canal therapy and dental pulp regeneration [113]. Long junctional epithelium, which is a common problem during periodontal therapy, can be corrected using regenerative therapies like guided tissue regeneration (GTR) [114, 115]. Electrospun nanofibre scaffolds made of the nanocomposite are now used to aid GTR due to their ability to enhance attachment, cell survival and reorganization of periodontal cells, in an ECM-like manner [116]. Not least, polymeric and nanohydroxyapatite nanocomposites have been well used for regeneration and reengineering of bone and other hard tissues [117, 118].

2.5 Nanobiotechnology and Stem Cells in Regeneration of Dental Tissue

Nanobiomaterials have been employed in the reconstructions and regeneration of both dental hard and soft tissues. Engineering of bone tissue involves the use of biomaterials that have functional properties for bone regeneration [119, 120]. Modified nanocrystalline materials with nanopores have been modified for protein

adsorption and improved regenerative properties in replacement of bone and bony defects [121–125]. A comprehensive discussion of novel applications of nanotechnology to dental hard tissues regeneration is covered elsewhere [120]. As the field of nanobiotechnology expands, the use of nanobiomaterials has widely increased and it is intricately applied for both hard and soft tissue engineering. These nanomaterials have constituted both soft and rigid scaffold matrices, and their use has been integrated with biological molecules such as ECM signalling molecules, growth factors, as well as stem cells biomodulation [82]. Although the combinatorial use of nanobiomaterials with stem cells for dental tissues regeneration holds ample promise in the future, there are currently several bottlenecks to this approach [93, 119]. It is conceivable that future management of dental soft and hard tissue loss might involve the use of autologous stem cells with nanotechnology for tissue regeneration and restoration.

3 Potential Obstacles, Complications and Ethical Issues in the Use of Nanobiotechnology for Dental Regeneration

Despite the invaluable promise of regenerative nanodentistry, ethicolegal, regulatory, privacy, metaphysical, equity, safety, social security and public acceptance issues, viz-*a*-viz its use still persists [49, 126, 127]. A host of controversial issues have been raised regarding all these aspects of concern, and some experimental evidences have been provided in defence of the use of nanobiomaterials. For instance, Libonati et al. (2011) have shown that components that leak from nanocomposites were responsible for *in-vitro* mouse blastocyst embryotoxicity, but this toxicity was reversed when implanted subcutaneously, in vivo [128]. Further, toxicity was observed to be minimized for adhesive orthodontic nanocomposites of titanium dioxide (TiO_2) (at ca. 1% TiO_2 *w/w*) as compared to TiO_2-free nanomaterials [129, 130]. On a general note, dentists practicing regenerative nanodentistry need to possess adequate technical knowledge to provide nano-based care using the right approach [131]. This would minimize health risk to the dental patients and promote the practice of evidence-based dentistry. Similar, but bigger ethical issues arise from the field of nanoethics (as compared to bioethics), because of the exponential gain of physicobiochemical property at the nanoscale [131]. Furthermore, allogenic and autologous regeneration of tooth buds via cell homing (which is the least complicated and applicable approach) cannot be ethically developed using embryonic stem cells, and using adult stem cells obtained from extracted teeth (especially 3rd molars) is procedurally laborious (and expensive) for routine commercial utility, and there is a risk of incorporation of microbial contaminants [132, 133]. Also, large surface area-to-volume ratio improves body fluid transport and absorption probability into non-target body organs [27]. When this happens, toxic bioaccumulation may ensue leading to undesired adverse side effects [126, 134]. Although the benefits of regenerative nanodentistry potentially outweighs its harms, its use must be applied in the light of best

available evidence. Important tasks include the improvement of biological properties of nanobiomaterials via green synthesis, and other emerging biocompatible and environmentally friendly approaches [35, 135, 136].

4 Conclusions and Future Perspective

Regenerative nanodentistry is presently a poorly explored field as there is inadequate interdisciplinary integration between nanobioscientists, stem cell (and regenerative medicine) experts, and the practicing dentists. The use of nanobiomaterials in dental medicine potentially provides dentists with an alternate mean of combating challenging oral health problems, with high degree of specificity, precision, therapeutic efficacy, and negligible complications. Not least, it can potentially reduce dental operation/consultation time, thereby making dental treatments appealing and cost-effective. This chapter discusses some of the current and potential application of nanobiomaterials in the field of dental and maxillofacial medicine. It is by no means and exhaustive discussion of the field but presents a compendium of resources that can be explored for building knowledge in this emerging field. Importantly, risk assessments and ethicolegal issues (nanoethics) in regenerative nanodentistry should be considered. Although the field of regenerative nanomedicine has gained significant popularity, nanodentistry and its manifold applications in the era of precision medicine still needs to be promoted, to bring much desired advancement to the field of individualized/precision oral health.

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Chapter 9
Nanotechnology in Dental Implantology

Biju Thomas and Amitha Ramesh

1 Introduction

Dental osseointegrated implants are becoming the treatment of choice for the replacement of lost natural tooth. Even in the cases of completely edentulous patients implant supported dentures are becoming increasingly preferred over conventional complete dentures. Since the introduction of the term "osseointegration" by Per-Ingvar Brånemark titanium and its alloys have become the favored materials in the fabrication of dental implants. The potential of the implant to sustain loads, achieved through primary stability soon after implantation, is critical for a successful clinical result. Long-term results, on the other hand, depend on the osseointegration of the implant with the host bone. The sustainability of the bone-implant system is largely determined by the biological and biomechanical features of the biomaterial that is used in the fabrication of the implant [1].

Failure of dental implants has been attributed largely to the absence of osseointegration or infection in the peri-implant area. Due to the Clinician's desire for shorter healing time after implantation as well as immediate loading, the majority of the advancements in the design and fabrication of the implants are aimed towards these objectives [2].

Implant surface chemistry and topography has been shown to directly influence osseointegration. Surface chemistries and patterns are typically regulated at the micron level while tissue responses to these features are predominantly driven on a nanoscale level. Understanding and directing these interfacial interactions is critical for constructing novel implant surfaces with increased adhesion and tissue integration and thereby can avoid rejection [3].

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2 Relevance of Nanotechnology in Dental Implantology

Surface energy, implant surface material composition, surface roughness and surface topography are the four material-related characteristics that might influence events at the bone-implant junction. There seems to be three tiers of surface topography namely macro, micro, and nano. Surface profiles in the nanoscale range play a significant role in protein adsorption and osteoblastic cells adhesion and thereby the rate, degree as well as quality of osseointegration [3].

It has been hypothesised that implant osseointegration would be improved if the implant surface resembled the surface topography of the extracellular matrix in natural tissue. Surface coatings like with hydroxyapatite, titanium oxide, silver and gold nanoparticles has been shown to improve osseointegration by strengthening the adhesion of fibrin clot and by serving as a bridge for osteogenic cells. The presence of mechanical features like nano-grooves and nano-pillars has also proven effective in enhancing osseointegration [3].

3 Materials Used in the Alteration of Topography of Implant Surface Using Nanoscale Interactions

3.1 Metal Based Nanostructured Implant Surfaces

Owing to their biomechanical qualities, nanostructured metals and metal alloys have been exploited in the fabrication of orthopedic as well as dental implants for many years. These are easier to process and finish, provide satisfactory strength and toughness, and can also be sterilised using standard sterilisation protocols. Novel dental implants have been developed incorporating nanostructured titanium and its alloys. Nanostructured Co-Cr, stainless steel, Ni-Cr and gold alloys, on the other hand, are the metals of choice for implant prosthetic components.

3.1.1 Titanium

Several nanotechnology approaches exist for altering titanium implant dental surfaces. It has been demonstrated that titanium surface coatings with nanopores of 30, 150, and 300 nm diameter aid in adhesion and osteogenic differentiation of human mesenchymal stem cells as well as in swift osseointegration [4]. Traditional titanium implants comprise of an alloy of various elements to ensure optimal strength and corrosion resistance. However, certain biomedical applications require nanostructured titanium implants with any alloying elements so as to prevent their toxic effects. Recent research has proven that commercially pure titanium (CpTi) can be nanostructured to produce superior mechanical qualities that can even outperform several titanium alloys.

Nanotechnology also has a lot of potential to functionalise the surface and offer them unique qualities like "self-cleaning". Bacterial adhesion to nano-modified titanium implants can be influenced by the topographical and chemical properties of titanium oxide nanotubes (TNTs). Titanium oxide coated structures can hinder bacterial adhesion and have direct antibacterial properties owing to their nanoscale surface roughness and elevated surface energy. TNT was thus introduced as a potential antibacterial coating over dental implants. TNTs have other qualities that are significant to dental implants, such as a highly established organisation, a large surface area, and roughness and ability to be laden with therapeutic chemicals. TNTs are appealing for improving osseointegration and bone regeneration owing to these abilities. TNTs enhance osteoprogenitor cell adhesion, proliferation, and differentiation, notably in nanotubes of lower diameters (30 nm) in comparison to larger diameter nanotubes (70 nm). This reaction is also influenced by the topography of the surface. One study reported that relative to smoother surfaces, bacteria cultivated on surfaces with TNTs (40–60 nm sizes) exhibited the highest drop in population. When compared to TNTs with lesser diameters, TNTs with a diameter of 60 nm have thin walls and stronger photocatalytic activities and at a diameter of 100 nm, antibacterial properties begin to fade. This could be the consequence of the bacteria's stress reaction to TNTs, which induces cellular membrane rupture. However, considering that the effect of inhibiting bacterial adhesion and growth on medication resistance in bacteria is not well known, more research is needed [5].

Conflicting findings exist on how well the hydrophilicity of a TNT-coated surface impacts microbes. Bacterial multiplication and adherence may be aided by a surface that is more hydrophilic. TNTs can be loaded with significant quantities of antibiotics, anti-inflammatory drugs, nanoparticles, and ions attributable to their design. Antibiotic preload could significantly improve the antibacterial activity. TNTs containing vancomycin and antimicrobial peptides were found to have improved antibacterial activity against Staphylococcus epidermidis and Methicillin-resistant Staphylococcus aureus, as well as reduced bacterial adhesion to implant surfaces. Using processes like as chemical reduction, spin-coating, sputtering, and drop-casting, TNTs can be laden with antibacterial nanoparticles and diverse ions like silver, gold, and copper [6].

The utilisation of ion/nanoparticle functionalisation of TNTs in dental implants with nanoscale characteristics has a lot of promise. Challenges with cytotoxicity caused by discharged ions or nanoparticles must be investigated further, as these must be assessed against their therapeutic efficacy.

3.1.2 Titanium Alloys

On exposure to air, a stable passive layer of titanium oxide forms on titanium surfaces, thereby inferring the metal better biocorrosion resistance when compared to other metals. However, Commercially pure titanium (CpTi) have too low static and fatigue strength to be used in load bearing situations. The surface composition of the alloy Ti–6Al–4V deviated from that of the Commercially pure titanium (CpTi). The surface

layers of this alloy comprise aluminum and vanadium, usually as metal oxides. In general, CpTi is marginally preferred, but in-vitro investigations have consistently found Ti–6Al–4V to be superior. In the establishment of osseointegration, the interfacial zone across the titanium alloy implant and organic bone is crucial. This thin (20–50 nm) area is where growth factors are released from bone cells, kicking off the stages that lead to bone formation. Proteins from the blood plasma are accumulated on the surface oxide layer at the very first step. The creation of a fibrin matrix framework serves as a scaffold for osteoblasts follows. This fibrin scaffold helps in the deposition of bone by osteoblasts leading to osseointegration. Although adding alloying elements boosts titanium's mechanical qualities significantly, the usual alloying metals like aluminum and vanadium are hazardous. Hence, tremendous effort is being put into developing vanadium and aluminum-free Ti alloys [7].

Whilst niobium has been explored in binary alloys with titanium, it is more commonly employed in ternary alloys like Ti–6Al–7Nb. Binary alloys with low quantities of niobium (less than 10% by mass) have been discovered to have excellent mechanical characteristics. They usually outperform CpTi in terms of tensile strength, yield strength, and hardness. There's also confirmation that their corrosion resistance has improved. There have been investigations on binary indium-titanium alloys, such as Ti–In–Nb–Ta, that have demonstrated to have good bioactivity. Tantalum and niobium sustain the β-phase of titanium, therefore their inclusion effectively substitutes vanadium in Ti–6Al–4V and improves the biological tolerance of the resulting alloy in the case of either metal. However, the majority of studies focus on CpTi, with a few mentioning Ti–6Al–4V and Ti–6Al–7Nb specifically [7, 8].

3.2 Ceramic Based Nanostructured Implant Surfaces

Titanium implants, while having the record of predictable clinical results and broadest commercial adoption, also has several disadvantages. Titanium is not totally bioinert and can cause allergic responses. It is able to diffuse not only in the peri-implant area, as evidenced by the higher quantities discovered in the peri-implant bone and regional lymph nodes, but also throughout the human body. Because of these reasons, for the past 4 decades ceramic materials are being studied and designed for oral applications [9].

3.2.1 Zirconia and Its Alloys

Zirconia based implants has been gaining favor in the dental implant industry due to their corrosion resistance and biocompatibility. Enhanced bioactivity of these implants allows for faster implant integration and improve long-term prognosis, especially in compromised patient conditions. The majority of zirconia used in dentistry is in the form of yittria-stabilised tetragonal zirconia polycrystals with a 3 mol%

yittria content (3Y-TZP). 3Y-TZP dental implants have been shown to be equivalent to titanium implants in terms of cell attachment, proliferation, and histopathological response in in-vitro and in-vivo experiments. The static fracture strength of the ye3Y-TZP implant ranges from 725 to 850 N, which is within clinically acceptable limits. However, in the oral environment, 3Y-TZP may succumb to low-temperature deterioration (LTD), resulting in the implant's catastrophic failure. Furthermore, the technique of preparation and cyclic loading may affect the fracture strength resistance of zirconia implants. Before zirconia dental implants may be produced as a clinically viable replacement for titanium implants, these flaws must be addressed. Nawa et al. developed a Ce-TZP-based nanostructured zirconia/alumina composite (NANOZR) in 1998. As a matrix, the composite is comprised of 10 mol% Cerium-dioxide (CeO_2) stabilised TZP and 30 vol% Al_2O_3 as a second phase. NANOZR outperforms 3Y-TZP in terms of flexural strength and fracture toughness, as well as being totally resistant to LTD. It has a cyclic fatigue strength that is more than double that of 3Y-TZP, indicating that it is appropriate for dental implants [10].

In a study conducted by Katunar et al., 2017, CpZr (Commercially pure Zirconia) cylinders were anodised at 30 and 60 V for 60 min followed by mechanical preparation and degreasing. Enhanced cell spreading, osteoblastic and osteoclastic morphology were seen in mouse macrophages, osteoblasts, and myoblasts cultured in vitro. Additionally, in vivo implantation of 60 V anodised Zr implants in a rat model revealed new bone growth surrounding the implants. In a comparable study, 60 V anodised Zr implants put in a rat model resulted in a considerably increased trabecular thickness and cancellous bone volume indicating that anodised implants initiate osseointegration sooner [11].

3.2.2 Hydroxyapatite (HA)

Hydroxyapatite can act as a source of calcium and phosphate and has generated a lot of interest as a dental material because of its ability to integrate osseous tissues to coated implant surfaces.

HA-coated implants appear to be more effective in areas that impact the quantity and quality of bone compared to pure or titanium alloy implants. Cellular adhesion to the implant surface is one of the most critical steps in tissue-implant interaction. These coatings have been demonstrated to dissolve and subsequently interact with biologic fluid when exposed to physiologic conditions. Interestingly, the release of calcium and phosphate ions from HA coatings upon degradation and dissolution might speed up the bony adaption of these surfaces. The degree of HA crystallinity, which plays a crucial role in the first cellular interface with the implant surfaces, appears to be the fundamental determinant in determining the rate of breakdown. The rate of dissolution of HA is inversely proportional to its crystallinity [12].

Biocompatibility, osteo-conductivity, direct bonding onto bone, bone formation and regeneration characteristics are all excellent with HA. It has a wider surface area and a high reactivity, allowing it to bond to enamel and dentine apatite, resulting in a biomimetic implant coverage. It also behaves as a calcium and phosphate source and

thus may improve in the remineralisation of the outer enamel lesion. In micro and nano forms, it promotes the density of osteoblast cells on the implant. Despite the reported positive findings, the HA/bone interface's long-term stability has been questioned. From a microbiological standpoint, HA-coated implants may pose patients at risk for bacterial plaque accumulation [12].

3.2.3 Beta Tricalcium Phosphate (β-TCP)

The chemical composition of calcium phosphate layer has attracted a lot of attention as it replicates the structure and chemical constitution of the surrounding bone. The rod-shaped crystal structure of calcium phosphate has been reported to boost the osteogenic activity of the area surrounding it. According to Ono et al., this rod-shaped structure improved the bone metabolism cycle with elevated osteoclast activity and progressive bone formation. Increased TRAP positive multinucleated cells were seen on the surface of the calcium phosphate blocks and the process was accompanied by active osteoblasts in the newly formed bone and high alkaline phosphatase (ALP) activity. Calcium phosphate coatings also improve the surface bioactivity by increasing calcium deposition and protein adherence on zirconia implants [13].

It is worth noting that calcium phosphate coatings has a poor binding capacity with Zr/ZrO_2 alloys, especially when fabricated through physical deposition. As a result, several methods to increase its adhesive strength have been attempted, including co-coating with HA before calcium phosphate and hydrothermal sintering after calcium phosphate.

Bao et al., 2013 prepared a titanium dioxide and Si-doped octacalcium phosphate (OCP) composite coating on zirconia substrate. The authors initially prepared a titanium dioxide coating on zirconia substrate. Following this, Si-doped coatings were synthesised on the titanium dioxide layer and TiO_2/Si-OCP composite coating was obtained. The evaluation of properties of the material was then performed which indicated that the dense TiO_2 layer prevented low temperature degradation (LTD) of zirconia substitutes and that the coating had a lamellar-like, uniform, continuous structure with no cracks [14].

Stefanic et al., 2012 also studied OCP coatings on zirconia implants and presented a two-step biomimetic procedure to prepare such coatings. The authors obtained a thick octacalcium phosphate layer by initially immersing the zirconia discs in a solution of pH 7.4 and in the second stage immersing the substrate in a solution of pH 7.0. The analysis of the material properties yielded good tensile adhesion strength [15].

3.2.4 Bioactive Glass

The thermal expansion coefficients of Si–Ca–Mg–Na–K–P–O system-based bioactive glass is similar to that of metallic alloys usually employed in the fabrication of implants. Bioactive glasses offer better biocompatibility and bioactivity. Bioactive

glass coatings can enhance osseointegration by facilitating formation of apatite at the implant-bone interface. They have also been found to indirectly alter osteoblast gene expression. Bioactive glass coatings can be obtained through techniques like laser cladding, electrophoretic deposition, enameling, sol-gel method, and thermal spraying [16].

3.3 Polymer Based Nanostructured Implant Surfaces

Polymers have emerged as health-care materials over time as a result of their ability to be adjustable in a wide variety of physical, chemical, and biological characteristics. Polymeric materials are being used in the fabrication of blood storage containers, parts of joint prosthesis, syringes, and catheters to name a few. Dental implants are also being developed with polymeric materials like polyurethane (PU), Polytetrafluoroethylene (PTFE), polyether-ether-ketone (PEEK), polymethylmethacrylate (PMMA), ultra-high molecular weight polyethylene (UHMW-PE), polysulfone (PSF), polymethylsiloxane (PDS), and polypropylene (PP) [17].

Polymeric materials have lower elastic moduli, are thermally and electrically passive, have higher elongation to fracture, lower cost of fabrication as well as better biocompatibility when compared to other categories of biomaterials. On the other hand, it is more challenging to sterilise polymer-based implants especially through ethylene oxide or autoclaving. Also, porous polymers can undergo elastic deformation to close the porosities that are intended for tissue ingrowth [17].

Nano-topographies of differing heights and depths and in the shape of pits, ribbons or islands can be constructed in a variety of ways, electron beam lithography (EBL) or photolithography being two recognised methods. They are, however, expensive and time-consuming, especially when considering large surfaces. Therefore, new technologies like polymer de-mixing are being scrutinised. During polymer de-mixing, a combination of polymers like polystyrene (PS) and poly(4-bromostyrene) (PBrS) undergo phase separation spontaneously during spin casting onto silicon wafers. Affrossman et al., in 1996 reported that the surface structure obtained on spin-coating blends of poly(p-bromostyrene) with poly(deuteriostyrene) varied based on the polymer composition and concentration as well as the speeds used in spin-coating process [17, 18].

3.4 Hybrid Implant Surfaces

Hybrid dental implants are fabricated from a combination of two or more materials, generally polymers, metals or ceramics modified by surface coatings or patterning. The surface modifications improve bone binding as well as provide occlusive surfaces with greater toughness. Additionally, antimicrobial and osteogenic effects are also provided by hybrid dental implants with a nanostructured surface. Study by Zheng

et al. using silver-implanted titanium with a nanostructured surface demonstrated good antimicrobial activity against porphyromonas gingivalis, Streptococcus mutans, and Candida albicans as well as an increase in osteoblast phenotype gene expression.

Nanostructured, light-stable, antibacterial coatings for dental implants and restorative materials may be created by depositing a silver co-ordination polymer compound onto implant titanium. Zinc (Zn) integrated into titanium oxide (TiO_2) coating improves antibacterial activity and bone marrow stem cell activities. To enable rapid osseointegration, HA/collagen nanocomposites coated on titanium rods were employed. The biocompatibility of dental implants is improved by a gelatin-gold nanocomposite coating on titanium. Antibacterial activity is achieved by coating titanium with ZrO_2-Silver (Ag) and ZrO_2-Copper (Cu) [17, 19].

4 Alteration of the Topography of Implant Surface Using Nanoscale Interactions

The capabilities of osteogenic cells and the degree of peri-implant osteogenesis are significantly affected by surface architecture. The surface architecture and chemistry of dental implants can in turn be modified through various physical and chemical methods. The prime objective of these approaches is to enhance some of the attributes of the implant surface, such as improving peri-implant osteogenesis by promoting bone formation for better wear and corrosion resistance, and removing the contaminants [17, 20].

4.1 Physical Methods

4.1.1 Plasma Spraying

Plasma spraying is one of the first technique employed to create a rough surface on titanium and involves the projection of precursor materials into the hot plasma jet generated by a plasma torch under vacuum or reduced atmospheric pressure. It can produce nanoengineered interfaces with dimensions lesser than a hundred nanometers. This procedure involves vacuum-assisted clearance of surface impurities, accompanied by kinetic energy mediated condensation of charged plasma or metal ions upon dental implant surface. This strategy permits the coating of different materials such as silver, gold, and titanium on a variety of substrates such as ceramics, polymers, and metals. This methodology is commonly used to encase dental implants with calcium phosphate coatings to alter their bioactivity. Despite the clinical benefit, plasma spraying method has several disadvantages, including variations in composition of the coating material and non-uniform thickness of coated layer, some of

which can pose health risks and jeopardise the long term stability of dental implant [17, 20].

4.1.2 Sputtering

It is a type of Physical Vapour Deposition (PVD) technology and involves the ejection of atoms or molecules into a vacuum chamber, which when bombarded with high energy ions become precursors for coating. This results in the development of thin bio-ceramic films. This approach enables better adherence between the coating and the substrate, as well as more control over coating qualities. It also increases biological activity and biocompatibility, as well as mechanical qualities including wear and corrosion resistance. The primary disadvantage of this approach is that it is extremely sluggish and has a poor deposition rate [17, 20].

1. Magnetron sputtering

A viable thin coating is deposited onto the substrate in this method. This method preserves the biological activity of hydroxyapatite layer at the same time preserving the mechanical traits of the metal. Coatings are deposited at room temperature in a custom designed sputter deposition unit [17, 20].

2. Radiofrequency sputtering

This method involves the deposition of thin layers of calcium phosphate onto the substrate metal surface. The chief advantages of this approach are the coating's high adherence to the metal surface, its crystalline structure, and the ease with which the calcium phosphate ratio may be modified [17, 20].

4.1.3 Ion Implantation

This approach involves projecting a beam of high-energy (10 Kev) ions onto a metal surface while within a vacuum chamber and allows any component to be injected near the exterior of any material. Atomic rearrangements are a part of this approach. The collision between incident ions and substrate ions enables incident ions to release electrons on the metal's approaching area. Because it is an ultra-clean method, it is feasible to synthesise layers with high purity, as well as regulate and identify the depth and concentration of the contaminants allowing for notable adhesion. Since this operation is carried out at low substrate levels, it has little impact on the substrate's bulk characteristics. This method allows for the incorporation of physiologically active sodium, calcium and fluoride ions. It is also readily repeatable and regulated. Modification of pre-existing nanomeric characteristics and development of superficial stresses must be carefully evaluated when utilising the ion implantation method [17, 20].

4.1.4 Laser Treatment

A laser can be utilised as a micromachining device to fabricate 3-dimensional structures at micro and nanometer scales. In this method brief pulses of a single wavelength are produced that allows the energy to focused on a single point on the substrate. It enables the creation of complex features with high resolution while still being extremely clean and fast. It is ideal for making selective alterations to implant surfaces, as well as for targeted, precise and guided surface roughening [17, 20].

4.1.5 Picometer to Nanometer Thin Titanium Dioxide Coatings

A layer of titanium dioxide approximately 300 pm to 6.3 nm in thickness can be deposited onto the implant surface using a slow rate sputter deposition. While this technique does not alter the surface topography, it improved the surface oxygen saturation. This can, in turn, increase the bioactivity of the implant surface [17, 20].

4.1.6 Ultaviolet (UV) Photofunctionalisation

This method utilises titanium oxide mediated photocatalysis and decomposition by UV light to remove hydrocarbon contaminants on the substrate surface. Like the previous methods, this technique also increases the biological activity of the implant surface [17, 20].

4.2 Chemical Methods

4.2.1 Anodic Oxidation or Anodisation

Anodisation can effectively transform smooth titanium surfaces into micro tubular structures with a diameter of less than 100 nm. Controlling the physicochemical qualities of surfaces, spacing, and nanotube diameter may be achieved by adjusting factors such as current density, voltage, and electrolyte chemistry. Anodisation generates pillar-like nanostructures with configurable diameters on titanium surfaces, as well as longer nanotube arrays. On titanium, for example, multi-walled nanotubes and nano-hydroxyapatite coatings (15–25 nm) have been deposited resulting in enhanced bioactivity [17, 20].

4.2.2 Acid Treatment

Acid treatment eliminates contaminants as well as the oxide layer, resulting in a more hygienic and uniform implant surface. Sulphuric acid, nitric acid, hydrochloric

acid and hydrofluoric acid are the most often utilised chemicals in this method. Acid treatment increases roughness thereby increasing the surface area and improving the contact between the bone and the implant [17, 20].

Composition of the etching solution, temperature and exposure time can all be adjusted to regulate various characteristics like wettability, surface topography, thickness of the protective oxide layer and most importantly nano-roughness.

Furthermore, by varying the composition of the etching solution desirable components like fluorine, which has antibacterial activity and can also lead to bone formation, can be added onto titanium nano-surface [17, 20].

4.2.3 Alkali Treatment

Alkali treatment involves immersing a titanium implant in sodium or potassium hydroxide, then heating it to 800 °C for 20 mins before washing it in distilled water. When sodium hydroxide is used, a nanostructured, bioactive sodium titanate layer is formed on the surface of the immersed implant in this method. This bioactive surface works as a substrate for calcium phosphate nucleation when saturated in Simulated Body Fluid (SBF), producing Ti–OH with the release of sodium ions from sodium titanate via ion exchange. Further, calcium titanate is developed when positively charged calcium ions from SBF react with negatively charged Ti–OH. Calcium and phosphate ions in calcium titanate can combine to form apatite crystals which can promote cell differentiation in the bone marrow [17, 20].

4.2.4 Combination of Anodisation and Chemical Etching

Polymer as well as metal-based composites with superior biological characteristics can be manufactured by coupling anodisation and chemical etching. Anodised nanotubular titanium with sodium hydroxide treated nanoporous poly(lactic co-glycolic acid) (PLGA) demonstrated increased cell activity. But no significant change was seen when this composite was compared to anodised titanium. Titanium surface can be altered with a variety of hydrothermal treatments and sodium hydroxide to produce a wide array unique nanostructures including nanorods, nanoflowers, and mesoporous scaffolds [17, 20].

4.2.5 Hydrogen Peroxide Treatment

The titanium dental implant surface undergoes oxidation and chemical dissolution and a titanium-peroxy gel layer is formed as a result of the reaction between titanium and hydrogen peroxide. The thickness of the titanium layer can be controlled by alteration of the treatment time [17, 20].

4.2.6 Sol-Gel Method

This method is a type of wet chemical deposition and is among one of the most extensively used procedure for coating implant surfaces with calcium phosphate, titanium dioxide, TiO_2-CaP composite and silica-based materials. Herein, a sol, which is a colloidal suspension of solid particles in a liquid solution, is deposited onto the implant surface through techniques like spraying, spin-coating or dip-coating.

The extent of adhesion between the substrate and the TiO_2 sol-gel coating is determined by factors like chemical pre-treatment, sintering temperature and surface roughness. The high electron density at the atomic level accounts for the high bond strength between the nanoscale coating and the implant surface [17, 20].

4.2.7 Chemical Vapour Deposition

In this method, unlike that of physical vapour deposition, a chemical interaction occurs between the substrate surface and the elements in the gas phase. This results in the deposition of a non-volatile compound on the implant surface [17, 20].

4.2.8 Combination of Chemical Vapour Deposition and Sol-Gel Method

Bioactivity of metallic surfaces, like that of titanium, can be improved by using a combination of techniques like chemical vapour deposition and sol-gel method and a combination of materials like diamond-like carbon and niobium oxide [17, 20].

5 Evaluation of Quality of Oseointegration in Nanomodified Implants

Osseointegration is an integral part for the success of any implant. Properties like biocompatibility, bioactivity and cytotoxicity can be assessed in vitro using simulated body fluid (SBF) and cell cultures. However, evaluation of osseointegration requires in vivo models. Two in vivo models commonly employed are the cranial bone onlay model and embedding the implant directly into the bone [21].

The cranial bone onlay model involves exposing the skull and elevating the periosteum. A dental bur is then used to make multiple perforations around the implantation site to access marrow space. The implant is then directly laid onto the cranial surface and the skin along with the periosteum is sutured closed. After a suitable post-surgical period, the animals can be euthanised and the implant-bone interface analysed on this method is fairly easy to perform and causes minimal trauma to the animals. However, the main disadvantage is that the number of implants that can be simultaneously tested is limited by the size of the cranium [21].

The second model requires implants to be surgically embedded into the bone and is much more invasive than the aforementioned technique. Typically used implantation sites include mandible, long bones like tibia, and pelvis. Like the previous method the animals are euthanised after a suitable post-operative period and the implant-bone interface is analysed. This technique is more advantageous than the cranial bone onlay model as it more closely resembles the typical clinical scenario and multiple implants can be tested. However, this technique requires more clinical expertise and is more invasive [21].

The harvested implant sites can be assessed through methods like histological analysis, microCT, and molecular analyses. Mechanical testing of the implant-bone interface can also be performed to assess the physical characteristics of the interface. The mechanical testing of the harvested calvaria in the cranial bone onlay model is a bit more challenging as compared to assessing the implant directly embedded into bone because of the shape of the harvested structure [21].

It is important to remember that even with these strategies, the implant-bone interface, amount of the new bone formed and osseointegration can be influenced by various other factors. Implant geometry, density of the surrounding bone (trabecular, spongy, cortical), choice of the model adopted (dog, sheep. mice), time of implantation, implant surface configuration, and load on the implant can affect the degree of osseointegration [22].

6 Mechanisms of Nanomodifeid Implant Surface Interaction with Haematopoietic Cells

Biomedical devices fabricated for sites contacting blood should not modify cellular elements like platelets or cause activation of intrinsic coagulation cascades. In such cases, the concept of biocompatibility should include leukocyte activation, complement activation, changes in plasma proteins, and thrombogenicity [23].

Multiple studies have demonstrated that topographical changes in a nanoscale level can direct alignment, orientation, and adhesion of cells, cytoskeletal orientation, activation of stem cells and differentiated cells like macrophages, epithelial cells and fibroblasts. Curtis et al., hypothesised these changes to be due to a variety of factors, including imbalanced or asymmetric distribution of interfacial forces across the nanostructure, patterns of surface chemistry possibly displaying binding sites for specific molecules, strains within the cells induced by their attempt to conform to non-planar surface, and stress relief in the substratum surface itself induced partly by random thermal events and partly by the cells themselves.

The first event following the implant placement is the blood–protein adsorption at the implant–blood interface. Depending on the exposure time, the composition of the adsorbed layer can vary and proteins with stronger adsorption will be favored. The proteins also undergo various conformational changes leading to different biological interactions which govern the interaction of platelets, their adhesion or activation,

leukocyte recruitment as well as activation of intrinsic coagulation and complement cascades. At the implant and platelet adhesion surface, activation of coagulation factors can lead to the formation of thrombin which converts fibrinogen to insoluble fibrin from which a fibrin network may be produced. The platelets may also trigger an inflammatory immune response, leading to either thrombosis and/ or fibrous encapsulation of the implant [23, 24].

7 Sequence of Biological Events in Relation to Mesenchymal Stem Cells on the Surface of the Implant

Adult MSCs are multipotent and can give rise to cells of different lineages including adipogenic (fat), osteoblastic (bone), and fibroblastic (connective tissue) lineages. The generation of osteoblasts around an implant will assist in the osseointegration and therefore increase the long-term prognosis.

Cells in their natural environment are present in the extracellular matrix which are of nanoscale dimensions. Stem cells cultured on substrates with nano-scale features can take on different shapes due to their effect on cytoskeletal mechanics [23, 24].

Dalby et al. demonstrated that on nanostructured surface MSCs can differentiate better without stimulation by osteogenic factors. The chemotactic factors produced after the blood-protein layer formation on the implant will help attract circulating MSCs. Focal adhesion formation as well as organisation of cytoskeleton is influenced by the interaction between human mesenchymal cells and the substrate and can be altered by material hardness and chemical patterning. Bone marrow cells exposed to nano-meter scale islands have been demonstrated to exhibit long filamentous processes called filipodia. They are considered to be points of interaction between the cells and nano-structured surface, and are hypothesised to be one of the cells' main method of gathering information about their surroundings [25].

The cytoskeletal changes in the stem cells causes changes in Rho A (Small G protein involved in signalling and cytoskeletal organisation) through indirect mechanotransductive pathways and thereby impose morphological changes on the cell [25].

Studies demonstrate an increase in the absorption of Bone Morphogenetic Proteins (BMP), fibronectin and osteopontin as well as an increase in the production of alkaline phosphatase, Transforming Growth Factor-beta (TGF-β), and prostaglandin E_2 (PGE_2) following placement of nano-modified implants [3].

MSc differentiation into osteoblasts involves a variety signalling pathways and extracellular matrix factors like fibronectin (FN) and vitronectrin. MSCs respond through Wnt pathways, signalling through Wnt5a and calcium as well as through integrin signalling to surface roughness and triggers changes in cytoskeleton. This results in a change of cell polarity, downstream activation of gene transcription, and osteoblast differentiation and maturation. Spatial localisation of fibronectin on implant surfaces can cause elongation of MSCs and stress the cytoskeleton further

inducing the expression of osteoblast genes. Work by Anselme et al., corroborated that while cells interact with surface topography on micrometer and nanometer scales, surface chemistry influences the attachment of cells on various substrates [3, 26].

8 Future Directions

Much research and product development is going on in dental nanomaterial technology. However, these materials need to be put tested in actual clinical situations. Hybrid dental implants with nanotopographical structure are also under research. However, it is difficult and costly to be manufactured on a large scale. 3-dimensional printing technology may answer this problem and provide customised dental implants that match the patient needs. 3-D printed implants can also enable rapid bone growth as the shape and porosity can be controlled during fabrication [27].

Dental implants with antibacterial coatings or drug release properties are also under study and may provide better prognosis with reduced incidence of implant-associated infections [27, 28].

9 Conclusion

The field of dental nanomaterials has grown exponentially since its introduction a few decades back. Dental nanotechnology can give us a new generation of materials with better biocompatibility, higher efficiency, better mechanical properties, and low cost. Nanomodified implants with antibacterial and osteogenic properties are also being developed to provide a better long-term prognosis. A combination of nanotechnology with stem cell and tissue engineering technology also provides multiple exciting avenues of research. However, more research is required to understand cell interaction on nano-topographied implant surfaces and the long-term effect of such an implant in the body.

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Chapter 10
Applications of Nanoscience and Nanotechnology in Oral Cancer: A Review

Mohammad Reza Kasaai

1 Introduction

Nano-science and nanotechnology are growing in a variety of areas of science, technology, and engineering, leading to important changes in the world in the near future. Different research topics including preparations, properties, characterizations, and applications of nano-materials (NMs) in vitro and in vivo are under study since 2000 [58–60, 62, 86, 113]. Nanomedicine is the applications of nano-science and nanotechnology in biology and medicine. It is a new field of research as well as a new branch of medicine that deals with application of nanomaterials (NMs), nanoparticles (NPs), or nano devices in medicine [44, 49]. Nanotechnology has gained recognition in its application in the diagnostics of oral cancer (OC). It can be also employed in several combinations in the therapeutics purposes of OC. The changing trends of diseases and improvements in science and technology have led the world to look toward different procedures including nanotechnology (diagnosis and treatment of oral malignancies) [143]. Abbreviations appearing in this chapter are presented in Table 1.

Today, cancer is a critical health issue and the second most common cause of death worldwide (just after cardiovascular), its incidence increases and its treatment remains a significant challenge as the number of cases continues to rise [19, 48, 111].

Oral cancer is a common type of cancer. It occurs through genetic modification and environmental influences like the smoking of tobacco, smoke-free tobacco (snuff or chew-able tobacco), drinking alcohol, consumption of areca nuts, viral infection [human papilloma virus (HPV) infection], and excessive sunlight exposure [29–31]. About 90% of all oral malignant tumours are squamous cell carcinomas (SCCs). Oral squamous cell carcinoma (OSCC)/oral cavity cancer is one of the most common

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Table 1 Abbreviations appeared in this chapter

Abbreviation	Expression, term	Abbreviation	Expression, term
CE	Conventional emulsion	OCs	Oral cancers
CEs	Conventional emulsions	ODD	Oral drug delivery
Cisplatin-CDDP	Cis-diamine-dichloro-platinum	OSCC	Oral squamous cell carcinomas
D	Diameter (size) of nano-particle	PDT	Photodynamic therapy
DNA	Deoxyribonucleic acid	PEG	Poly(ethylene) glycol
DLS	Dynamic light scattering	PTT	Photo-thermal therapy
EGF	Epidermal growth factor	PIT	Phase inversion temperature
GIT	Gastrointestinal tract	PIC	Phase inversion composition
LNPs	Lipid nanoparticles	PLGA	Poly(lactic-co-glycolic acid)
NE	Nano-emulsion	PS	Photosensitizer
Nes	Nano-emulsions	PSs	Photosensitizers
NIR	Near infrared	ROS	Reactive oxygen species
NP	Nanoparticle	SANPs	Self-assembled nanoparticles
NPs	Nanoparticles	SCC	Squamous cell carcinoma
NRS	Numerical rating scale	SEM	Scanning electron microscopy
OC	Oral cancer	TEM	Transmission electron microscopy

cancers that has a high mortality rate [13, 26, 29, 90, 97, 112, 132, 143–145]. OSCC is a common malignant tumour of the head and neck area. It has a comparatively poor survival rate, and in some regions, the incidence of the disease is rising [3]. Statistically, 354,864 cases, and 177,384 deaths in 2018, i.e., approximately 2% of cancer deaths occurred from OC. The incidence and death rates in men were greater than that of women [17]. No significant changes in the tumour stage of OSCC were reported for young and old patients [25]. The comparison was performed between younger than 40 and equal to or older than 40. They concluded that: (i) SCCs of the OC in young patients were not at an advanced level, and (ii) Young patients have a possibility of survival.

SCC, which results from the oral mucosal epithelium, is a fatal and debilitating disease, due to tumour infiltration, orofacial destruction, cervical lymph node metastases, and final blood spread [127]. These tumours can invade the mucous membranes of the tongue, the oral cavity, the floor of the mouth, the alveoli, and the hard palate. The tongue has been reported to be the most common subsite of poor prognosis [88, 111]. OC that often generated from long-term exposure to different potential

risk factors can lead to the accumulation of multiple gene mutations [125]. Over 95% of people with OSCC, smoke tobacco, drink alcohol, or both [10, 126]. OC was significantly reduced by smoking cessation and drinking [12]. OC is the sixth most common cancer and has an overall 5-year survival rate of around 50% (all sites and stages combined) [20]. OC is an aggressive cancer, primarily affects epithelial cells, and its development results in metastases and is ultimately fatal [125]. Major risk factors associated with OC include smoking, drinking, human papillomavirus infection and habitual use of areca nut. Among them, smoking is the main cause of death [7, 56, 131].

NMs/NPs are of interest for research groups in medicine, and pharmaceutical fields, this is because, the properties of NMs are superior in comparison with the conventional ones [11, 78]. The nano-sized materials/NPs possess a high efficiency, which can be used for the protection and delivery of micronutrients and drug components [39].

This chapter is mainly focused on the detection, diagnosis, and treatment of OCs using nano-science and nanotechnology. In this chapter, an effort has been made to provide a review of several research groups on: (i) applications of diagnosis, detection, devices, drugs, and treatments by employing nano-science and nanotechnology; and; (ii) clinical applications of nanotechnology in diagnosis, care and treatment for OCs.

2 General Aspects of Nanomaterials/Nanoparticles

2.1 Characteristics and Properties of Nanomaterials/Nanoparticles

NMs (with the size 1–00 nm; larger surface area/volume) generate unusual properties and phenomena different from bulk materials with the same ingredients [60, 86, 113]. The most characteristics of NMs are their sizes and shapes. There is a relationship between the structure of a NM and its properties. Materials with nano sized and high surface areas possess superior chemical, physical, and mechanical properties in comparison with bulk counterparts and create significant interactions and effects [49, 55, 61, 95, 110, 136, 137]. Reduction in particle size yields in an increase in surface interactions, and leads to an increase in solubility. Nano-sized carriers are more efficient than of large-sized ones. The NPs remained in the human body for a longer period than that of larger particles for achieving sustained therapeutic effects [77].

Solubility of drugs in water is an important property for their medicinal applications. Their low solubility can be an issue. For instance, the use of a greater amount of a drug could result in side effects [120].

2.2 Applications of Nanomaterials/Nanoparticles in Medicine

Nanoscience and nanotechnology have been used in health care practices including prevention, identification, relief, healing, treatments, and therapies. The drug delivery involves their use of NMs, image contrast agents, targeting molecules, and therapeutic drugs [28, 84, 98]. NPs are efficient bioactive components for cancer chemoprevention, due to their enhanced permeability and retention (EPR) effect, explaining for the properties of certain molecules that preferentially localize to tumour tissue over normal ones, and yield in tumour cells to grow quickly [130]. The applications of nanotechnology in medicine mainly alter pharmacokinetics. By employing nanotechnology, the percentage of injected dose to reach the tumour increases accomplishes target-specific delivery and uptake, which in turn decreases dose requirement [2]. NPs can pass through (skin, lungs, gastrointestinal tract, GIT) of the human body easier and faster than that of larger particles. Lungs and GIT are more permeable to the foreign particles than that of skin. NMs are able to cross tissue and cell membranes and enter into cellular level and have the ability to cause adverse biological effects at the cellular, sub-cellular, and molecular levels, and result in cellular injury [21, 69, 89].

These potentially harmful effects could be enhanced by the ability of NPs to be taken up and travel through the body, deposit in target organs, penetrate cell membranes, lodge in mitochondria, and trigger injurious responses [21, 69, 89]. The detrimental effects can be enhanced by NPs, and this is due to their ability to travel faster and easier than larger sized particles through the body, deposit in target organs, penetrate cell membranes and finally yield in cell injury. Drugs and bioactive compounds encapsulated in NPs resulted in an increase in their bio-availabilities and bioactivities [99].

Different techniques associated with NE formulations were employed in drug delivery systems to treat different cancers. Oral delivery is useful, due to the direct delivery of a particular medication to a desirable organ, and targeted cells [1, 40, 116, 128, 129].

NEs are employed in a diverse range of biomedical sectors (development of pharmaceutical formulations for topical, ocular, intravenous, drug delivery, health care,, diagnostic identification, cancer therapies, drug therapies and biotechnologies) [50, 70, 103, 107]. NE formulations can be also improved for oral delivery of drugs with low solubility [128, 129]. NEs serve as a template to generate hydrophobic active pharmaceutical ingredients, for biomedical applications [50]. NEs can be used to encapsulate drugs, reduce toxicity and enhance the delivery of drugs. Conventional treatment strategies by emulsions have a low efficiency on tumours, and they affect normal cells too.

NE formulations show several benefits over conventional emulsions (CEs) for oral administration/ODD such as amplified absorption, enhanced clinical potency, and as the ideal way for ODD [50]. The therapeutically active forms for conventional formulations are less than that of NEs ones [43, 114, 122, 151].

Diindolylmethane has shown activity against different types of cancer cells (antioxidant and pro-apoptotic activity) [57]. Some studies have examined the link between cruciferous-derived compounds consumption and OC treatment and prevention using lipid nanotechnology [47, 119]. Lipid nanoparticles (LNPs) have been extensively used as oral drug deliveries. LNPs as a part of pharmaceutical formulations have several advantages (improvement in drug stability, great encapsulation efficiency for lipophilic drugs, and improvement for oral drug absorption) compared to liposomes [87, 101]. LNPs can be used in oral treatments as a part of pharmaceutical formations to deliver hydrophilic and hydrophobic compounds at the same time. LNPs' chemotherapeutic formulations are optimal delivery systems for OC treatments, this is due to low doses of anticancer drugs that are given on a continuous (for a long time) basis. Delivering low doses of the drug could stop the growth of new tumours [85]. Use of lipid-based nanotechnology eliminates daily administration by delivering of low doses of the particular drug for a long period. Use of lipid-based nanotechnology as a promising tool results in a reduction in the frequency of daily administration by increasing drug exposure in a sustained/controlled release manner. By employing LNPs, the oral bioavailability of anticancer drugs in vitro and in vivo was considerably improved. LNPs have high efficiency to be absorbed by human body and good stability in environments. Their Muco-adhesive/penetrative properties are suitable for oral administration [85].

2.3 Description of Oral Cancer

Oral organs for a human body include lips, Jaws, tongue, mouth, gums, the roof of the mouth, palates (the hard palate, the bony front portion of the roof of the mouth, and soft palate, the muscular back portion of the roof of the mouth), retro-molar trigone (the area behind the wisdom teeth), front two-thirds of the tongue, gingiva (gums), buccal mucosa (the inner lining of the lips and cheeks), the floor of the mouth under the tongue, cheeks, and uvula. Unusual growth of mouth cells could lead to non-cancerous tumours (warts and fibromas) [80]. OC/oral cavity occurred between the vermilion border of the lips and the junction of palates or the posterior one-third of the tongue. The Oral cavity is a transition zone between the skin and gastrointestinal system, usually lined by epithelium formerly known as oral mucosa. Based on its adaptive functions, oral mucosa categorized into three types (masticatory mucosa lining the hard palate and gingiva to resist masticatory forces, specialized mucosa lining the dorsal surface of the tongue for taste sensation; and lining mucosa that covers the rest of the regions, which are comparatively less subjected to the masticatory forces) [51]. The oral cavity has an overall 5-year survival rate for OSCC [83]. Oral cancer has high mortality and morbidity [65]. SCC is a common malignant OC that resides in the cells of the epidermis or adnexal.

A cancerous (malignant) tumour is a group of cancer cells that can grow into and destroy nearby tissue. It can also spread (metastasize) to other parts of the body [8, 80, 96]. The most common place of oral cancer spreads to the lymph

nodes in the neck. Early detection is of importance for cancer prevention and disease management. Tissue biopsy remains the gold standard for oral cancer diagnosis, but it is invasive, which may cause patient discomfort. NPs generate localized surface plasmon resonances at near-infrared (NIR) wavelengths, providing higher image contrast and resolution. Therefore, using nano-based techniques can help clinicians to detect and better monitor diseases during different phases of oral malignancy [22]. SCC is a common malignant OC that resides in the cells of the epidermis or adnexal [65].

Early diagnosis is the best way: (i) to control and cure OC in the first stage of the disease; (ii) to reduce its rate of death; (iii) to provide a suitable treatment; and (iv) to improve the quality of life for the patients [32, 97, 105, 148]. Various diagnostic procedures with light-based for early identification of OC are now available [97]. Point-of-care (POC) are good devices to improve the quality of life and survival rates for OC patients. Conventional procedure of biopsy by needle has a limited accuracy [63]. Over the past few decades, conventional diagnostic and treatment methods for OC have been improved, However, these methods still are far from the nano-based techniques.

Nanotechnology is a cutting edge and rapidly evolving technology in medicine. NPs have the potential to be used as a diagnostic tool, as well as therapeutic agents in OC. Nano-diagnostics promise increased sensitivity, multiplexing capabilities, and reduced cost for screening and imaging of OC [4]. Nano-techniques and nano-devices detect and identify cancer cells, and provide targeted delivery of anticancer drugs to tumour cells. Nanotechnology plays an important role to control OC by: (i) elevating sensitivity of detection; (ii) development of therapies and devices; and (iii) increasing in efficacy of drug delivery [4]. Use of NPs for identification and treatment of OC are the primary steps of progressing. NPs/NMs have the potential to replace by conventional materials for diagnosis and treatment of OC.

Human epidermal growth factor receptor 2 (HER2)/neuprotooncogene (neu) protein is a tyrosine kinase receptor and it is a member of transmembrane tyrosine kinases. These proteins play an essential part in cellular growth and differentiation [83]. HER2/neu is associated with the pathogenesis of various cancer types (breast, ovarian, gastric, and ovarian cancers). In head-and-neck cancers, the expression of HER-2/neu in OSCC as a potential biomarker to target antigens for specific immunotherapy in OSCC has been evaluated [82, 102, 135]. The expression of HER-2/neu in OSCC, may be due to multiple reasons. Therefore, therapy against HER-2/neu in OSCC is debatable. Her-2/neu has been associated with advanced disease, metastasis, poor clinical outcome, and survival in various carcinomas. Over-expression of Her-2/neu leads to increased basal tyrosine kinase activity and transforms the cells by persistently stimulating the signal transduction pathway [34].

2.4 Nano Materials in Dental Medicine

NMs were also used to restore the crown portion of damaged teeth, tooth enamels, anticancer tooth enamel agents, cleaning and polishing agents for dental care, and implant materials [71]. In nano dentistry, NMs such as toothpastes, rinsing solutions for better oral healthcare services, dental filling, polishing the enamel surface to prevent cavities, and implant materials were used. The NMs are more effective than that of conventional ones. NMs with antibacterial properties prevent bacterial growth. Use of NMs in dentistry also could be cost-effective, time-saving and prevent mental trauma [31].

Use of NMs in dentistry is vast: (i) making artificial teeth; (iii) repairing materials for teeth; (iv) bone graft nano-composites; (v) joint-materials; (vi) anticancer polishing materials; (vii) implant materials; and (vii) the new dental materials are under commercialization. The NMs with superior properties were more efficient, more resistance and have longer shelf-life compared to traditional dental materials [71].

2.5 Administration of Different Medications

In the pharmaceutical field, targeted drugs can be delivered by various routes (oral, ocular, and dermal) of administration [41, 46]. Among different types of administration, the oral route is the most convenient one [108, 138]. Administration of different medications, via oral route eliminates pain induced by injection [1, 40, 116]. The oral administration delivers a specific medication to a desirable organ directly. NE formulations can be administered by various routes of the body particularly by the oral route [46].

3 Use of Nano-materials in Oral Cancer Therapy

Natural products derived from plants play a significant role in the health care of many cultures, both ancient and modern [36, 79, 94, 115, 117]. Natural compounds with antitumour activity are receiving notice in medical research [79]. Many natural products are in clinical development, particularly as anticancer agents [94]. Biopolymer-based materials such as chitosan-based materials incorporated by natural compounds (curcumin, phenolic compounds, ellagic acid) with different useful properties have been used in OC therapies [9, 81].

Curcumin, a chemical constituent of turmeric, possesses anti-inflammatory, antimicrobial, antioxidant, anti-amyloid, and antitumour effects [64, 66]. The applications of curcumin remain limited, because of its short biological half-life, low aqueous solubility, poor absorption, and low bioavailability via the oral route [14,

15, 64, 73]. It has been applied in treatment of inflammatory disorders and cancer. It is a principal chemosensitizer for chemotherapy. It helps to care for patients from the side effects of treatment [6, 35, 36, 92, 117, 134]. Curcumin with anticancer properties has been applied for cancer therapies. Curcumin inhibits the growth of OSCCs. Biopolymer-curcumin nano encapsulated systems were used for administration of hydrophobic drugs in aqueous dispersions [65].

Poly (ε-caprolactone) (PCL) NPs coated with chitosan were prepared by using the nano-precipitation technique [81]. Mucoadhesive NPs loaded with curcumin were developed to deliver curcumin for local treatment of oral cancer. Free curcumin and curcumin loaded into the NPs coated with chitosan caused a significant reduction of human oral cancer cells (SCC of the oral tongue, SCC-9). No significant cell death was observed after 24 h of treatment with unloaded NPs coated with chitosan. Curcumin-loaded NPs showed reduced cytotoxicity when compared with the free drug. In conclusion, chitosan coated by (PCL) NPs may be considered as a promising strategy to deliver curcumin directly into the oral cavity for the treatment of oral cancer.

Mucoadhesive NPs-curcumin systems were prepared to deliver curcumin for OC treatment. Use of free curcumin and curcumin-chitosan NPs yielded in the reduction of OC cells (SCCs of the oral tongue, SCC-9). Curcumin-chitosan systems showed reduced cytotoxicity in comparison with free curcumin. The assembly of chitosan-curcumin is a reliable system for delivery of curcumin directly into OC cells [81].

Poly lactic-co-glycolic acid) (PLGA)-poly(ethylene) glycol (PEG) (PLGA-PEG) NPs, a complex biomaterial, exhibited good transport properties and inhibited the growth of cancer cells. PEG-PLGA polymer was synthesized and conjugated with NR7 peptide (NSVRGSR), to improve the above-mentioned properties [143]. NR7 peptide is based on the alignment of the tripeptide motif with the EGF binding domain [67].

Cis-Diamine dichloro platinum (cisplatin, CDDP)-PLGA-PEG/NR7 NPs systems having cytotoxic effects with high efficiency were used as targeted delivery systems. The assembly is a desirable system to inhibit OSCCs. The targeted delivery system, CDDP-PLGA NPs, was prepared by solvent evaporation and its size, shape and physico-chemical properties were characterized [143]. Cisplatin, CDDP was employed to treat various cancers (ovarian, testicular, colorectal, and OC) [16, 33]. The CDDP was approved for cancer therapy due to the synergistic effect as a single medication or in combination with other compounds having anticancer properties. The CDDP inhibits the growth of cancer cells via inducing cross-linkage with DNA, by intervention for activation of cell division and generation of new cells [42].

Polymeric NPs with active targeting moiety increase the target specificity. The epidermal growth factor (EGF) receptor is recognized as an important target for the development of cancer treatment. EGF receptor is highly expressed in human epithelial cancer cells such as OSCC. Several EGF-targeting therapeutic agents such as cetuximab and erlotinib have already been approved by the USFDA.

A major nano-medicine approach is targeted cancer therapy. Polymeric NPs possessing active targeting moieties improve the target specificity. Epidermal growth factor (EGF) was known as a target for cancer treatment. EGF receptor (EGFR) is

considered in human epithelial cancer cells such as OSCC. Various EGF targeted therapy compounds (cetuximab and erlotinib) were approved by USFDA [67, 91].

Anticancer and targeted effects and good transport properties were reported for PLGA/NR7 NPs. The life/death assay showed higher levels of red fluorescence in targeted PLGA/NR7 NPs in comparison with the PLGA NPs. The presence of NR7 targeting moieties on the surface of the PLGA support allows a strong binding of a ligand to a specific cell surface, enhanced carrier properties and a great cell killing power. The targeted micelles deliver anticancer agents gradually over a period of time to cancer cells than that of the non-targeted ones. Targeted cancer therapy using nanotechnology in medicine is an efficient method to treat OSCCs [143]. The target specificity in vitro was performed on targeted and non-targeted NPs in OSCCs. Cytotoxic effects of targeted and non-targeted NPs were evaluated by life/death assay, MTT assay, and apoptosis analysis [143]. The MTT reagent is a mono-tetrazolium salt that consists of a positively charged quaternary tetrazole ring core containing four nitrogen atoms surrounded by three aromatic rings including two phenyl moieties and one thiazolyl ring [45]. The apoptosis analysis detects and quantifies the cellular events associated with programmed cell death [37].

Graphene and its composites are interesting materials with unique characteristics (nano-sized, very large surface area per volume) and excellent mechanical properties. These materials can be transferred or deposited onto different support materials [30, 31]. The composite materials having various formulations, structures, and properties are used in several branches of medicine (with a particular attention in dentistry and oral cancer diagnosis and treatment). The chemo-therapy efficiency of platinum (Pt)-graphene quantum dot (QD) composites for treatment of OSCC was improved by modification with PEG. The composites promote inclusion in the synthesis phase of the cell cycle and lead to cell apoptosis [30, 31, 72, 147].

Polymer-based, lipid-based, and metal-based nano-carriers are main chemo-therapy families to treat OC. PH-sensitive poly [(2-methacryloyloxy) ethyl phosphorylcholine] (PDPA) and poly [(2-diisopropylamino) ethyl methacrylate polymersomes] (PMPC) were used to distribute chemotherapy agents to the tumour cells and to improve collective anticancer therapy. The cytotoxic effect of PMPC-PDPA was improved by encapsulation. Doxorubicin (DOX), and Paclitaxel (Taxol) as individual drug or combined medication delivery systems were used to treat different types of cancer [38].

Combination therapy is an efficient method to treat different types of cancer. Anisamide-targeted lipid-calcium-phosphate (LCP) NPs were employed to deliver HIF1α siRNA. Combination of photo-dynamic therapy (PDT) and photo-thermal therapy (PTT) was also a combination procedure to treat OC [153]. Applications of different types of Nano-materials in different branches of cancers are presented in Table 2.

Table 2 Applications of different types of nano-materials in different branches of cancers

Type of nano-materials	Branch of biomedical	Functions and applications	References
GO, RGO	Oral cancer	Graphene oxide (GO) and reduced GO (RGO) are used as biomarkers for oral cancer; RGO is highly conductive, and this property makes it a perfect material to form NPs; it can be used along with cerium oxide for the detection of cancers, and oral cancer treatments	[30, 31]
Platinum (Pt) loaded-graphene quantum dot composite, modified via polyethylene glycol	Oral cancer	The composite enhances chemotherapeutic efficiency for treatment of oral cancer	[30, 31]
Nano-emulsions	Different types of cancers	NEs serve as a good carrier system for chemotherapeutic drugs; NEs can be used in target cancer therapy to deliver active ingredients; NEs can be delivered in the deepest of tissues; NE formulations are more effective than that of CEs for cancer therapy	[104, 109, 133]
Curcumin	Oral cancer	Curcumin inhibits the growth of OSCC	[65]
Chitosan coated by (PCL) NPs		NPs of curcumin is a desirable choice to treat OC/oral cavity	[81]
Gold NPs	Different types of cancers	The NPs have been used in cancer diagnostics and therapeutics	[93, 95, 121]

4 Treatment Methods for Different Cancers

Multiple strategies including surgery, chemotherapy, radiotherapy, freezing, targeted drug therapy, photodynamic therapy (PDT), and immunotherapy have been used to treat various cancers. Chemotherapy is the best way to reduce the risk of death, when cancer cells spread and metastases occurred [5, 18, 53, 68].

Surgery removes tumour tissues, whereas radiotherapy and freezing destroy tumour tissues. Significant wounds and psychological disorders are major side effects for the three methods employed for OSCC treatments. PDT as a simple non-invasive procedure that has been also applied to treat different cancers including OSCC [5, 68].

In PDT therapy process, a photosensitizer (PS) initially is activated by light with an appropriate wavelength and intensity. PDT is a three-way process consisting of PS, light, and molecular oxygen, followed by reactive oxygen species (ROS) created from energy of the activated PS. The ROS plays a significant role in destroying tumour tissues [53, 75]. The efficiency of the PDT process depends directly on the photo-oxidation ability of PSs and concentration of the created ROS [18]. Various techniques associated with NE formulations have been also utilized in ODD for the treatment of different cancers. Chemotherapy slows down the growth or kills the tumour cells with a reasonable efficacy. However, it may cause healthy cells to destroy or die [143]. The delivery of RNA NPs is an alternative therapy procedure to treat OC [140, 141].

Targeted delivery and controlled release of inactive platinum (Pt) prodrugs may offer a new approach to improve the efficacy and tolerability of the Pt family of drugs, which are used to treat 50% of all cancers [33]. The efficiency of targeted therapies as well as targeted micelles delivery systems using nanotechnology are significantly greater than that of non-targeted ones. The targeted processes provide/deliver more anticancer medications to desirable cells (OSCCs) than that of the non-targeted ones.

4.1 Oral Chemotherapy

Chemotherapy and radiotherapy are considered as the main OC treatments. However, these treatments may induce mouth tissues swelling [149]. Use of nanotechnology in different treatments and therapies (surgery, chemotherapy, and radiation) for OSCC healing/curing in comparison with the traditional ones would yield in: (i) to deliver more medications to tumour tissues, (ii) to increase the efficacy of treatments significantly; (iii) to employ targeted treatments without any effects on healthy cells or with a low level of side effects on healthy cells; and (iv) to reduce systemic toxicity [65]. Advantages and disadvantages of oral chemotherapies compared with parenteral chemotherapies, the risks and benefits of oral chemotherapy from the patient, physician, health care system perspective have been described elsewhere [146].

Oral mucositis (OM) is a common painful side effect of chemotherapy and radiation therapies. Bulk curcumin as well as curcumin nano-micelles were effective medications to treat and prevent chemotherapy and radiation therapy induced OM. It is an alternative way of palliative and local treatments. Curcumin nano-micelle capsules have advantages (stability, bioavailability, resistance to deterioration, increase in solubility) over bulk curcumin [52, 106]. These capsules are efficient medications to treat and prevent OC by radiotherapy and chemotherapy induced OM [66]. The core–shell structure of nano-micelle protects the inner core from water. It can be an appropriate alternative to deliver curcumin. The curcumin nano-micelles have advantages, including easy development, resistance to degradation, and improved solubility, bioavailability, and stability [52, 106]. Nano-micelle curcumin capsules

are effective in prevention and treatment of head and neck radiotherapy and especially chemotherapy-induced OM [66].

Oral chemotherapeutic drugs including the familiar agents [chlorambucil, cyclophosphamide, methotrexate, and 6-mercaptopurine (6-MP)] have been available for decades [146]. Some oral chemotherapies may be associated with fewer side effects than parenteral alternatives. However, the promise of oral chemotherapies will only be realized with careful attention to the safety and monitoring requirements [146].

4.2 Photo-therapy

Photo-thermal therapy (PTT) refers to efforts of using electromagnetic radiation (most often in infrared wavelengths) for the treatment of various medical conditions, including cancer. This approach is an extension of photodynamic therapy, in which a photosensitizer is excited with specific band light. This activation brings the sensitizer to an excited state, where it then releases vibrational energy (heat) and is able to kill the targeted cells. The major challenge in PTT is to develop nanocomposites that simultaneously exhibit bio-imaging and PTT under a single NIR irradiation, with high therapeutic efficiency [139].

The temperature-dependent up-conversion luminescence (UCL) property was used to determine the local temperature of the composite NPs. The UCL was used to select the irradiation dose for the PTT process. The performance of the composite NPs in the PTT process was found for OML-1 OC cells [139].

Multifunctional nanocomposites were synthetized by linking $NaYF_4$:$Yb^{3+}Er^{3+}$ upconversion nanoparticles (UCNPs) with gold nanorods (AuNR). The nanocomposites exhibited fluorescence labelling, local temperature sensing and photothermal functions accompany with a single NIR laser excitation. The AuNR-$NaYF_4$:Yb^{3+}, Er^{3+} composite NPs exhibited superior photo-thermal properties in comparison with the pure AuNR NPs or a blend of AuNR NPs and $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs [139].

The nanocomposite particles caused the destruction of cancer cells by up to 70% dead cells under 976 nm laser irradiation for only one min at 0.3 W/cm,^{2} which is below the maximal permissible exposure of human skin deeper penetration in tissue. The temperature of the aqueous solution after 1 min of laser irradiation increased by just 0.5 °C. It is the reason, why the laser exposure dosage had a negligible effect on the OML-1 oral cancer cells [139]. AuNR coated with a silica layer of ~20 nm gives off more heat to the surroundings compared to uncoated AuNR. The local temperature of Au NR has reached ~45° C. It is sufficient for destruction of cancer cells just 1 min, after laser irradiation, which is higher than that of the ambient temperature [139]. Au NR-UCNPs would yield in a great cancer efficacy (~70% of OML-1 cells death/min) employing low intensity irradiated at 976 nm. The results indicate that a great efficacy for targeting tumours is via a highly efficient photothermal effect combined with UCL labelling. AuNR-SiO_2-UCNPs composite NPs

are a multifunctional tool for UCL imaging, thermal sensing, temperature sensing, and PTT applications to achieve a controllable cancer therapy [139].

Using fibre optics makes it easier to irradiate the tumour tissue with an excitation laser beam. OC tumours close to the surface of the skin do not require high penetration. The benefits of using PTT to treat OC are: (I) treatment of the process is easy to visualize. (i) no general anaesthesia is required. Streptavidin molecules were bound with hybrid multifunction composite NPs by indirect linkage to OC cells via biotinylated secondary antibody and anti-Her2 antibody to determine human epidermal growth factor receptor 2 (Her2). A cancer marker on the surface of OC cells was bound by promoting specific linkage between streptavidin and biotin [54, 150]. As a result, AuNR-SiO_2-UCNPs composite NPs are able to detect OC cells.

Hou et al. [54] reported that when the OC cells progressed and reached to metastasis level, a gradual increase in neu expression was achieved. The authors suggested that neu may be involved in the development of OC and its early assessment may be useful for diagnosis and treatment of OC. Monoclonal antibody PAb3 to c-erbB-2/neu protein was utilized in the immune-peroxidase staining of 86 human specimens from oral mucosa [54].

Semiconductor quantum dots (QDs) are among the most promising emerging fluorescent labels for cellular imaging. However, it is unclear whether QDs, which are NPs rather than small molecules, can specifically and effectively label molecular targets at a sub-cellular level [150].

Up-conversion NPs (UCNPs)-gold NMs (AuNMs) systems as hybrid (UCNPs-AUNMs) systems have been employed in bio-imaging and PTT process [23, 24, 76, 100, 154]. The nanocomposites required excitation intensity higher than 0.6 W/cm,^{2} and long excitation time to perform PTT [27, 118, 124, 142]. The maximum permissible exposure to human skin, for the above-mentioned nanocomposites is not applied for the PTT process [152]. In contrast, the nanocomposites required low excitation intensities (0.3 W/cm^2) and short excitation times to achieve a high efficiency for PTT process [123, 139, 155]. The composite NPs required a single NIR 976 nm laser source to perform bio-imaging, local temperature sensing, and PTT process simultaneously. It is more practical than that of dual-wavelength excited composite NPs [139, 155].

PDT is not an invasive therapy method, and has been employed to treat OCs. Traditional PDT therapy met some difficulties [low quantum yield for singlet oxygen ($^1O_2$), and long-term photo-toxicity during PS application]. Nano-PSs [sulphur-doped carbon dots (S-CDs)] with a great quantum yield for singlet oxygen ($^1O_2$), and a high efficacy of therapy] were synthetized to overcome to the difficulties. The S-CD NPs were characterized (size, surface morphology, quantum yield, surface potential) [74]. The biocompatibility and therapy effects of PSs (S-CDs NPs) in vivo, were compared with 5-ALA. The safety of low dose (nmol L^{-1}) of SCD NPs was confirmed, indicating that the S-CD NPs are safe, useful and reliable compounds as PDT agents, and can be employed for OC therapy [74]. The S-CD NPs demonstrated a strong binding ability, high therapy efficiency, and low cytotoxicity without any light irradiation. The high efficiency of S-CD NPs could be due to a great generation

rate of $^1O_2$. The safety of low dose (nmol L^{-1}) of S-CD NPs was confirmed, indicating that the S-CD NPs are safe, useful and reliable compounds as PDT agents, and can be employed for OC therapy [74]. The biocompatibility and therapy effect of PSs (S-CDs NPs) in vivo, were compared with 5-ALA. It was more effective than that of the traditional PS 5-ALA.

Gold contains electrons that are free to move throughout the metal. These electrons act as conductors of current when a voltage is applied. In metallic gold, the free electrons absorb the energy from a particular wavelength of light (wavelength range from 700 to 800 nm) and convert it into heat. This property is utilized in treating large tumours in the human body. This is known as hyperthermia therapy [95]. Gold NPs have opened new avenues in the various fields, especially in diagnostics and therapeutics [93, 121].

5 Conclusions

Oral Cancer (OC) is one of the most prevalent diseases in the world, it has a significant death and morbidity rate. This chapter gives a review of oral cancer to diagnose, detect, prevent, and therapy by employing nanoscience and nanotechnology. The strong points of nanomaterials over bulk materials for OC (diagnosis and therapy) were highlighted. The following conclusions were made: (i) OC/oral cavity is a common malignancy of the head and neck areas. About 90% of all oral malignant tumours are squamous cell carcinomas (SCCs). Oral squamous cell carcinoma (OSCC) originates from epithelial cells in the oral mucosa. Oral cancer is an aggressive cancer that mainly affects oral epithelial cells. OC is an invasive cancer that primarily affects oral epithelial cells. SCC is a common malignant OC that resides in the cells of the epidermis or adnexal; (ii) the incidence and death rates in men are greater than that of women; (iii) the use of nanotechnology and helps health care experts to identify and determine the stage of cancer cells during different phases of oral fatality; (iv) various types of NMs such as toothpaste, mouth rinses, materials used to restore the crown portion of damaged teeth, tooth enamels, anticancer tooth enamel agents, and cleaning and polishing agents for dental care were used to prevent cavities. Respecting hygienic principles prevents the creation or advancement of cancerous cells or tumours. In other words, prevention is better than curing; (v) NM has been used with significant success in identifying and treating multiple types of fatality including different types of cancers particularly oral cancer; (vi) early diagnosis through screening is the best way to control oral cancer in the first stage of the disease, to provide prompt treatment, and improve the quality of life for OC patients. Nanotechnology plays a significant role by elevating sensitivity in the first stage of identifying of OC, and enhancing the efficacy and delivery of treatment; (vii) natural compounds possessing pro-apoptotic effects such as chitosan carrier of curcumin, chitosan-based delivery systems for curcumin, phenolic compounds, and ellagic acid have been used in oral cancer therapeutics. Curcumin inhibits the growth of OSCC; (viii) graphene and gold NPs were employed to treat oral and other types of cancer;

(ix) chitosan-based delivery systems for (PCL) NPS can be used to treat OC/oral cavity; (x) PLGA-PEG showed great cell uptake and excellent apoptotic properties on OC; (xi) Nano-targeted therapy can be an efficient way for the treatment of OSCC. The targeted therapy delivers more amount of desirable drugs to cancer cells than non-targeted ones; (xii) Various methods (surgery, chemotherapy, radiotherapy, freezing, targeted drug therapy, photodynamic therapy, and immunotherapy) have been used to treat various cancers, when cancer spreads and becomes metastatic; (xiii) chemotherapy is the best way to treat various cancers; (xiv) RNA-based nano delivery systems are favourable therapy procedures for OC treatment; (xv) PDT was used for the treatment of human oral cancers; and (xvi) different techniques associated with NE formulations have been utilized in oral drug delivery for treatment of different cancers.

6 Future Perspectives

The toxicity of NMs is needed to be explored before they are employed for human use. Due to a limited knowledge on the safety of NMs, investigation on the toxicological evaluation and examination on NMs, should be considered as a priority in the field of nano-medicine and health care. In vitro, nano-toxicity studies should be carried out in a way that has some prediction of in vivo nano-toxicity at the tissue and organ levels. The toxicity of NMs should be determined before marketing in order to obtain a clear information from their safety/toxicity.

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Chapter 11
Nano Structured Materials in Dental Medicine—From Laboratory to Industry: New Opportunities, Challenges and Risks

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1 Introduction

With the gradual understanding of the performance and fine structure of natural organisms, scholars have combined nano structure with commercial materials and developed a batch of new materials that are more consistent with the biological conditions of oral cavity. These new nano structured materials undoubtedly provide a huge development space for the improvement and innovation of dental medicine. As an emerging interdisciplinary industry, the research of dental nano structured materials involves biology, oral medicine, materials science and chemistry, etc., with the knowledge-intensive and technology-intensive characteristics. Due to its multi-disciplinary characteristics, the research of biomaterials must involve the participation of different academic backgrounds, so as to solve the complicated problems in clinical application. From lab to industry, the research process is characterized by large investment and long period. A new nano structured materials will go through

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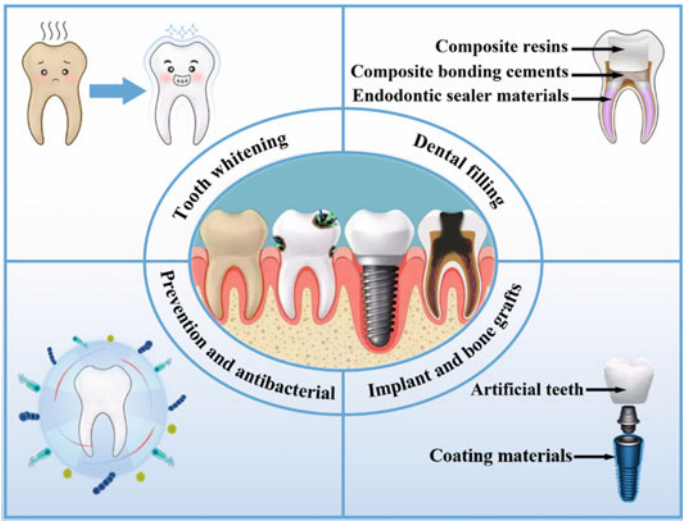

Fig. 1 Schematic diagram of the classification of the common dental nanomaterials. Nano structured materials in dental medicine can be roughly divided into the following categories: dental filling materials, implant and bone graft materials, artificial teeth, preventive materials and tooth whitening materials. Therein, dental filling materials can be further divided into composite resins, composite bonding cements and endodontic sealer materials. Meanwhile, coating materials additionally derived from the implants endow the implants with different properties

many steps from concept to product. This chapter will discuss the challenges and risks in the industrialization of nano structured materials in dentistry from three aspects, including commercialized dental nanomaterials, dental nanomaterials with industrialization prospects (Fig. 1) as well as the challenges and risks during the industrialization process.

2 Commercialized Dental Nanomaterials

2.1 Preventive Dentistry Materials

Dental caries, mainly caused by the colonization of local organisms, is a common and epidemic oral disease that can even seriously jeopardize overall health. In the pathogenesis of caries, *Streptococcus mutans* is the main microorganism, plaque is the main cause, and enamel crystal demineralization matters the most. Therefore, bacteria inhibition and enamel remineralization are the major preventive measures.

In view of the problems aforementioned, NanoCare Gold, a dental caries antibacterial solution containing spherical silver nanoparticles, is considered to have promising antibacterial effect. However, the aggregation of silver nanoparticles may affect the

later repair materials [1]. Hydroxyapatite within teeth is a needle-like or columnar crystal matter of 40–60 nm, about 20 nm wide and 3–5 nm thick, which belongs to the category of nano-hydroxyapatite (nHA), so nHA is widely used to promote tooth remineralization. Due to its excellent biocompatibility and bioactivity, nHA can be applied directly to repair minor cracks on the tooth surface. Meanwhile, nHA is a soft material with high whiteness and a Mohs hardness of 5, which can be applied alone as a toothpaste friction agent or combined with other abrasives. Besides, it also can play its part in friction polishing with no harm to tooth enamel, and in stain and dental calculus elimination with excellent whitening performance. Therefore, many toothpastes containing nHA have been developed in recent years. Those products can stimulate and activate teeth and periodontal tissue during brushing to facilitate tooth mineralization, alleviate tooth allergy and promote tooth whitening, which effectively prevents gingivitis and periodontal disease. The benefits of toothpaste containing nHA were first reported in Japan in the 1980s. The gathered results revealed a 56% reduction in caries incidence in school children brushing with nHA toothpaste in comparison with the control group [2].

Oral hygiene products such as mouthwash solutions are also nano-modified fluid. For instance, the mouth products added with fluoride nano-calcium can reduce caries activity and dentine permeability, as well as increase labile fluoride concentration in oral fluid [3].

2.2 Composite Bonding Cements

In 1998, a bonding cement using nanotechnology was introduced to the dental market, namely Dentsply's Prime & Bond NT fifth-generation dentin luting cements. It combined nanotechnology with dentin luting technology by mixing amorphous silica fillers of only 7 nm in diameter with luting cements. It also significantly enhanced the strength of the luting cement between dentin tubules and hybrid layer, with a bonding force of 25 MPa, effectively improving the luting effect and prolonging the service life [4–6]. Compared with conventional cements, nanoparticle impregnated luting cements have proven to be very effective in increasing the adhesive strength of enamel and dentin due to the deeper penetration of these small sized particles into the dentinal tubules, which increases the elastic modulus and reduces polymerization shrinkage [7]. Researchers have also found that the size scale of composition determines the strength of luting cements. In 2011, researchers created a novel approach to improve compressive and tensile strength of zinc poly carboxylate by incorporating zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles. Results revealed the excellent physical and mechanical strength when compared with the conventional zinc poly carboxylate cements [8].

2.3 Composite Resins

Composite resin is a bonded restoration material made of resin matrix with surface-treated inorganic filler and initiation system. Over the past three decades, composite resins have been tremendously improved in resin matrix, inorganic filler and curing method [9]. The mechanical property and operational performance have been greatly improved, but there are still some shortcomings, such as significant shrinkage, poor wear resistance and low strength. Currently, some of the commercial nanocomposite resins can solve these problems to some extent. For example, it has been found that 3M ESPE's commercially available Nano-Resin modified glass ionomer cement (GIC), Ketac™ Nano, with the addition of zirconia, silica nanofillers or nanoclusters, gives it higher shear bond strength to enamel compared with the conventional GIC and glass carbomer [10]. Due to the addition of nano-silica, the micro-hybrid composites Herculite XR has a high gloss value. Filtek Supreme, 3M ESPE's nanocomposite resin, which combines the characteristics of microfilled and micro-hybrid resins with a blend of silica/zirconia nanoparticles of 20 and 70 nm diameter, can be used as a universal restorative material for anterior and posterior restorations with superior compressive strength, wear volume and polishing property [11].

2.4 Endodontic Sealer Materials

Root canal therapy is the most effective method of treating pulp infections. The infection is eliminated by tightly sealing the root canal system with a sealer. The endodontic sealers currently used in the clinical application are clove oil, resin, apatite and calcium hydroxide, which have the limitations such as high tissue irritation, difficulty in forming, poor sealing ability and easy degradation. A new endodontic sealer with nanosilver, GuttaFlow, has solved these problems perfectly [12, 13]. Meanwhile, nHA-PA66 is a nanocomposite material consisting of nHA and polyamide 66, which has been nano-modified based on hydroxyapatite to overcome the disadvantages of brittleness, coarseness and poor flowability. It also has antibacterial property, while exhibits non-cytotoxic to osteoblasts and promotes periapical tissue repair, which has a promising application prospect in root canal treatment [14].

2.5 Artificial Teeth

The two major types of artificial teeth used in the clinical practice are resin teeth and porcelain teeth. Resin teeth are flexible, tough, easy to grind and polish, and bond well with resin base. While the disadvantages are obvious, such as significant coefficient of expansion, low hardness, poor wear resistance, easy aging and discoloration. With the introduction of nano structures, a new type of denture artificial teeth made of

nanocomposite resin has been introduced into the clinical application, which is highly polishable and has good resistance to pressure and impact. The nanocomposite resin used in denture artificial teeth consists of a copolymer of urethane dimethacrylate, methylmethacrylate, polymethylmethacrylate and homogeneously dispersed spherical pre-polymerised silica nanofiller particles. Due to the unique polymer structure, the nanocomposite resin artificial denture is superior to the conventional materials in terms of hardness, smoothness and stain resistance [15]. Compared with 12 kinds of commercially available denture artificial teeth, Suzuki found that nanocomposite resin artificial teeth of Shofu's Veracia had better hardness and abrasion resistance [16].

2.6 Bone Graft Materials

After periodontal surgery or tooth extraction, alveolar ridges are often low and flat for progressive and irreversible absorption, which makes it difficult to repair dentures or place implants in the later period. Despite the acceptable outcomes of autologous bone grafting, it still has several disadvantages, such as difficulty in accessing materials and additional surgical trauma. Similarly, allogeneic bone or xenogeneic bone has potential risks in immune rejection. Recently, many kinds of bone grafts with nanocrystalline hydroxyapatite have been emerging. For example, NanoBone® has low cytotoxicity, good biocompatibility and superior proliferation ability compared with BioOss® [17].

2.7 Implant Coating Materials

Due to the favourable stability, low mobility, high chewing efficiency and non-destruction to adjacent teeth in tooth repairing, the implantable denture has gradually become one of the best solutions for dentition defect and loss. However, there is still a noticeable failure rate in the long-term follow-up for the low osseous binding ability and extensive peri-implant inflammation. Currently, surface modifications are commonly used in clinical practice to promote the integrating capacity with bone and avoid implant failure. BIOMET 3i NanoTite implants with nHA implant coatings (approximately 50% of the total surface area) exhibit the sound clinical bone-binding capacity with minimal marginal bone loss [18].

3 Dental Nanomaterials with Industrialization Prospects

3.1 Antibacterial Materials

Bacterial biofilm is the main cause of dental caries and periodontitis, so the applications of antibacterial materials in oral cavity have broad prospects. Current antimicrobials have low efficacy and cannot target the protective matrix and acidic pH within the biofilm. David et al. reported dextran-coated iron oxide nanoparticles termed nanozymes (Dex-NZM) as biomimetic catalysts for localized and pH-activated biofilm disruption [19]. Compared with other similar materials, Dex-NZM has the characteristics of low cost, easy synthesis and excellent biocompatibility, for which its potential application is fantastic.

The pectin-gelatin hydrogel patch doped with phage lyase (ClyR) and metronidazole was proposed for the treatment of periodontitis [20]. Compared with the conventional treatment of periodontitis, ClyR is mild, non-toxic and harmless for its protein property. What's more, ClyR targets pathogenic bacteria and kills bacteria in 1–5 min without damaging the symbiotic bacteria, which is of great significance for maintaining the ecological balance of bacteria to keep oral health.

3.2 Bonding Materials

The limited durability of dentin bonding harshly shortens the lifespan of resin composites restorations [21]. Bisphenol-A diglycidyl methacrylate (BisGMA) adhesives with new monomers can effectively extend the lifespan of dental resin materials for its favourable physicochemical property and leaching characteristic [22]. A real-time magnetic motion of dental adhesives may open new paths to enhance the longevity of resin-based restorations. In detail, a new dental adhesive with superparamagnetic iron oxide nanoparticles (SPIONs) is sensitive to magnetic field for bonding optimization, which can enhance penetrability into etched dentin guided by magnetic field [23].

3.3 Resin-Based Dental Filling Materials

At present, the short-term bonding strength between resin cement and dentin interface can meet basic clinical needs. However, poor durability and biocompatibility often result in microcracks and secondary caries, which represents the failure of the treatment in the end. Therefore, some desirable nanomaterials are proposed to be added into denture powder to improve the wear resistance, glossiness and biocompatibility of dental resin.

The successful synthesis of hollow mesoporous silicon microspheres has made a certain contribution to the progress and innovation of enhanced dental resin materials [24]. This resin filler has the advantages of stable structure, uniform particle size, excellent dispersion and simple operation, which can effectively improve the biocompatibility, mechanical property and anti-aging property of resin cement. Whereas a novel dental light-cured composite resin containing the antibacterial component Nano-MgO can reduce its shrinkage and improve its antibacterial property for secondary caries prevention [25]. As for antibacterial resin filler, nano-silica modified with long-chain alkyl quaternary ammonium salt shows admirable antibacterial performance without strength reduction, which makes it viable for clinical application. Beyond that, barium titanate ($BaTiO_3$) piezoelectric nanoparticles can be used as multifunctional biological active fillers in dental resin composites for its dual effect of antibacterial property and remineralization prompting, which provides a new direction for the selection of biological filler [26].

3.4 Implant Materials

The composite ceramic material containing calcium sulfate ($CaSO_4$) and beta-tricalcium phosphate (beta-TCP) is an osteoinductive material [27], which has the characteristics of excellent biosafety, mechanical property and adjustable degradation rate. Organisms can be used as a reactor without growth factors, and a certain volume of bone implants constructed in bone or non-bone environment can repair the defect of autogenous bone without affecting the body function. Compared with conventional guided bone tissue regeneration (GTR) membranes, the hydrogel composite doped with cubic cuprous oxide (Cu_2O) and polydopamine-coated titanium dioxide (TiO_2@PDA), denoted as CTP-SA, can perfectly automatch variform bone defects [28]. In addition, CTP-SA can switch antibacterial and osteogenic modes to address the requirements of patients at different healing stages by blue light and near infrared (NIR) laser, thereby realizing the customized GTR procedures.

3.5 Coating Materials for Bone Implants

Titanium (Ti) and its alloys are widely used in the orthopaedic applications. However, the lack of bioactivity and osteointegration makes surface treatment necessary to obtain an effective osteoinduction or osteoconduction. In view of the seriousness of the problem, low energy consumption micro arc oxidation technology can be used to form an oxide inner layer on the surface of titanium alloy substrate to enhance the bonding strength between coatings and implants. The coatings containing perfluorosilane or polytetrafluoroethylene emulsions can inhibit bacterial adhesion owing to the hydrophobic property. In addition, macroporous tantalum (Ta) can be coated on titanium alloy implant for bone repair [29]. A tremella-like ZnO doped with

college-I (ZnO@Col-I) composite can be applied to coating on Ti implant surfaces, which can respond to yellow light and NIR, as well as enables the implant to realize light-controlled switching (antibacterial or osteogenic) functions on demand [30].

3.6 Materials for Promoting Wound Healing of Oral Mucosa

The wet and highly dynamic environment of the mouth makes the local treatment of oral mucosal diseases challenging [31]. Therefore, the development of materials with satisfactory adhesion and sufficient drug bioavailability under wet conditions has attracted extensive attention. An adhesive hydrogel based on a phototriggered S-nitrosylation coupling reaction (denoted as HA-CNB) can gel and bond firmly with the host tissues in just a few seconds after light irradiation [32]. Compared with currently available materials used in clinics which perform poorly in the oral environment, the thin, elastic, safe and tissue-adhesive layer of HA-CNB hydrogel serves as a stable barrier for more than 24 h to protect the oral mucosa from destructive actions. As a result, it has great potential for commercialization.

A viscosity-adjustable film (PVA-DOPA film) composed of polyvinyl alcohol (PVA) and mussel adhesive proteins (DOPA) can extend residence time and enhance the adhesion strength, which can improve the efficiency of buccal drug delivery and inspire the rational design of tissue adhesives and wound dressings in the near future [33].

3.7 Materials for Tooth Whitening

The application prospect of materials in the field of tooth whitening is great and bright. Chemical bleaching is based on the degradation of chromogen by reactive oxygen species (ROS) [34]. Oxidative bleaching agents with blue light, especially hydrogen peroxide (H_2O_2), have been widely applied to tooth whitening in clinical practices. However, H_2O_2 and high intensity of blue light are harmful to teeth and normal tissue respectively, causing corrosion to tooth surfaces and irritation to oral mucosa, eyes and skin. Therefore, there is an urgent need to transfer some less-destructive tooth whitening materials to products.

Photodynamic therapy (PDT), an invasive approach with high spatiotemporal accuracy, can efficiently produce ROS when photosensitizer (PS) is excited by appropriate light in the presence of molecular oxygen. By manipulating the light source as well as applying location and time, the amount of ROS can be precisely controlled to achieve on-demand production and avoid normal tissue damage. This technique has been studied extensively in many fields [35]. For example, PDT is effective for teeth whitening and can promote the remineralization of tooth enamel with boron-dipyrromethene (BODIPY) as a photosensitive drug [36]. PDT is expected to become

a new method for tooth whitening without harm on normal tissue. Phthalimide peroxyhexanoic acid can be excited under long-wavelength light (570–590 nm), which is safer and less destructive, avoiding eye and skin irritation. ZnO with high specific surface area and narrow energy band gap can effectively remove pigment on tooth surfaces through physical adsorption and ROS generated under the irradiation of warm light [37].

Besides the photocatalytic materials, pyroelectric materials-potassium sodium niobate can release oxygen free radicals through temperature changes. It is a simple and low-cost strategy to whiten teeth by the changes of oral temperature caused by factors such as diet, breathing, conversation and exercise in daily life. In addition, the piezoelectric property of $BaTiO_3$ can be activated by force, resulting in the generation of ROS to degrade stains on tooth surfaces, achieving the effects of tooth whitening [38]. Unlike existing techniques, piezo-catalysis tooth whitening has the potential to be widely adopted for household use.

4 Challenges and Risks During the Industrialization Process of Dental Nanomaterials

A new type of dental nanomaterial must go through many links from the concept to a product, including laboratory research stage, pilot production stage, clinical trial stage, large-scale production stage and drug administration approval process stage and commercialization stage [39]. Meanwhile, each stage must be subject to strict and complex supervision.

However, there are still many problems in the process of industrialization, such as the insufficient practicality of scientific research and innovation, the imperfect technical structure of products, the low grade of products, the shortage of industrialization funds and the incomplete level of talent structure. In the long process from the research to the industrialization of dental nanomaterials, the tasks and difficulties faced by different stages are various, which should be solved by effective organization and management in a targeted manner [40]. From the scientific research stage to the engineering stage, its core subject will be transferred from scientific research institutes to enterprises. In the enterprise operation stage, there are numerous difficulties and challenges in market cultivation and development of dental products, which have their own particularities [41]. As a result, it is necessary to comprehensively analyze difficulties, identify problems and propose effective measures in order to efficiently promote the industrialization of nano structured materials in dental medicine.

4.1 Market-Oriented Research and Development Based on Actual Clinical Needs

The research and development of dental nanomaterials must be carried out based on clinical practical applications and market orientation to solve the shortcomings of the existing nanomaterials. New nanomaterial products have high technical added value and returns. Nevertheless, due to the long cycle, long industrial chain and complex administrative approval links of research and development, the investment of dental nanomaterials cannot get returns in a short time. The large amount of capital invested often leads to high risks. Therefore, it is necessary to comprehensively consider various factors, especially the level and scale of engineering and industrialization. What is more significant is to firmly grasp the market, understand the clinical and market demand in detail as well as actively develop marketable products, which will obtain rich returns through longstanding and unremitting efforts [39].

4.2 Pay Attention to Engineering Development and Technology Integration Research to Improve the Overall Performance of Products

The clinical applications of any research involve a wide range of issues, and pure scientific innovation usually cannot be directly applied to the clinical practice. The safety, effectiveness and operability of clinical applications must be considered. The industrialization process must integrate material research and clinical application. Besides, relevant comprehensive supporting research is also indispensable, such as the formulation of product standards, operating skills and production standardization [39]. The strengthening of comprehensive supporting research will be conducive to the performance advantage and comprehensive competitiveness of the final product.

The successful research and development of the laboratory is an important step in the industrialization of engineering, but also the foundation of industrialization, which is widely accepted by the public. However, people tend to ignore the problems of process amplification in later industrialized production. It is difficult to transform the small-scale test process into large-scale production and develop products with low energy consumption, high environmental protection and controllable cost under the guarantee of biosafety and effectiveness in the subsequent engineering, which is one of the key factors for the success of the industrialization process [39]. Consequently, it is necessary to closely integrate scientific researchers in professional fields with business activities of enterprises to achieve an effective combination of technical strength and capital strength, thus ensuring that project results are effectively transformed into productivity.

4.3 Introduce Capital Elements to Solve the Capital Problems Synergistically in the Process of Industrialization

From research to translation of dental nanomaterials, the whole process is very long (generally 3–10 years) with multiple risks such as technology and market. However, a lot of funds needs to be invested in the period of industrial production and market exploitation. Regrettably, most scientific research institutions are unable to independently bear this funding. In consequence, the capital shortage becomes a restrictive factor for the industrialization of dental nanomaterials. How to effectively introduce capital elements is the top priority. Taking tooth whitening in our team as an example, we have established a company with China National Pharmaceutical Group Corporation to jointly carry out the industrialization of tooth whitening materials. After the introduction of capital, it is possible to integrate the support of the government for technological innovation with the enterprises to effectively solve the funding problem and vigorously promote the process of industrialization.

4.4 Establish a Multidisciplinary Science and Technology Management Talent Team

The research and development of dental nanomaterials is an emerging industry that covers multiple disciplines including dental medicine, chemistry, biology and materials science. At the same time, the industrialization is also inseparable from talents in operations, management and scientific research. The establishment of a multidisciplinary research and management team is the guarantee for the success of industrialization [39]. If the operator were not familiar with the technical field, the scientific research personnel and the operating personnel would often encounter friction due to the gap in understanding of technology and management, which would even affect the process of transformation of project results. Therefore, it is necessary to coordinate and give full play to the innovation ability of scientific research talents in the team, the management and balance ability of management personnel, as well as the operation ability of business personnel in marketing. Based on the above model, our project has formed a high-level talent team that includes humanity, science, engineering, medicine and other disciplines, which integrates talents from a variety of fields in scientific research, management and operation. Our team has a strong ability to tackle key problems and is committed to jointly carrying out the industrialization of photoresponsive ZnO for various applications in dental and other biomedical fields.

5 Conclusion

This chapter separately describes commercialized dental nanomaterials and dental nanomaterials with industrialization prospects, as well as analyzes the challenges and risks in the industrialization process. As referred to the current successfully commercialized nanomaterials, this chapter aims to provide some inspiration and enlightenment to dental researchers and enterprises on the transformation of nano structured materials in dental medicine. To better develop the industrialization of research innovation which should adapt to the current market industrialization, scientific researchers should have an understanding of the world's cutting-edge technologies and the relevant market, enhance communication with the research and development personnel of enterprises and seek the compatibility of existing technologies with the market to solve the difficulties of the market. It is necessary to give full play to the research expertise of scientists and the rich experience in business operations of enterprises, conduct research from the perspective of the market and attract more high-quality social capital, further expanding and strengthening the industrialization of scientific research results.

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Chapter 12
Bioceramic Dental Inserts Based on Calcium Phosphate Nano-particles

Djordje Veljović and Vesna Miletic

1 Introduction

Despite significant improvements in prevention, diagnosis and treatment, dental caries remains the most common noncommunicable disease worldwide, not only expensive to treat, but with possible complications that require hospitalization [1]. It has been estimated that a total of US$442 billion was global expenditure related to caries in 2010 and that 2.3 billion people and 560 million children were affected by this disease worldwide, according to 2015 Global Burden of Disease Study [1].

Dental caries leads to the loss of tooth substance (enamel and dentine) due to the detrimental action of metabolic acids produced by oral bacterial biofilm over time (Fig. 1). Enamel contains 90–92 vol% (96 wt%) hydroxyapatite (HAp) mineral and the rest being organic proteins and water, while dentine contains about 40–50 vol% (70 wt%) HAp, 30 vol% (20 wt%) organic material (collagen) and 20–25 vol% (10 wt%) water [2, 3]. The loss of tooth substance *i.e.* demineralization is gradual and reversible only in its initial stages when no cavitation in enamel and dentine is present. Once the cavitation occurs, the process becomes irreversible in that tooth mineral cannot be produced to reverse the cavitation and restore the original shape, form and aesthetics of the tooth [4]. At this stage, an oral health professional must intervene and restore a carious tooth by choosing from a wide range of dental restorative materials.

For more than 150 years, dental amalgam has been the restorative material of choice. Disadvantages of dental amalgam mainly include extensive removal of healthy tooth substance for the desired cavity design. Health and environmental concerns related to mercury release from dental amalgam and the "Minamata

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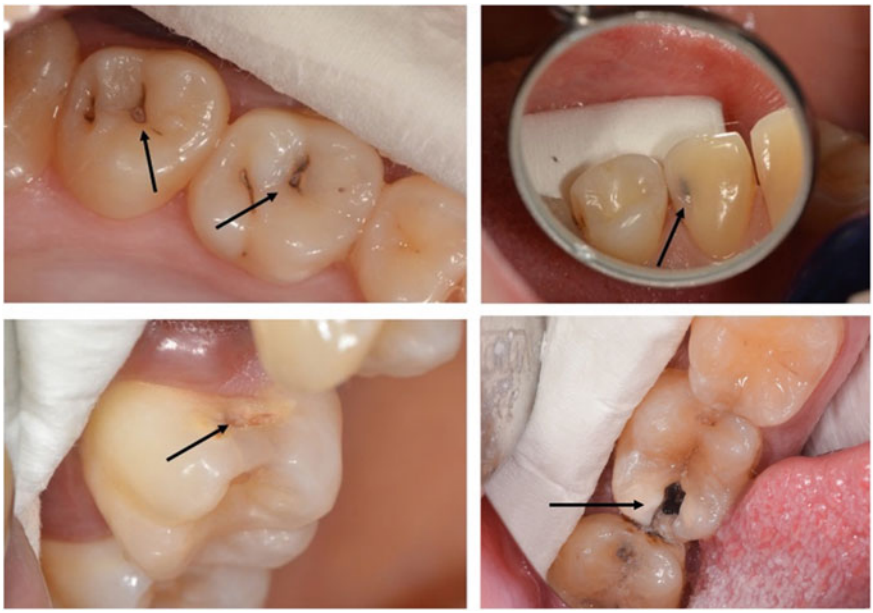

Fig. 1 Caries lesions in different stages

Convention", an international treaty signed in 2013,[1] have led to formal initiation of phasing-down amalgam as dental restorative material.

The process of phasing-down amalgam in dental practice has begun much earlier, with constant improvements of dental resin-based composites. Composites have become the materials of choice for direct anterior and posterior restorations over the past few decades. In addition to composites, glass-ionomer cements have been developed and improved since early 1970-ties for restoration of mainly root caries and lesions in high caries risk patients, not directly exposed to occlusal masticatory forces. A steady decrease in amalgam for posterior restorations has been reported over the past decade, from about 13% to less than 1% [5]. At the same time, dental composites have increased to 99.5% direct posterior restorations placed by both dental students and professionals [5]. Studies looking into clinical performance of dental amalgam and composites show similar survival rates and clinical efficiency [6]. Factors that significantly affect restoration survival are related to patient's age, number of restoration surfaces, molar and endodontically treated teeth, but not material (composite vs. amalgam) [6]. This means that composites can successfully replace amalgam even in large posterior restorations.

[1] https://treaties.un.org/Pages/ViewDetails.aspx?src=IND&mtdsg_no=XXVII-17&chapter=27&clang=_en

2 Current Material of Choice in Restorative Dentistry

Dental composites contain organic resin (continuous phase) in which inorganic filler particles are dispersed (dispersed phase) (Fig. 2). Resin matrix is a mixture of dimethacrylate monomers, whilst the filler phase contains barium/strontium glass, colloidal/fumed silica, zirconia or quartz particles. An organic–inorganic coupling agent (silane) is used to coat the filler particles. As a bifunctional molecule, silane forms strong covalent bonds with the filler particles and resin monomers, thus bonding the organic and inorganic phase and improving mechanical properties of dental composites [7].

There is no universal classification of dental composites which comprise a range of formulations for a variety of clinical indications. Composites are classified according to various parameters, e.g. (1) filler size to macrofill (10–50 μm), microfill (40–50 nm) and hybrid (~1 μm + 40 nm) [8]; (2) filler volume content to ultra-low fill (<50 vol%), low-fill (50–74 vol%) and compact (≥74 vol%) [9]; (3) consistency (viscosity) to flowable and sculptable or (4) recommended increment thickness to incremental (~2 mm) to bulk-fill (~4 mm).

Filler volume content was found to be a better indicator of mechanical properties of dental composites than filler size [9]. This influences the choice of composites for certain clinical indications, e.g. compact composites are recommended for complex, cusp-capping restorations instead of ultra low-fill or low-fill due to better mechanical properties, higher elastic modulus, flexural strength and modulus, hardness, fracture resistance. Conversely, ultra low-fill composites would be a better choice than compact for cervical abfraction lesions due to lower modulus elasticity to match tooth deformations in this region and lack of occlusal forces.

Flowable composites are generally considered to have inferior mechanical properties than sculptable due to lower filler content. While this is true for some flowable composites in which lower viscosity is achieved by reducing the filler content, in others high filler content is maintained and low viscosity is achieved by adding modifying agents (surfactants) [8]. The latter widens the range of clinical indications for flowable composites in posterior teeth, in addition to their most common use as liners under sculptable composites.

One of the most important recommendations for proper composite placement is adherence to recommended increment thickness. This allows optimal depth of cure [10, 11], i.e. consistent polymerization throughout increment thickness. It is widely

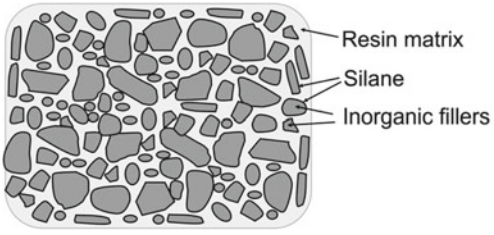

Fig. 2 Schematic presentation of dental composites

known that the quality of polymerization significantly affects a wide range of material properties and restoration longevity.

Incremental technique of composite placement was widely adopted in early 1990-ties [12] and is still used for composite materials recommended in 2 mm-thick increments. This technique comprises placement of multiple composite increments, each individually adapted against cavity walls, sculpted and light-cured, until the entire cavity is filled. While this approach demands relatively short clinical time in small cavities requiring 1–2 increments, incremental technique is much more time-consuming in large cavities, involving 3 or more tooth surfaces.

Bulk-fill composites were developed and marketed in early 2010s, in efforts to reduce the number of increments in posterior restorations, and hence shorten clinical working time [13]. These materials are recommended in 4–5 mm thick increments. Bulk-fill composites come in 2 viscosities, flowable and sculptable. Flowable bulk-fills are generally recommended as base materials to be covered by a top layer of a sculptable incremental or bulk-fill composite. Sculptable bulk-fill materials are recommended for restoring the entire cavity with the exception of cusp capping, due to better mechanical properties than flowables but still inferior to highly filled incremental nano-hybrid composites [14]. A cavity up to 4–5 mm which does not exceed the diameter of the light-curing tip may be restored in a single increment using sculptable bulk-fill composites. Bulk-fill composites have comparable clinical efficiency as incremental composites in clinical trials [15].

The previous sections illustrate that dental composites are a heterogeneous group of materials and that there is no “one size fits all” approach. Not even a single “class” of composites has a clear advantage over others for the same indications (e.g. incremental vs. bulk-fill, microhybrid vs. nanohybrid, compact flowable vs. low-fill sculptable etc.).

3 Dental Inserts

Dental inserts are prefabricated glass–ceramic or leucite-reinforced ceramic fragments of different size, shape and colour for restoring occlusal and occluso-proximal cavities in posterior teeth. Inserts were introduced in the 1990-ties with an idea to reduce the amount of polymerizable resin composite in the cavity in order to limit the polymerization shrinkage, the associated stress and coefficient of thermal expansion [16].

Inserts are designed to be placed inside the unpolymerized composite resin, thereby reducing the amount of resin in the restoration. They may come with compatible burs to match tooth reduction to insert size or may be used without specific burs in which case the amount of tooth reduction and resin composites may be greater. Examples of bur-insert systems are SonicSys inserts (Vivadent)-SonicSys Approx/SONICflex (Kavo) and Cerana (Nordiska Dental).

There is limited research on ceramic inserts as they were not widely received in clinical practice. An eight-year prospective clinical trial showed that 23 out

of 25 ceramic inserts were retained, clinically acceptable and in function, indicating an acceptable alternative for single-visit aesthetic restorations [17]. An in vitro study showed lower gingival microleakage scores after thermal cycling for leucite-reinforced Cerana than Beta-Quartz glass–ceramic inserts and resin-based composite control [18]. Similarly, lower enamel gap formation was reported for leucite-reinforced Cerana and SonicSys but not Beta-Quartz glass–ceramic inserts [19]. In terms of proximal contacts, Cerana inserts performed similar to dental composites [20]. Leucite-reinforced SonicSys and Beta-Quartz glass–ceramic inserts were found to have significantly worse bonding to the surrounding composite and increased dentine gap formation after thermal cycling [19].

4 Bioactive Dental Restorative Materials

A new trend of restorative materials has emerged—"bioactive" restorative materials, which does not only include filling materials, but also adhesives, and cements. Bioactive restoratives have been described as materials that, "while restoring the damaged structures, exert a biological effect on their tissues and surroundings with which they are in contact" [21]. Potential forms of "bioactivity" of restorative materials include the formation of reparative tissue or biological-like calcium phosphates, antimicrobial activity and cell attachment. In terms of restorative dentistry goals, bioactive materials would be expected to produce apatite-like crystals to close the interfacial gaps, seal restoration margins and remineralize partially demineralized enamel and dentine.

Taken together, the main expectations of modern restorative materials are to provide quick and easy direct, mechanically and aesthetically stable restorations with positive bioactive properties.

Bioactive dental restorative materials are produced by adding bioactive glass, calcium phosphates or silicates as particles in the filler content [22]. Since bioactivity implicates material-environment interaction to produce a certain biological/chemical effect, developing dental restoratives with this property requires ion release from the material. This may influence a diverse range of material properties.

Adding ion-releasing fillers to dental composites requires a careful selection of particle type and balance within the material composition. For example, an early commercial self-adhesive restorative material claimed to have bioactive properties, inducing HAp formation and contributing to interfacial gap closure by release and recharge of significant amounts of calcium, phosphate and fluoride was Activa (Pulpdent). A clinical trial was stopped after only a year due to unacceptably high failure rate of 24% with the main reasons for failure being restoration loss, postoperative sensitivity and secondary caries [23]. This led to the changes in the manufacturer's instruction of use, now requiring an adhesive layer prior to the placement of Activa.

Another clinical trial associated higher postoperative sensitivity with another potentially bioactive composite Cention N (Ivoclar Vivadent) compared to Activa over a month post-restoration [24]. Containing patented alkaline fillers, Cention N

is claimed to buffer the environmental pH by increased release of hydroxide ions, thus preventing demineralization of tooth tissues. Fluoride and calcium ion release from Cention N was shown to lead to the formation of small amount of apatite in orthophosphate-containing artificial saliva [25].

Basic research shows that bioactive glass, calcium phosphate or HAp fillers may affect the quality of polymerization [26], mechanical properties of dental composite mixtures initially and after degradation [27]. This may be due to lower mechanical strength of these particles compared to conventional glass/silica/zirconia fillers, water dissolution and/or changes in refractive indices between the filler and resin phase. Bioactive particle type and amount as well as resin matrix have a significant effect on the quality of polymerization and mechanical properties of composite formulations [26, 27]. Furthermore, functionalization of bioactive particles may affect their properties. For example, ion substitution of CaO in the original composition of bioactive glass with ZnO or SrO increased and CaF_2 reduced the refractive index, thus modifying it to match that of organic resin matrix [25].

Bioactive fillers in dental adhesives could improve a range of properties, hardness elastic modulus, radiopacity, degree of conversion, as well as bond strength. Possible mechanisms of action are ascribed to remineralization of adhesive-dentine interface, through the formation of a HAp-like layer around bioactive fillers serving as nucleation sites [28]. Calcium-fluoride fillers contribute to antibacterial and acid resistant properties of CaF_2—containing adhesives. The presence of fluoride promotes the formation of fluorapatite from calcium and phosphate ion precipitates, which is known to be more acid resistant than HAp, naturally present in tooth mineral [28].

HAp filler-containing adhesives may improve bond strength to dentine albeit this effect was found to be dependent on the nanoparticle shape and content [29]. A commercial universal adhesive with incorporated HAp nano-sticks showed higher shear bond strength to dentine after phosphoric acid etching (total-etch protocol) compared to nano-rods (50–100 nm) in concentrations of 0.5–1.0 wt% but not 1.5 wt% [29].

5 Calcium Phosphate Bioceramics: From Nano-particles to Functional Sintered Materials

Bioceramic and biocomposite materials intended to replace damaged parts of bone and tooth tissues, which were used also in some cases for therapeutic and diagnostic purposes, were developed continuously in recent decades and the level of their sophistication increased with the development of the biomaterial science. One of the main goals of the biomaterial science is to obtain materials that are similar to natural tissues in terms of microstructure, chemical composition, physicochemical and mechanical properties, before and/or after incorporation into the organism. The development of bioceramic materials in modern research implies a high level of multidisciplinarity, which, in addition to the development of materials science and medicine, implies

the development of chemistry and chemical engineering, physics and biophysics, bioengineering, biomechanics, nanotechnology, etc. Bioceramic materials based on calcium hydroxyapatite and calcium phosphate, due to their bioactive properties, chemical composition, excellent biocompatibility, structural similarity to the bone and tooth minerals, osteoconductivity and osteoinductivity, stimulation of osseointegration processes, have a very important role during the processing of implants for application in dentistry, maxillofacial surgery and tissue engineering [30–34].

Since HAp, the main inorganic phase of bones and teeth, gives satisfactory mechanical properties to human hard tissue and provides necessary stability and appropriate function of skeleton, the logical first step for the processing of nanostructured bioceramic materials for hard tissue regeneration and reparation is the synthesis of HAp nanoparticles of different forms and shapes [33–39]. Chemical formula of HAp, $Ca_{10}(PO_4)_6(OH)_2$, predicts the precursor composition for synthesis of HAp, but nonetheless to a large extent particle size depends on the processing conditions such as pH, temperature, atmosphere and ambient pressure, duration of the reaction and aging, concentration and purity of the precursors, rate of the reactants addition to the reaction vessel, etc. Different modifications of chemical precipitations, hydrothermal methods, sol–gel techniques, modified precipitation methods in the presence of urea, glycine, various carboxylic acids, controlled calcination and suitable treatment of biological waste (shells, bones, egg shells), spray pyrolysis, application of microwaves and ultrasound etc., were used to synthesize nano-size particles of HAp, as starting materials for the processing of dense and control porous bioceramics [33, 35–40]. The properties of HAp powders, obtained by different techniques, such as crystal size, particle shape and size, agglomerate size distribution, type of agglomerates, phase composition, Ca/P ratio, specific surface area, elemental composition, presence of dopants in the structure, etc., determined the possibility of their application in the processing of bioceramic and biocomposite materials for hard tissue replacement [32, 38, 41–43]. Successfully sintered bioceramic and biocomposite dental inserts found in the literature, based on calcium phosphates with adequate mechanical properties, were processed starting from nanostructured HAp synthesized mainly by precipitation and hydrothermal methods [44–48].

Calcium hydroxyapatite powders in large number of cases were obtained by relatively simple precipitation methods from aqueous solutions. The theoretical Ca/P mass ratio in the stoichiometric calcium hydroxyapatite is 2.151, while the Ca/P molar ratio is 1.67. In a number of cases the calculation of precursors for HAp synthesis was based on the theoretical Ca/P ratio, but only in some cases the powder with stoichiometric Ca/P ratio were obtained. In other cases, calcium-deficient HAp powders were synthesized, with the Ca/P molar ratio less than 1.67 [36–38, 41–43]. The reasons for obtaining calcium-deficient hydroxyapatites, although the stoichiometric Ca/P ratio was in the precursor solution, could be in relation to different variation of parameters during synthesis. In addition to the initial stoichiometric Ca/P ratio, the route of the reaction, nucleation and formation of HAp particles are influenced by a number of parameters such as temperature, initial pH value and its change during reaction, starting concentrations and forms of calcium and phosphorus, the rate of reactants addition, time and temperature of aging of the obtained precipitate,

etc. [49, 50]. It is necessary in some cases to establish an inert atmosphere in the reaction vessel in order to avoid the absorption of CO_2, which is present in the air and can be easily incorporated into the HAp structure. Carbonated type of hydroxyapatite can be spontaneously formed in these instances. Carbonated hydroxyapatite is present in natural bone tissue, but if the goal is to obtain a stoichiometric HAp powder, the substitution of PO_4^{3-} in the structure of hydroxyapatite with CO_3^{2-} must be prevented. Syntheses of hydroxyapatite from aqueous solutions start mainly at the pH values around 10–11. The pH value continuously decreases during the synthesis of HAp due to the removal of OH^- ions from the solution during the precipitation of hydroxyapatite. At the same time the suspension of synthesized nano-particle aspires to the pH values which are a guarantee for the stability of the stoichiometric hydroxyapatite nanoparticles [36, 41, 50].

The aging of the precipitate after the reaction is desirable in order to increase the probability for obtaining stoichiometric HAp powder, but also during aging small crystals become larger and also increase the tendency of HAp nanosized particles for agglomeration. As a consequence of high surface energy and due to metastability of nanoparticles, the HAp nano-powders are often agglomerated without control. During the synthesis of HAp from aqueous solutions, spray-drying of the final suspension is the key step in order to control the agglomeration process toward the fabrication of soft agglomerated nano-particles, which was well known as a very important step in the processing of dense nano-structured bioceramic dental inserts [36, 41]. The relatively low crystallinity degree is characteristic for a large number of hydroxyapatite powders synthesized by various modified precipitation methods. During the processing of high-density dentine substitutes, low-crystalline HAp powders composed of soft agglomerated nano-particles with a stoichiometric Ca/P ratio are especially interesting as starting material, due to the possibility for processing of fine-grained and nanostructured monophasic hydroxyapatite inserts [41, 43, 45].

Hydroxyapatite powders obtained by hydrothermal processes have a high degree of crystallinity, clearly defined and often uniform crystal structure, in contrast to the powders obtained by precipitation methods. Hydrothermal syntheses are performed by treating the precursor solutions in autoclaves at a well-defined temperature and pressure. The hydrothermal synthesis can greatly influence the characteristics of the obtained powders by varying the previously mentioned parameters of synthesis. From 1960-ties onwards, hydroxyapatite powders composed of whiskers, rod-like and spherical particles, controlled agglomerated, from the nano to the micron levels in the size, were synthesized starting from different precursors as a source of calcium and phosphorus [44, 51–53]. During the processing of dentine substitutes, powders composed of spherically agglomerated nano-particles are of special importance, due to the possibility to obtain controlled porous microstructures via sintering, with spherical pores of micron dimensions which have a similar cross-section compared to dentinal tubules [44, 51, 54].

Bioceramic particles synthesized by the aforementioned synthesis methods, whether suspended in the liquid phase or in the form of dried or temperature-treated (calcinated) powder, can be consolidated by various methods in order to bring them

into intimate contact. The aims of consolidation are to provide the optimal green density, uniform microstructure, desired shape and dimensions of formed compact before sintering. Deagglomerated calcium phosphate nano-particles, or converted by optimal drying treatment into soft agglomerates, need to be consolidated into green bodies of high density, in order to obtain nanostructured bioceramic materials with optimal properties required for biomedical application, by sintering at relatively low temperatures. HAp nanoparticles that have a considerable tendency for agglomeration, must be transferred by pressing, molding from suspensions or molding from gels into an energy-efficient green compacts, where the particles are in intimate contact, which will allow sintering at the lowest possible temperatures and at the same time enabled full control of microstructural parameters [33].

Despite great possibilities for application of dense HAp-based bioceramic materials in medical practice, insufficient values of some mechanical parameters, especially under physiological conditions, limits the use of this material in some clinical applications. Various sintering techniques have been used to obtain calcium phosphate bioceramic materials with adequate mechanical properties, especially fracture toughness and hardness, for use as dentine substitutes—dental inserts. The main disadvantage of calcium phosphate bioceramic materials obtained at high temperatures is their brittleness, and fracture toughness similar to human dentine in the range of 1.1–2.0 $MPam^{1/2}$ is necessary for potential application of bioceramic material as dental insert [55]. In addition to the required mechanical properties which enable the application of sintered HAp based bioceramic material as substitute for dentine, and also appropriate biocompatibility, it is also necessary to examine the shear bond strength of the insert with different restorative composite materials and dental adhesives, by applying of different clinical protocols in order to optimize and simplify clinical procedure during restoration of large cavities.

Selected calcium phosphate bioceramic materials sintered by different techniques, which can be potential dentine substitutes due to the fracture toughness and hardness values, are shown in Table 1. Controlled grain growth during the final stage of the temperature treatment, is a consequence of the optimized synthesis of HAp nano-particulate powder, green body properties and sintering parameters. Prominent sintering techniques are listed in the table, i.e. single-step and two-step conventional sintering, microwave sintering, spark plasma sintering and hot pressing [41, 44, 45, 50, 56–58]. By limiting the grain growth at the nano level, similar structure of implants as perfect natural hard tissue can be achieved, which means structural similarity with the inorganic phase of natural bones and teeth. The nanostructure of the bone implants provides a similar environment as natural bones to the surrounding human cells, which were attached to the surface of bioceramic implants.

Following this concept, various nanostructured bioceramic materials based on calcium hydroxyapatite and tricalcium phosphate as precursors of nano-crystalline hydroxyapatite have found specific applications in orthopaedic surgery and dentistry, as well as in tissue engineering and various fields of regenerative medicine, as solo implants or parts of complex systems. The presence of nano-sized grains or particles in bioceramic implants results improves fracture toughness, hardness and compressive strength of bioceramics, as well as biocompatibility, cell proliferation, spreading

Table 1 The processing parameters and mechanical properties of calcium phosphate bioceramic materials sintered by various techniques

Sintering method	Temperature; time	Applied pressure (added additive)	Grain size, nm	Density, g/cm^3	Vickers hardness (*HV*), GPa	Fracture toughness, MPam$^{1/2}$
Conventional sintering [50]	850 °C; 24 h	300 MPa	67	3.13	5.0[a]	1.06
Conventional sintering [44]	1200 °C; 2 h	400 MPa	≈1000	2.64[b]	3.05	1.30
Conventional two-step sintering [56]	1050 °C; 20 h	400 MPa	375	3.07	4.9	1.11
Conventional two-step sintering [45]	980 °C; 24 h	400 MPa	1400	3.11	5.22	1.02
Hot pressing [41]	950 °C; 2 h	20 MPa	50	2.93	4.30	1.52
Spark plasma sintering [57]	1100 °C; 5 min	60 MPa	≈550	3.00	7[b]	1.25
Microwave sintering [58]	1000 °C; 20 min	345 MPa, NH_4PMA	168	3.08	8.40[a]	1.90
Microwave two-step sintering [45]	850 °C; 10 min	400 MPa	78	3.06	5.00	1.58

[a]Knoop hardness, [b]Controlled porous material

and their attachment [58, 59]. Limiting grain growth to the nano-scale during the processing of different nano-crystalline bioceramic materials based on calcium phosphate, due to its approved biocompatibility and osteoconduction, chemical similarity and possibility for bonding with the inorganic part of hard tissue and also its evident osteogenic potential, can be one of key steps in regeneration and reparation of bones and teeth, and also during the formation of new hard tissue in vivo and in bioreactors.

Modern sintering techniques enabled a significant reduction of temperature and shorter sintering time. Table 1 shows that the average grain size of sintered bioceramic materials ranged from nano- to sub-micron levels. Lower sintering temperatures, in addition to reducing grain size, affect the change in the fracture mechanism from trans-granular to intergranular in the case of HAp based bioceramic materials. In the case of inter-granular fracture, the main contribution to higher resistance to crack propagation is provided by the grain boundaries, and a significantly larger number of grain boundaries are in the crack pathway in the case of nanostructured material in comparison to sub-micron one. Crack propagation in the case of intergranular fracture type implies crack pathway along the grain boundaries, whereby the crack in a large number of places in nanostructured material loses energy, which

reduces its length and improves fracture toughness. Increasing hardness of bioceramic materials with decreasing grain size in dense bioceramic is in compliance with the expectations derived from the Hall–Petch equation, which precisely indicates the possibility of increasing the hardness of ceramic materials with decreasing grain size [42, 50, 59]. Recent calcium phosphate materials processed using different sintering concepts, cover practically the entire range of fracture toughness for human dentine and also have adequate hardness values. Based on these properties, calcium phosphate materials are potential candidates for dentine replacement in the form of dental inserts.

In addition to the grain size control, it has been shown that it is possible to achieve adequate mechanical properties of calcium phosphate bioceramic materials by controlling the shape and size of pores. Calcium phosphate materials can be used in biomedical practice in the form of micro-porous and macro-porous sintered forms. Pores in the bioceramic material enhance the quality of tissue-material bond. While macro-porosity has a great influence on vascularization, osteoconductivity and contact of biological fluids, cells and tissues to the implant surface, micro-porosity has an effect on cell adhesion, and partially controls the rate of calcium phosphate resorption. Sub-micron porosity with defined shape and size of pores can affect the mechanical properties of bioceramic materials [44, 51, 54, 60, 61].

Aforementioned dense and controlled porous bioceramic HAp-based materials could be used as bone replacements in the maxillofacial region, for different purposes in dental practice and also in orthopaedic surgery. In dental practice dense and controlled porous HAp-based bioceramic materials have been used as an immediate tooth root replacement to minimize alveolar ridge resorption and to maintain the dimensions of the ridge, and also can be used as dental inserts to reduce polymerization shrinkage and improve the stability and mechanical properties of the insert-containing restoration in large cavities [46–48].

6 Bioactive Dental Inserts

The idea behind bioactive dental inserts is to combine bioactive potential of inserts (compacts) produced from apatite-based particles with aesthetic restorative materials. Irrespective of the material, prefabricated inserts reduce the amount of uncured resin composite, thereby controlling polymerization shrinkage and stress. Based solely on apatite particles, inserts would mimic high mineral content of tooth tissues with controllable and tailored properties.

HAp inserts were first developed for dental application by Lezaja et al. in 2015 [46]. Table 1 shows the properties of conventionally sintered controlled porous material [44, 46], which was the first calcium phosphate-based material tested as dentine substitute, i.e. dental insert of regular cylindrical shape. Standardized inserts were 1.6 mm thick and 4 mm in diameter.

Controlled porous material used in this study was obtained starting from spherically agglomerated nanoparticles of carbonate-substituted HAp nano-particles 50–100 nm in length. Crystallite size calculated using the Scherer equation was 50 nm. The specific surface area of mesoporous HAp powder determined by the BET method was 39 m^2/g. In the centre of spherical agglomerates were spherical intraagglomerate pores, whose role was of key importance for the formation of controlled porous bioceramic materials with adequate mechanical properties. Hydrothermal synthesis of used HAp powder has been described in the literature in details [35, 44, 51]. Starting from the mentioned HAp particles, phase composition and porosity were investigated, as well as the influence of pore shape and size on the mechanical properties of bioceramic materials, processed by conventional and microwave sintering. After the spherical structure of HAp agglomerates was confirmed to be stable, despite the application of relatively high pressure, the initial idea was to obtain controlled porous materials. Maximum pore size would be determined by the dimension of spherical intraagglomerate pores, while irregularly shaped interaglomerate pores would become spherical as their dimensions decreased during sintering [44].

Applying this concept, Veljovic et al. processed bioceramic materials of controlled porosity by microwave and conventional sintering of carbonate-substituted HAp powders with different Ca/P ratio composed of hard spherically agglomerated nanoparticles [44, 51]. The presence of HPO_4^{2-} and CO_3^{2-} ions in HAp crystal structure instead of PO_4^{3-}, which was confirmed by Fourier-transform infrared spectroscopy, decreased Ca/P ratio and affected deviation from HAp stoichiometric composition. As a consequence of this ion change in the crystalline structure, a lower amount of Ca^{2+} ions in the HAp structure was needed to compensate the negative charge of the crystal lattice, and the powders had a calcium-deficient Ca/P molar ratio. Calcium-deficient hydroxyapatite powders (CDHAp) started partial phase transformation into β-tricalcium phosphate (β-TCp) already in the initial sintering stage, followed by phase transformation into α-TCp as a high-temperature phase at temperatures above 1100 °C, according to the following equations:

$$\text{CDHAp} \rightarrow Ca_{10}(PO_4)_6(OH)_2 + \beta\text{-}Ca_3(PO_4)_2$$
$$\beta\text{-}Ca_3(PO_4)_2 \rightarrow \alpha\text{-}Ca_3(PO_4)_2$$

The main advantage of β-TCp compared to HAp is higher solubility and bioactivity, while α-TCp has even more pronounced bioactivity. Veljovic et al. showed by sintering of spherically agglomerated HAp powders with different levels of calcium-deficiency, that the phase composition has an inferior effect on the fracture toughness of the obtained controlled porous bioceramics in comparison to their specific microstructure with spherical micron-sized pores and strong necks established between spherical agglomerates. Controlled porous bioceramic material with the dominant HAp phase was obtained by conventional sintering at 1200 °C for 2 h, starting from green compacts obtained by isostatic pressing at 400 MPa, achieving density of 2.64 g/cm^3, hardness 3.05 GPa and fracture toughness 1.30 $MPam^{1/2}$. In addition to HAp as the dominant phase, α- and β-tricalcium phosphates were

detected by x-ray diffraction analysis. Fracture toughness of sintered controlled porous bioceramic materials obtained in the mentioned studies were higher compared to the values available in the literature for sintered dense and porous materials based on calcium phosphates, with the pores of micron- and nano-levels [50, 56, 57]. The authors confirmed by scanning electron microscopy (SEM) the assumption that crack bypasses spherical intraagglomerate pores, encountering strong necks formed between resistant spherical agglomerates. This finding supports the theory that the presence of spherical pores contributes to greater fracture toughness of controlled porous bioceramic materials. In the same study, the finite element method confirmed that the presence of spherical pores in the controlled porous microstructure is one of the preconditions for good mechanical properties of bioceramic materials of controlled porosity. In short, if micron-sized pores are unavoidable in bioceramic materials, spherical shape would be ideal. Lezaja et al. examined the potential application as dental inserts of the above controlled porous material based on hydroxyapatite, α- and β-tricalcium phosphate (Fig. 3) [46]. SEM micrographs of insert surfaces after etching with 37% phosphoric acid revealed spherical pores around one micron in diameter.

Bond strength between the prefabricated inserts and a range of restorative materials was tested: universal, microhybrid Filtek Z250 composite (3 M), flowable composite Filtek Supreme Flowable (3 M) and resin-modified glass-ionomer cement Vitrebond (3 M). A universal adhesive (Single Bond Universal, 3 M) was used for composite build-ups according to either total-etch or self-etch protocol. The highest shear bond strength between the inserts and restorative materials was observed for the total-etch adhesive protocol and universal composite (range *cca.* 10–14 MPa) whilst

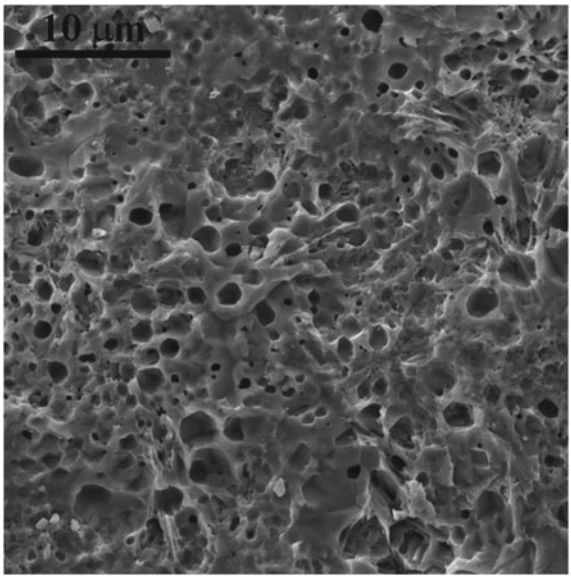

Fig. 3 SEM micrograph of acid-etched surface of a controlled porous calcium phosphate dental insert

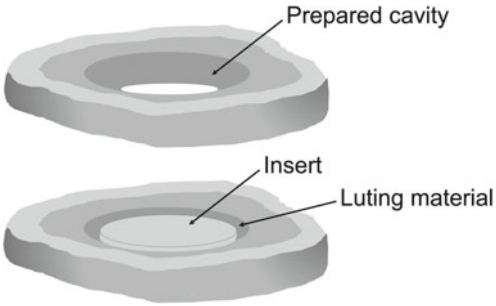

Fig. 4 Schematic illustration of insert-containing restoration in a simulated cavity (5 mm in diameter and 1.6 mm deep) in standardized dentine disks. Insert dimensions: 4 mm in diameter and 1.6 mm deep. Cavity diameter was 1 mm wider than the insert diameter to create space for luting material

the lowest bond strength was found in groups with no adhesive present, Vitrebond, flowable and universal composite (below 3 MPa). Insert etching for 15 s with 37% phosphoric acid in the total-etch protocol improved bonding compared to un-etched surface in the self-etch protocol by superficial demineralization and increased pore diameter to 3–4 μm.

The same article [46] investigated the shrinkage of insert-containing restorations compared to control composite restorations in simulated cavities in standardized dentine disks (Fig. 4).

Digital image correlation, based on a 2-camera system (Aramis 2 M, GOM), was used to visualize and quantify strain and displacement during light polymerization of the restorative material (Filtek Z250, Filtek Supreme Flowable or Vitrebond), i.e. polymerization shrinkage. HAP inserts reduced polymerization shrinkage of the insert-containing restorations to below 0.5% in central parts of the restoration and acted as a possible central stress reducer. Shrinkage vectors changed with the addition of inserts in that the restorative material displaced vertically in unrestricted direction as opposed to the displacement in the restricted direction without inserts (Fig. 5). This could reduce stress development due to the elimination of opposing forces.

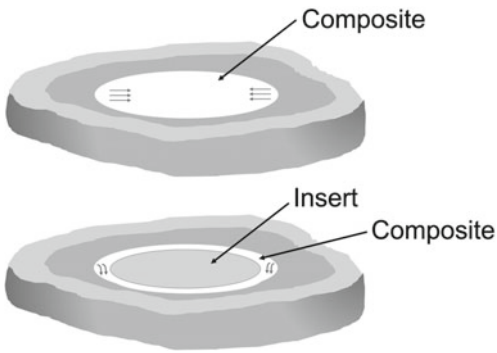

Fig. 5 Shrinkage vectors with and without an insert in a Class I cavity

The ratio of bonded and unbonded surfaces in a Class I cavity clinically was calculated to be lower in the insert-containing restoration compared to composite restoration without an insert. In this particular situation, 'C' factor with an insert was calculated to be 1.65 compared to the 'C' factor without an insert (2.28). Significantly greater shrinkage of the flowable (2.1–3.4%) than universal composite (*circa* 1.3%) suggests that the latter would be a preferred material in insert-containing restorations [46].

Given that the composite is expected to fill the gaps between an insert and cavity walls, excellent adaptability of a universal composite would be required. It is warranted to further investigate the effect of pre-heating composite on material adaptability, shrinkage and degree of conversion in insert-containing restorations. Pre-heating dental composites is known to significantly reduce viscosity and increase flow, allowing better adaptability [62].

In addition to investigating the mechanical characteristics of insert-containing dental restorations, aesthetics was tested in a series of experiments measuring optical properties initially and following staining [63, 64]. HAp inserts are completely non-transparent and intensely white due to the nature of hydroxyapatite particles. This could have a negative effect on overall restoration aesthetics.

Marjanovic et al. [63] investigated optical properties of 'sandwich' restorations containing un-aesthetic dentine replacement materials (HAp inserts, calcium silicate cement Biodentine, Septodont and fiber-reinforced bulk fill composite everX Posterior, GC) in relation to control composite restorations. In this study, 2 mm-thick dentine samples (insert, Biodentine, everX, and control universal composite) were covered with enamel shades of the two control composites in two shades (A1 and A3) and three thicknesses (0.6, 1 and 2 mm). Control composites were universal nanohybrid Filtek Z550 (3 M) and microhybrids Filtek Z250 (3 M) and Gradia (GC) which differ in the presence/absence of BisGMA, respectively. Colour and translucency were measured using a clinical spectrophotometer. Colour was significantly affected by the type of dentine replacement materials as well as the shade and thickness of the 'enamel' layer. HAp inserts and Biodentine affected both colour and translucency of the final restoration with increasingly adverse effects associated with thinner 'enamel' layer. The effect on restoration translucency was especially pronounced. While the composite alone showed translucency parameter between 10 and 30, depending on the shade and thickness, both inserts and Biodentine resulted in highly opaque 'sandwich' restorations with translucency parameter below 2. Similar decrease, albeit to a lesser extent, was seen in control specimens with composite 'dentine' and 'enamel' layers. Somewhat higher translucency parameter (2.5–10) was found in everX-containing specimens, likely due to the increased light transmittance by glass fibers.

It was found that generally a 2 mm-thick layer of universal microhybrid or nanohybrid composite could counteract the unfavourable aesthetics of HAp inserts and calcium silicate cement Biodentine, resulting in a final 'sandwich' restoration that is aesthetically comparable to a control composite restoration. As for the fiber-reinforced everX, increment thickness of the 'enamel' layer could be reduced to 1 mm without an adverse effect on colour, but with generally higher translucency

than control composite restorations. The effect on translucency may not be clinically as important in posterior restorations as in anterior unless there is severe discoloration of dental tissues due to the presence of caries or old metallic fillings.

Miletic et al. [64] investigated the effect of wine staining on color differences of bulk-fill and universal composite restorations with dissimilar dentine replacements. Dissimilar dentine replacement materials refer to the materials with substantially different optical properties than universal composite materials. HAp inserts, calcium silicate cement Biodentine and fiber-reinforced bulk fill composite everX Posterior were used to restore 4 mm-deep Class I cavities in extracted teeth. The cavities were modified in that the cusps were cut off and teeth were sectioned 1 mm below the bottom of the cavity to allow standardization. Bottom 2 mm of each cavity were restored either with a HAp insert, Biodentine or everX whilst the top 2 mm were restored with a universal composite Gradia, Filtek Z250 or Filtek Z550. Additional test groups were prepared using bulk-fill composites, Filtek Bulk Fill (3 M) or Tetric EvoCeram Bulk Fill (Ivoclar Vivadent). Colour measurements of the whole restoration were performed before and after staining in red wine for 48 h at 37 °C. Decrease in lightness, ΔL, in the insert-containing group was between 2.6 and 5.7 after staining which was generally lower than for Biodentine and everX groups (5.4–9.0 range). While ΔE_{00} was in the range of 3.5 and 9.0 for composite_Biodentine, composite_everX or control composite groups, ΔE_{00} of Filtek Z250_insert combination was significantly lower (1.5–3.0 range). This combination seems the most favourable after wine staining. Pooled ΔE_{00} showed that HAp inserts and Biodentine resulted in lower ΔE_{00} of the restoration observed as a whole after wine staining compared to everX posterior. Inserts generally resulted in the overall ΔE_{00} of the restoration in the range of 2.0–6.0 and the control composite showed ΔE_{00} in the range of 5.0–6.0. These changes were all above the CIEDE2000 50:50 perceptibility and acceptability thresholds of 0.8 and 1.8, respectively presenting variable levels of colour mismatch [65].

With respect to the applied protocols and the conclusions reached by Lezaja et al., Ayoub et al. examined the possibilities for application of monophasic dense HAp inserts for dentine replacement [47]. The potential application of two-step sintered HAp-based dental inserts with density close to the theoretical value for HAp was investigated using different protocols. The inserts used in this study were obtained from HAp powder with a stoichiometric Ca/P ratio synthesized by the precipitation method, composed of controlled agglomerated hydroxyapatite nanoparticles 50–100 nm in the length and of crystallite size of approximately 35 nm, calculated using the Scherer equation. Controlled obtaining of soft agglomerates was performed by the spray-dry method, and the specific surface area of the obtained mesoporous powder, determined by the BET method, was 59 m^2/g, which was significantly higher than for previously mentioned hydrothermally synthesized HAp powder. The precipitation method for synthesis of used HAp powder has been described in the literature in details [36, 41]. Nano-dimensions of particles and the soft nature of the agglomerates enabled sufficiently intimate contact between the particles and relatively high green density reached by isostatic pressing at 400 MPa, which allowed full densification by two-step sintering.

Two-step sintering, as a concept which enables control of grain growth at the nano-level, implies long holding time at the final sintering temperature in conventional furnaces. During the first step, the green compacts are first heated to a higher temperature in order to achieve the activation energy necessary for densification. After a short break at the temperature of the first step, the temperature decreases rapidly to a lower value, but is sufficient to allow complete densification in the second step over a relatively long period, in order to limit the grain growth to the desired level. In order to examine the possibility of two-step sintering to obtain dense monophasic bioceramic materials, Veljović et al. obtained optimal microstructure by heating green compacts from room temperature to 1075 °C with a heating rate of 20 °C/min, by holding in the first step for 15 min and then cooling to 980 °C and holding at the second step for the next 24 h. Two-step sintered HAp was used to produce cylindrical inserts. Full dense microstructure after etching with 37% phosphoric acid can be seen in the Fig. 6 [45].

SEM micrograph shows etched grain boundaries at the surface and further acid-treated inserts were used in the total-etch adhesive application protocol. The phase transformation of HAp to TCp was avoided during the sintering, due to the stoichiometric Ca/P ratio in the initial HAp powder and the relatively low temperature of the second step during the final temperature treatment. Fully dense two-step sintered monophasic HAp inserts were composed of grain size from 200 nm to 1.8 μm, had a significantly higher hardness value of 5.22 GPa compared to previously used controlled porous biphasic bioceramic inserts. This was a direct consequence of the higher density and absence of phase transformation of HAp into TCp. Hardness of monophasic HAp inserts was higher than hardness of human enamel and

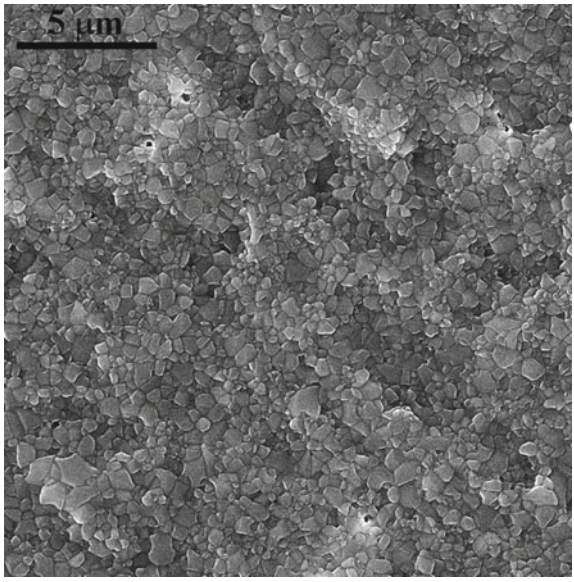

Fig. 6 SEM micrograph of acid-etched surface of a two-step sintered HAp dental insert

certainly higher compared to human dentine, and fracture toughness was close to values for dentine [45, 48].

In addition to optimizing grain size, porosity, phase composition, fracture toughness and hardness of dense HAp-based inserts, strong insert-restorative material bonding is crucial for dental application. Shear bond strength between the monophasic two-step sintered HAp inserts and a range of restorative materials were tested: universal, microhybrid Filtek Z250 composite (3 M), auto-cured resin-modified glass-ionomer cement GC Fuji VIII (Fuji VIII) and self-adhesive resin luting cement Maxcem Elite (Maxcem). A universal adhesive (Single Bond Universal, 3 M) was used according to either total-etch or self-etch protocol. Z250 composite, bonded with Single Bond Universal adhesive in both total-etch and self-etch protocols, showed significantly higher bond strength compared to Maxcem and Fuji VIII. Phosphoric acid etching improved bond strength of Maxcem compared to Maxcem without etching, but the difference did not reach statistical significance. The highest shear bond strength between the inserts and restorative materials were observed for self-etch adhesive protocol and universal composite (mean value 18.51 MPa), and the lowest bond strength was found in group with resin-modified glass-ionomer cement GC Fuji VIII (mean value 4.50 MPa) [48].

A number of studies focused on strengthening bioceramic matrix with the addition of different nanoparticles of superior mechanical properties compared to hydroxyapatite and tricalcium phosphate. For that purpose, different composites based on HAp/TCp and different nanoparticles such as SiO_2, TiO_2, ZrO_2, Al_2O_3, carbon nanotubes, graphene, etc. were obtained [57, 60, 66–68]. However, during bioceramics strengthening two important aspects must be taken into account for dental application: colour and shear bond strength to commercial composites, cements and adhesives.

The possibilities for strengthening HAp-based bioceramics with the addition of yttrium stabilized zirconia, classified as no-cytotoxic bio-inert material, have been investigated in the literature. Due to its excellent mechanical properties, ZrO_2 is a frequently used material in dentistry, mainly as tooth crowns and dental implant material. The main disadvantages of application of ceramic materials based on zirconia in dentistry are its superior mechanical properties in relation to the human teeth. Initial cracks and further fractures can appear on the antagonist tooth over time. On the other hand, zirconia nano-particles as filler of polymer-based dental composites or as reinforcer in ceramic matrix can be of great benefit, yet without risk to antagonist teeth. Zirconia particles were often doped with yttrium due to the controlled phase transformation during the sintering of zirconia-based ceramics in the direction of the stabilization of mechanically superior tetragonal zirconia (t-ZrO_2) [47, 48].

Potential use of bioceramic dental inserts based on HAp and yttrium-stabilized t-ZrO_2 was investigated by Ayoub et al. [47, 48]. Bioceramic matrix in the mentioned study was reinforced with 20 wt.% of zirconia nanoparticles synthesized by the plasma method [69]. Needle-like HAp nanoparticles synthesized by precipitation and spherical nano-particles of zirconia after homogenization and formation of composite green compacts by isostatic pressing at 400 MPa, were sintered at higher temperatures compared to calcium phosphate inserts. HAp/t-ZrO_2 composite with optimal

mechanical properties was obtained by conventional sintering at 1300 °C for 2 h. Complete phase transformation of monoclinic (m-ZrO_2) to t-ZrO_2 was performed at the indicated temperature. On the other hand, incorporated stabilized zirconia nanoparticles significantly increased the fracture toughness of HAp-based inserts (1.85 $MPam^{1/2}$) compared to the previously mentioned calcium-phosphate bioceramic inserts. Fracture toughness was close to the upper limit for human dentine, while hardness of the obtained insert was 3.95 GPa.

SEM micrograph in Fig. 7 shows acid-etched surface of a HAp/t-ZrO_2 composite insert after etching with 37% phosphoric acid. Figure 7 confirms clearly a discernible effect of etching of HAp matrix, and uniform distribution of stable zirconia nanoparticles. Shear bond strength between HAp/t-ZrO_2 composite inserts and the following restorative materials was tested: universal, microhybrid Filtek Z250 composite (3 M), auto-cured resin-modified glass-ionomer cement GC Fuji VIII (Fuji VIII) and self-adhesive resin luting cement Maxcem Elite (Maxcem) [47, 48]. A universal adhesive (Single Bond Universal, 3 M) was used for composite build-ups according to either total-etch or self-etch protocol as in the case of monophasic two-step HAp based inserts. Adhesive bonding following both protocols yielded the promising adhesion after the application of Filtek Z250 composite, and the highest shear bond strength between the HAp/t-ZrO_2 inserts and restorative materials were observed in the case of self-etch adhesive protocol and universal composite (mean value 10.50 MPa). Dentine etching with phosphoric acid increased contact angles of universal adhesives, creating a surface less susceptible for adhesive uniform distribution compared to the self-etch protocol [70]. This could be the potential explanation for slightly higher shear bond strength associated with the self-etch protocol between inserts with dominant HAp crystalline phase and Filtek Z250 composite [48].

7 Conclusions and Future Perspectives

Bioactive particles hold great potential to improve dental restorative materials. The main characteristics of bioactivity are ion release/exchange with the oral environment, dentine and enamel remineralization and the formation of apatite-like crystals able to close interfacial gaps, thereby reducing the loss of tooth minerals and increasing the longevity of dental restorations. Successful future bioactive dental restorative materials will be a careful balance between bioactive particles and conventional resin composition. Generally, bioactive particles are added to dental restorative materials as a small part of the inorganic filler content to achieve bioactivity and maintain favourable material properties. Conventional inorganic filler particles are responsible for mechanical properties whilst bioactive glass, HAp or calcium phosphate particles add bioactivity to the restorative material.

Dental inserts in general decrease dimensional changes of the restoration by reducing the amount of uncured restorative material. Furthermore, prefabricated inserts may reduce the number of clinical steps in large cavities thus saving clinical

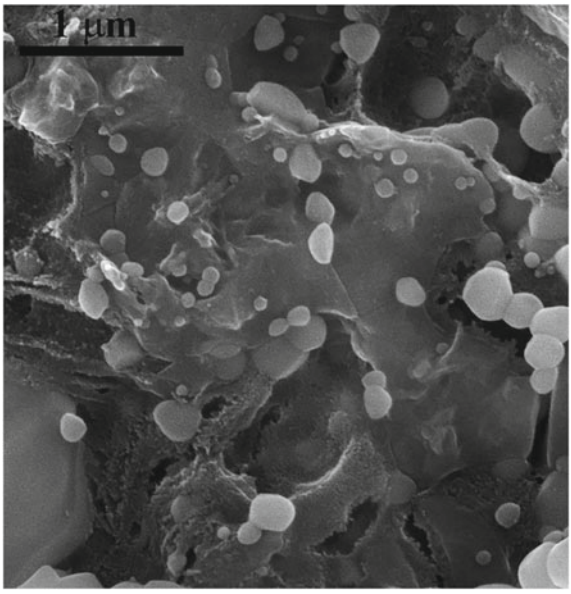

Fig. 7 SEM micrograph of acid-etched surface of a sintered HAp/t-ZrO_2 dental insert

time. Unlike bio-inert ceramic, HAp-based inserts exhibit bioactivity and may chemically interact with adhesives via functional groups. Apatite-based inserts were shown to reduce shrinkage of the restoration and change shrinkage vectors of universal composites. Total-etch or self-etch protocol of adhesive application may be used to achieve comparable bond strength between universal composites and apatite-based inserts. Despite unfavourable optical properties, HAp inserts may not have an adverse effect on restoration aesthetics despite its dissimilar properties to composites. A 2 mm-thick capping layer of a universal composite is required to produce a restoration with an overall aesthetics similar to that of an all-composite restoration. Colour differences after staining in an intense discolorant such as red wine are generally comparable in insert-containing and all-composite restorations.

Evolution of bioceramic materials based on calcium phosphates for insert processing will follow further sophistication of synthesis techniques and modern sintering methods, in the direction of nanostructured bioceramic and biocomposite dental inserts with optimal microstructure, improved mechanical properties and stronger adhesive bonding to the existing and new restorative materials. Optimization between HAp nano-particles and reinforcement nano-particles such as SiO_2, TiO_2, ZrO_2, Al_2O_3 can also improve the quality of insert-containing restorations. Incorporation of different cations instead of Ca^{2+} and anions instead of PO_4^{3-} i.e. doping of calcium phosphate nano-particles with ions present in human bone tissue, can also affect the control of microstructure and phase composition of newly formed inserts, in order to modify mechanical properties and quality of bonding with restorative materials. Generally, a better understanding of microstructural effects and chemistry

of bonding with restorative materials is necessary for further improvement of the mechanical properties of bioactive dental inserts and insert-containing restorations.

Future bioactive restoratives will likely be a heterogeneous group, suitable for a variety of indications, from minimal cavitations to large caries lesions endangering pulp integrity, from anterior teeth with highly aesthetic requirements to posterior teeth subjected to occlusal masticatory forces. Bioactive inserts tailored to match the physical characteristics of dentine and prefabricated for large cavities may simplify the placement procedure. If luted with bioactive restoratives, such inserts hold the potential for combined positive effects in efficient clinical working times.

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Chapter 13
Potential Hazards of Nanostructured Dental Materials

Sreelakshmi M. Ravindran

1 Introduction to Nanotoxicology

Ever since the introduction of the concept of nanotechnology by American Physicist Richard Feynman in 1959, this science has witnessed a period of great prosperity. Nanotechnology, also known as molecular nanotechnology or molecular engineering defines a technology that enables complete control of matter at nanoscale dimensions. Efficiency of the nanoparticle compared to its conventional counterpart, along with its global appreciation has led to a boom in their development. Nano revolution has seized the human life be it in health care, food sector, cosmetics, textiles, renewable energy, aerospace, and sports [1]. The growing interest in the application of engineered nanomaterials (ENM) in medicine and dentistry has opened new possibilities in diagnosis, treatment, and prevention of pathologic conditions. However, the drive for manufacturing nanoparticles is much rapid than its biosecurity research counterpart. Even though the contributions of nanotechnology to medicine have been enormous, there are heightened concerns on the toxicity of nanoparticles involved.

Nanotoxicology analyses the effects and consequences of particulate structures less than 100 nm in size. This nanoscale size facilitates them to get to places in the environment and the human body that are inaccessible to larger particles leading to unexpected exposures. Nanotoxicology can be thus defined as an aspect of nanoscience that deals with the study of adverse effects of engineered nanomaterials or nanoparticles on living organisms [2].

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2 Routes of Exposure

2.1 Inhalation Exposure

Inhalation is one of the crucial routes of entry for the nanoparticle as they can travel a long way in air by brownian diffusion. Inhaled nanoparticles are cleared by mucociliary transport as they proceed through the bronchial tubes. Fine particles (less than 2.5 μm) can be transported along with the air into alveoli. Unlike bronchial tubes, alveoli lack mucociliary clearance thus removing the foreign particles with help of macrophages. Evidence from animal studies have displayed that nanoparticle deposited in the lungs can translocate to the pulmonary interstitium [3]. Translocation of inhaled nanoparticles to secondary sites like spleen, liver, brain, and heart has also been reported [4].

Certain airborne nanoparticles which are combustion-generated tend to agglomerate. Due to the greater size of these agglomerated nanoparticles, they might settle out of the air lowering inhalation exposure. However, it may increase the possibility for dermal exposure, object-to-mouth, or hand-to-mouth entry.

2.2 Dermal Exposure

The corneal layer, the outer layer of the epidermis is a highly resistant mechanical barrier to nanoparticles and is much thicker than the epithelium of lung [5]. Usage of nanoparticles in cosmetics, sunscreens and stain resistant clothing has paved way for increased dermal interaction. Few studies reported that nanoparticles, primarily titanium oxide, did not penetrate past the stratum corneum whereas other studies demonstrated their capability to penetrate epidermis and dermis. Accumulation of nanoparticles in the hair follicles were also observed [6].

Limited dermal penetration of quantum dots were also appreciated in certain studies. This penetration was dependent on shape, size, and surface charge of the quantum dots, with smaller, spherical particles manifesting greater penetration than larger, ellipsoidal particles [7]. There are also studies describing penetration of quantum dots through viable epidermis after removal of stratum corneum in excised human skin [8].

The studies concerning dermal exposure and skin penetration of nanoparticles are contradictory especially with respect to titanium dioxide.

2.3 Uptake via Gastrointestinal Tract

Oral exposure occurs from ingestion of nanoparticle-containing food, swallowing of inhaled particles or from hand to mouth exposure. Migration of nanoscale

particle from gastrointestinal tract into the blood stream is difficult owing to the anatomic location of blood vessels which are situated several cell layers below the intestinal epithelium. However, there are animal studies describing the absorption of polystyrene nanoparticles from intestinal epithelium into lymphoid tissues contradicting the above theory [9]. Certain other studies point out that no such uptake into lymphoid system is possible from the intestinal epithelium [4, 10].

Like other sites, absorption of nanoparticles from gastrointestinal tract is dependent on particle size and surface characteristics. Smaller, hydrophobic neutral particles are thus effortlessly absorbed. Also, about 98% of the nanoparticles absorbed orally are excreted, unlike intravenously administered nanoscale materials which are found to accumulate in the liver.

2.4 Intravenous Route

Imaging methods using nanoparticle containing contrast agents, novel vaccines incorporating nano objects, nanoparticle containing drugs injected into target tissue, are some of the intravenous routes by which nanoparticles gain access into human body. Dermal as well as gastrointestinal route can be bypassed through injection, while other barriers such as placenta or blood–brain barrier become significant in this regard.

In an animal study assessing toxicity of intravenously administered titanium dioxide, there were no significant changes in the cytokines and enzyme levels measured in blood [11]. However, there were also studies reporting acute toxicity to brain, lung, spleen, liver, and kidney with high doses of titanium dioxide nanoparticles [12].

2.5 Neuronal Translocation

Studies in rats have advocated the translocation of inhaled nanoparticles from the nasal epithelium to the olfactory bulb, from where the nanoparticles can be directly transported to the brain [13]. Extrapolating the results from rat model into human research may not be desirable due to the existing anatomic and physiologic differences. 50% of the rat's nasal mucosa is olfactory (compared to 5% in humans) and the weight of their olfactory bulb is 177 times greater than that of humans [14].

However, due to significant deposition of nanoparticles in nasopharyngeal region of humans, there is high predisposition for neuronal translocation [15]. Detrimental effects on neurons including induction of oxidative stress, loss of cell viability and dopamine depletion were seen with CNS uptake of manganese nanoparticles in invitro studies [16]. Nanoscale carbon particles and gold particles were also postulated to have transsynaptic transport [17]. The neuronal and circulatory translocation

of inhaled incidental nanoparticles has been associated with neurodegenerative CNS diseases, although conclusive evidences are limited.

3 Biological Barriers to Nanoparticles

Barriers like skin, gastrointestinal tract (GIT) and lungs offer protection to organism from environment. Additionally, brain and placenta are protected by extra set of cells contributing to blood–brain barrier (BBB) and blood-placenta barrier.

Two types of cells are commonly involved in the formation of barriers. They include (i) A layer of epithelial cells that are protective in function and (ii) the adjoining cells forming the blood capillaries [1]. The epithelium can be perfused by either passage through the cell (transcellular pathway) or between the cells (paracellular pathway).

Epithelium of GIT is composed of various types of cells. They include keratinocytes in oral cavity and esophagus, gastric cells in stomach, and enterocytes in small and large intestines. The layer of mucous covering these cells offers additional protection. For molecules greater than 1 nm, permeation through paracellular pathway in GIT is difficult.

The multiple layers of skin have a collection of dead tissue on the top, further reinforcing the barrier. The studies pertaining to dermal exposure and penetration of nanoparticles have contradictory inferences and are mentioned under the previous section on ‘routes of exposure’.

BBB is made of cerebrovascular endothelium which has tight junctions. Pericytes, discontinuous basal membrane, and astrocyte end feet are other supplementary structures involved in BBB. There is also a blood-CSF barrier formed by the choroid plexus of epithelial cells and they also include tight junctions. These tight junctions of BBB and blood-CSF barrier prevent the entry of large hydrophilic molecules as well as nanoscale particles.

Maternal–fetal transfer of organic and inorganic molecules are determined by placental barrier. This barrier is composed of trophoblast cells on the maternal side and capillary endothelium on the fetal side. In humans, there are two trophoblast layers namely the syncytiotrophoblast and the cytotrophoblast. The syncytiotrophoblasts provide space for few paracellular pathways. The fetal endothelial cells are bound tightly blocking particles above 300 nm from crossing the placenta [1].

4 Toxicological Targets and Endpoints

Nanoparticles act at various levels in the biologic system. There are molecular, cellular and organ level targets for these nanoscale particles [1].

4.1 Molecular Level

Molecular level interaction of ENM can be attributed mainly to the production of reactive oxygen species (ROS). Certain metal containing ENM also release toxic metal ions that bind to functional group on macromolecules thereby disrupting their structural and functional equilibrium. Cationic nanoparticles interact with the negatively charged phospholipid membrane causing membrane lysis. Another molecular level interaction of nanoparticle is the formation of 'corona' which is a nanocoating formed by the attachment of protein on the nanoparticle.

4.2 Cellular Level

Particles of size approximately 500 nm are engulfed by the phagocytes thus protecting the organism from exposure to nanoparticles. Overburdening these cells by increased rate of phagocytosis over degradation results in local inflammation. Another interaction at cellular level is the formation of antibodies against nanoparticles. This immune mediated reaction occurs when these ENMs are recognized by antigen presenting cells. Some ENMs also cause mechanical disturbance by interacting with cell nucleus.

4.3 Organ Level

Accumulation of nanoparticles affects blood flow in capillaries, impairs renal filtration, and causes arrhythmias. Once inhaled, nanoparticles cause inflammation, fibrosis, and cancer of the lungs. Titanium oxide (TiO_2) nanoparticles are found to invoke mucus hypersecretion and emphysema like conditions [18]. There is also a possibility where the inhaled nanoparticle crosses the blood- brain barrier to reach the brain via the olfactory tract. Their potential to accumulate in liver has led to hepatic toxicity [19] (Fig. 1).

5 Properties of Nanoparticles Determining Toxicity

Size, surface area, shape, aspect ratio, surface coating, crystallinity, dissolution, and agglomeration are various properties of nanoparticles determining toxicity.

Size and Surface Area. As size of nanoparticle decreases, the ratio of surface area to volume exponentially increases. This enhances biological and chemical reactions. In other words, nanoparticles have higher toxicity in comparison to their bigger sized counterpart. In separate animal studies, size depended toxicity of silver

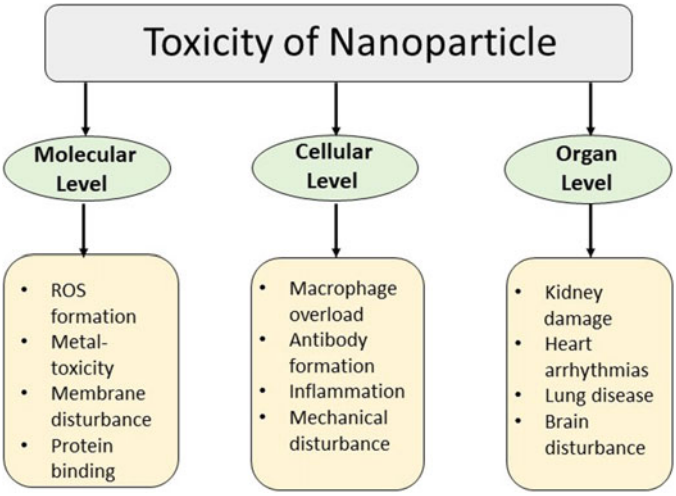

Fig. 1 Levels of interaction of nanoparticle. Redrawn from 'Engineered nanomaterials and human health: Part 2. Applications and nanotoxicology', Gubala et al. [1]

nanoparticles, amorphous silica nanoparticles and gold particles were assessed and was discovered that higher surface area led to higher reactivity inducing cytotoxicity and genotoxicity [20].

Shape. Another parameter determining nanoparticle toxicity is its shape that influences the degree of cellular uptake. Toxic effect of silver nanoplates over silver nanospheres [21], gold nanorods causing less cumulation of autophagosome than gold nanospheres [22] are few studies to substantiate the same.

Aspect Ratio: The width to height ratio of the nanoparticle or the aspect ratio is determinant of the toxicity. With increase in the aspect ratio, the toxicity of the nanoparticle is found to heighten. Spherical particle has an aspect ratio of 1 whereas nanotube has an aspect ratio close to zero.

Crystallinity: Crystalline structure affects toxicity of the nanomaterial. The ability of a substance to exist in different crystalline phases but are otherwise identical in terms of chemical content is called as polymorphism. Polymorph dependent toxicity of titanium dioxide nanoparticles was reported by Andersson et al. [23]. Thus, identification of polymorphic form of nanoparticles is considered significant in the estimation of toxicity.

Surface Coating or Surface Functionalization: The objective of the surface coating is to improve stability, wettability, dissolution, and functionality of nanoparticle. Surface coating can change toxic particles to nontoxic. At the same time, harmless particles may become more toxic due to bioavailability. When iron oxide nanoparticles were coated with polyethyleneimine (PEI), remarkable increase in uptake was observed than PEGylated iron oxide nanoparticles [24]. This resulted in severe cytotoxicity in both cancer cells and macrophages. Despite having the same

nanoparticle sizes presence of surface coating was found to affect the efficiency of internalization.

Dissolution: Despite having similar composition, two identical nanoparticles have contrasting behavior in dissolution, depending on different surface modifications. Safety, uptake, and toxic mechanism of nanoparticles are influenced by the property of dissolution.

Agglomeration. The size, surface charge and agglomeration state of nanoparticles under physiological conditions are fundamental parameters to be assessed prior to their application in toxicological studies. Nanoparticles are prone to clustering either via aggregation (irreversible) or agglomeration (reversible) processes due to their high free surface energy [25]. Agglomeration involves adhesion to each other with help of weak forces and aggregation includes fusion of particles by means of strong forces [26]. The agglomeration of nanoparticles could be a potential inducer of inflammatory lung conditions in humans.

6 Principles of Nanotoxicology

There are broadly four principles that determine the interaction of ENMs with biological system [5]. They can be summarized as following.

Transport principle: Transport of ENMs in the tissue is a property of their small size. Only small particles have access to the vesicular structures on the cell surface, allowing the use of the small vesicular transport pathways that are unreachable to larger particles with sizes above 300–500 nm.

Surface principle: Higher surface area-to-volume ratio is associated with smaller size of particle. With reduction in size of particle, there occur two significant changes. Firstly, the surface itself becomes larger and offers high probability for surface reactions. Secondly, as the particle size becomes smaller, there is increase in potential for appearance of surface defects within the crystalline structure.

Material principle: Difference in reactivity and behavior in biologic system is noted with difference in composition of ENM. Different binding capacities and distribution patterns are seen towards various biological molecules such as proteins, lipids, or DNA with change in composition of the nanoparticle.

Fibre principle: This principle states that, length and aspect ratio are determinants that help to predict the pathogenicity of a fibre, especially after inhalation. The fibre-paradigm applies only to fibres with a key length larger than 5 μm where the diameter does not determine the biological effect, provided it is less than 1–3 μm, and the aspect ratio is a minimum 3:1 [27].

7 Nanoparticles Used in Dentistry

With its wide range of application, nanomedicine has revolutionized the field of medicine. Broad array of nanomaterials also find application in dentistry and has led to the emergence of a subspeciality, widely accepted as nanodentistry [28]. A very brief overview of nanoparticles used in dentistry is included here. A detailed description of the same can be appreciated in previous chapters.

Homogenous distribution of nanoparticles into composite resin has brought about the development of nanocomposites. These includes nanoparticles of zinc oxide, silica, titanium oxide, calcium phosphate and calcium fluoride, added to impart superior attributes to the conventional composite resin. Significant improvement in microhardness, flexural strength, anticariogenic and antibacterial properties are seen with nanocomposites.

Nano-hydroxyapatite, nano-silver, nano-calcium phosphate and nano-silica are added to modify dental adhesives. Apart from its use as dental adhesive, nano-hydroxyapatite also finds application in root canal fillings, bone repair materials and as coating for dental implants owing to its improved osteogenic, antibacterial, and bacteriostatic effect. Nano-zirconium oxide also finds application as bone graft material.

Better resistance to crack propagation and improved fracture toughness are seen when nanoparticles of zirconium oxide, aluminium oxide and silver are added to bioceramics. Similarly, addition of nano titanium, zinc and cerium oxide to silicone elastomer materials and addition of nano-silver to denture base materials are found to modify mechanical properties and biocompatibility of the original material.

Nanotechnology modified coating materials for dental implants include nano-porous alumina, nano-zirconia, nano-calcium phosphate, nano-zinc oxide, and nano-hydroxyapatite. Higher bioactivity potential, cell adhesion and rapid osseointegration can be achieved with nanoparticle coating.

Sustained and controlled release of anticancer drugs are achieved by incorporating nano-silica particles in drug delivery system. Also, superparamagnetic iron oxide nanoparticles are used in tumor imaging due to enhanced optical properties.

8 Individual Nanoparticles and Their Associated Toxicity

8.1 Gold Nanoparticles

Gold nanoparticle is an interesting nanomaterial that finds application in nanomedicine as nanocarriers, cancer drug delivery and in biosensors. Their toxic effects are size dependent and found to affect human and animal embryonic stem cells, human lung fibroblasts, human lymphocytes as well as neural cell lines. Alteration in DNA methylation and hydromethylation in stem cells were seen with gold nanoparticles of size less than 20 nm [29]. Chromosomal aberration, oxidative DNA damage,

micronuclei formation, and strand lesions in invitro studies were noticed with gold nanoparticles [30]. There are also studies demonstrating that gold nanoparticles are lethal only to cancer cells and not to normal healthy cells [31].

8.2 Silver Nanoparticles

The substantial antimicrobial property and production of reactive oxygen species makes silver nanoparticles superior in inhibiting the growth of microorganisms. Their capability to induce toxic reactions towards microbes is also responsible for causing genotoxicity and mutagenicity in other cells. These nanoparticles have the potential to cross the cell membrane and reach the nucleus thereby causing genotoxicity. The silver nanoparticle-mediated oxidative stress and DNA damage can be controlled with the antioxidant N-acetylcysteine [32].

Cytotoxicity to keratinocytes and fibroblasts were assessed by various laboratory and clinical studies. Dermal biocompatibility was verified by certain studies [33] whereas some other studies suggested inhibition of keratinocyte proliferation and alteration of morphology of fibroblasts and keratinocytes on exposure to nanosilver [34]. Toxicity of nanosilver crystals to intestine and liver via oral exposure [35], to lung, brain, liver, reproductive and vascular systems of mammals [36], are few other studies that needs mention in this regard. Furthermore, the accumulation of silver is important in the pathogenesis of Silver-Russell syndrome, Parkinson's disease, and Alzheimer's diseases.

8.3 Copper Nanoparticles

Copper oxide nanoparticles finds application as antimicrobials, anticancer agents and in bioimaging. Despite these impressive biomedical properties, multiple studies reported that copper oxide nanoparticles were highly toxic to normal and healthy human cells. Various studies demonstrated that nano copper particles exhibited cytotoxicity in airway epithelial cells by inducing oxidative stress [37], genotoxicity towards human skin keratinocyte cells [38], neurotoxicity by accumulation in astrocytes [39], toxic reaction in cardiac microvascular endothelial cells, HepG2 cells, and human skin organ culture [40].

8.4 Zinc Oxide Nanoparticles

Like copper nanoparticles, nanoscale zinc oxide also finds application as anticancer and antimicrobial agent and is also used in optical imaging. Nanotoxicological assessment of zinc oxide particles revealed that they disrupt normal homeostasis of zinc ions

in the body. Increase in concentration of zinc ion was seen with disintegration of zinc oxide nanoparticles, affecting function of mitochondria and activation of caspases leading to apoptosis [41]. Apoptotic toxicity due to ROS triggered mitochondrial damage and genotoxicity due to DNA damage were proved in certain studies [42]. Animal studies have also pointed out inflammation and damage to liver, kidneys, lungs, neurons, and retinal cells induced by zinc oxide nanoparticles [43, 44]. In an invitro model using human lung fibroblasts, it was observed that nanoparticles of zinc oxide activated the extracellular secretion of lactate dehydrogenase, which was suggestive of cellular damage and reduced lung cell viability [45].

8.5 Iron Oxide Nanoparticle

This metal oxide nanoparticle due to its superparamagnetic property forms an indispensable part of magnetically targeted delivery system. They find application in magnetic resonance imaging, hyperthermia agents and targeted delivery of genes or drugs. The superparamagnetic iron oxide nanoparticles (SPIONs) help in generating heat in the presence of altered magnetic field. With the help of an external magnetic field, these particles can be easily delivered into tissues as well as organs.

Despite these properties, these nanoparticles are found to accumulate in various tissues and induce toxicity by damaging cell nucleus, mitochondria, and DNA. This leads to heightened production of ROS, micronuclei, apoptotic bodies, and condensation of chromosomes. Damage to actin cytoskeleton, alteration in gene expression, disruption of iron homeostasis, and oxidative stress are few other cytotoxic effects of these nanoparticles [46]. Few in vivo studies have pointed out that iron oxide nanoparticles can cross BBB, and precipitate acute neurotoxicity, immunotoxicity, and genotoxicity.

The toxic behaviour of SPION is also dependent on their surface modifications. Materials such as dextran, silicon, PEGylated starch, and citrate are commonly used as a surface coating. The stability of these coatings along with their cytotoxic nature needs further exploration.

8.6 Aluminium Oxide Nanoparticles

The biomedical application of aluminium oxide nanoparticles encompasses drug delivery, biosensing, cancer therapy, antimicrobial activity, and immunotherapy. However, like other nanoparticles, they also have exhibited biotoxicity and cytotoxicity. Studies revealed cytotoxic effect of nano alumina towards murine fibroblasts, human skin fibroblasts and human lymphocyte cell lines. Genotoxicity towards human mammalian cell lines were also assessed. The results emphasized that cytotoxicity depends on the dose, time of exposure, agglomeration, sedimentation, and enhanced cellular uptake. In an animal study on *Drosophila melanogaster*, inhibition

of neuronal activity resulting in neurotoxicity was observed with aluminium oxide nanoparticles.

8.7 Titanium Dioxide (TiO_2) Nanoparticles

Titanium dioxide nanoparticles, porous TiO_2, TiO_2 nanotubes, and TiO_2 nanocomposite are various configurations of titanium used in nanomedicine. Oral exposure to titanium dioxide may occur from food additives, medicines, toothpastes, and corrosion of titanium implants. There are studies reporting adverse effects to liver and intestine following oral exposure to titanium dioxide. Postmortem study of human liver and intestine confirmed the same [47]. The potential of TiO_2 to stimulate the development of colorectal tumors, and intestine-related autoimmune diseases such as inflammatory bowel disease including Crohn's disease, liver fibrosis, steatosis and edema are described in the literature. However, certain researches reported no pathological changes in liver and intestine following ingestion of titanium nanoparticles.

The pathogenesis of intestinal and hepatic effects can be assigned to the production of reactive oxygen species and associated inflammation. Few studies have also qualitatively detected presence of titanium in gut associated lymphoid tissue and kidney. In vitro and animal studies have reported genotoxicity to titanium dioxide particles observed as DNA deletions, strand breakage and production of micronuclei [48]. Percutaneous absorption of titanium nanoparticles from sunscreens were confirmed by a handful of studies where absorbed titanium particles were detected in hair follicles, epidermis, and dermis.

8.8 Silica Nanoparticles

Silica-based nanoparticles finds application in drug delivery, gene transfection and cell tracking. Despite their significance in biomedical research, injuries to the liver, kidney, spleen, and lungs following oral exposure and intravenous administration were detected in animal studies. The distribution, clearance, and biocompatibility of absorbed nanoscale silica were primarily dependent on particle shape. Yu et al. have demonstrated that silica nanoparticles can be excreted through the renal system as they cause damage to the glomerulus thereby increasing its permeability [49].

9 Interaction of Nanoparticles with Saliva

Saliva is a complex mucous secretion formed by the assembly of various electrolytes, immunoglobulins, mucins, proteins, enzymes, and nitrogenous products that helps in buffering, lubrication, protection, bacterial clearance, gustation, and digestion.

The interaction between nanoparticles and saliva is regulated by the electrolyte composition, pH, and viscous properties of the mucous components.

It is worth to consider the fundamental principles of colloidal chemistry and their application in biologic system to better understand nanotoxicity. A stable dispersion of nanoparticles in liquid is called a colloidal system or colloidal dispersion, the aggregation of which is determined by the ionic strength of the medium. Agglomeration or aggregation of nanoparticles, dispersion of nanoparticles by interaction with natural organic matter and the ability of nanoparticles to adsorb onto surfaces need to be analyzed to understand nanotoxicity. Aggregates of ENM settle onto the surfaces of the oral cavity and saliva is found to assist the same.

Certain solutes present in micromolar concentration in saliva (such as fluoride) can be adsorbed on the surface of ENMs. This can be attributed to the high surface area to volume ratio of the nanoparticle. Generally, the fluoride ions in saliva react with the calcium and phosphate ions in hydroxyapatite resulting in the formation of fluorapatite which shows superior resistance to acid attack. However, in the presence of ENMs, the fluoride adsorbed on its surface is prevented from organizing into fluorapatite crystals.

Certain immunoglobulins and complementary factors also adsorb onto the surface of ENM. The interactions of nanomaterial with the functional biomolecules like lysozyme, lysine, fibrinogen, albumin, and DNA have been the subject of research in the past years. A reduction in antibacterial action was seen in lysozyme adsorbed on the surface of nanoparticle due to alteration in spatial arrangement of the enzyme [50].

The precise interaction of nanoparticles with components of saliva is still under research. There is paucity of evidence to exactly assess the behavior of ENM with salivary biomolecules. The effect of ENMs on bioavailability of salivary anions, secretory activity of salivary gland, interaction with organic components like mucin, glycoproteins, proline-rich proteins, agglutinins, statherins, histatins, and cystatins are few among the many areas that remain obscure [51].

10 Interaction of Nanoparticles with Tooth Structures

The interaction of ENMs with enamel, dentin, cementum, and pulp is still not clearly understood. The current knowledge about the interplay between different tooth structures and nanoscale particles is incomplete and many ongoing investigations aim to understand the interactions and toxicological targets of nanoparticles used in dentistry.

Enamel: The ability of ENMs to permeate enamel is poorly understood. However, there is a report indicating the use of 20 nm hydroxy apatite particle to repair dentinal enamel. The bond formed between ENM, and the tooth structure is not clear, even though van der Waals forces and electrostatic forces are suggested to play a role.

Dentin: Larger diameter ENMs were found to infiltrate dentin than enamel. In various studies, infiltration of dentinal tubules with 100 nm hydroxy apatite particle, nanorods, spherical hydroxyapatite and silica nanoparticles were assessed, and they

were found to occlude dentinal tubules. Hence employing these nanoparticles in the treatment of dentin hypersensitivity can be a sensible strategy. Most studies used a partially demineralized dentin model.

Another study confirmed the remineralization potential of fully demineralized dentine following infiltration with hydroxyapatite and silica nanoparticles. Thus, the use of artificial nano HA in toothpastes can bring about remineralization, improve microhardness and can reduce bacterial colonization. Nanoparticulate bioactive glass, nanosized carbonated apatite alone or in combination with silica, nanosized calcium fluoride, carbonate hydroxyapatite nanocrystals, and nano precursors of amorphous calcium phosphate are also found to improve remineralization of dentin and enamel.

Cementum and Pulp: Very few studies address the interaction of ENMs with pulp and cementum. However, one prospective area of research can be the use of pulp stem cells to regenerate pulp tissues and vasculature of head and neck using nanofiber scaffolds.

11 Toxicology Testing

Various approaches are used to test the toxicity of ENM on cells and environment. They can be broadly categorized into in vitro and in vivo assessment methods. In vitro methods are quick and efficient techniques that are cost effective and has minimum ethical constrains. In vitro assessment can be further subdivided into proliferation assay, apoptosis assay, necrosis assay, oxidative stress assay and DNA damage assays [52].

Proliferation assay measures metabolically active cells with the help of tetrazolium salt [53]. Another assay where cells proliferating after nanoparticle exposure, are counted after visual inspection is known as cologenic assay. The release of apoptotic marker is measured by apoptotic assay. Various techniques employed to assess apoptosis includes Annexin-V assay, Comet assay, TdT-mediated dUTP-biotin nick end labelling (TUNEL) assay and inspection of morphological changes [52]. Comet assay and TUNEL assay are also used to calibrate the extend of DNA damage. Necrosis assay measures the integrity of cell membrane with the help of dyes like neutral red and trypan blue. Oxidative stress assay measures the level of reactive oxygen species, reactive nitrogen species and lipid peroxidation.

The in vivo assessment methods are performed on animal models and includes biodistribution, clearance, hematology, serum chemistry and histopathology. Biodistribution assesses the localization of nanoparticles to various tissues and organs. Nanoparticles are detected in the killed or live animals through radiolabels that helps to evaluate biodistribution. Examination of excretion and metabolism helps to appreciate clearance of nanoparticles. Nanoparticle exposed tissues can also be subjected to histopathologic examination. Micro-electrochemistry and microfluidics are recent advancements in invivo toxicologic testing [54].

Additionally, there are microscopic and spectroscopic methods like scanning electron microscopy or energy dispersive X-ray spectroscopy (SEM–EDX), transmission electron microscopy (TEM), atomic force microscopy (AFM), video enhanced differential interface contrast (VEDIC) microscopy and fluorescent spectroscopy that aids in nanotoxicological assessment.

12 Limitations Associated with the Study on Nanotoxicity

Knowledge on concentration, size, and charge of ENM in biologic media is considered as an essential prerequisite to understand their toxic behavior in biologic environment. Most of the data on nanotoxicology are based on in vitro studies or from studies on isolated cell cultures. Influence of blood flow, enzymes, and renal filtration are largely ignored in a cellular test system. Also, ENM degeneration in cultured cells may differ from that of healthy organism. Cells in vitro divide faster than cells in healthy organism and is considered as another limitation.

Other obstacles in nanotoxicology experiment are lack of characterization, lack of standardized methods and missing comparability. Many studies lack the use of reference nanomaterial to verify a specific biologic response.

13 Conclusion

Nano-technology has changed the face of modern medicine and dentistry. It has provided novel solutions to improve health and to diagnose and treat diseases. However, one major problem that has arisen is the concern on toxicity of nanoparticles. The research on nanotoxicology is still in its infancy. There are gaps in understanding about the human and environmental risks that the nanoparticles pose. It is still too early to draw explicit conclusions regarding the inherent danger of nanomaterials, let alone their exact mechanism of toxicity. There could be novel mechanism of injury that a nanoparticle may induce requiring distinctive tools, assays, and approaches to detect, that are yet to be developed.

The studies on nanotoxicology of dental materials per se are deficient and most of our understanding is based on extrapolation of data from other similar studies. Also, the clinical safety regulations for ENMs used in nano dentistry have not been regularly updated and warrants the need for occupational guidelines among health practitioners [51].

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