Chapter 3
Nanotechnology in Dental Therapy and Oral Tissue Regeneration

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Abstract The emergence of nanotechnology within dental fields has sparked great interest in their potential applications. This chapter focuses on the application of nanotechnology in dental therapy and oral tissue regeneration, especially in dental materials. Nowadays, various nano-additives have been introduced into many commercially available products, making it overwhelmingly difficult for both dentists and patients to properly choose from. Actually, the choice of nanomaterials is dependent on the clinical scenario and the tooth to be restored, paying close attention to esthetic demand, loading, and the presence of any risk factors. In this chapter, we will introduce "Nanotechnology in tooth defect therapy," "Nanotechnology in oral tissue regeneration," and "Nanotechnology in antibacterial for oral disease and therapy." Future direction is to develop more efficient and cost-effective nanobiosensing materials to treat dental diseases intelligently. For example, the materials have the potential to deliver drugs to disrupt biofilm formation in order to reduce the incidence of caries and periodontal disease and also can be used for tooth defect filling. Ultimately, it may be possible to achieve the pinnacle goal, tooth regeneration. Therefore, this chapter will help the readers gain a general grasp about the current application of nanotechnology in dental fields, relative benefits and limitations, and future trends.

Keywords Tooth defect · Oral tissue regeneration · Antibacterial

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3.1 Nanotechnology in Tooth Defect Therapy

3.1.1 Nano Tooth Filling Materials

Tooth defect is one of the main oral health problems. Its definition is that a part of a tooth is lost due to caries, trauma, or dysplasia, which will cause damage to the shape and structure of the tooth, leading to the loss of function. The most common cause of the disease is dental decay. Each year, about 200 million tooth defects are restored in the USA, costing a lot of money.

There are, of course, many details and variations that are specific to one problem. For a small defect, it is commonly restored by direct filling to restore the damaged tooth immediately. And for a large one, it is more suitable to employ prosthetic treatment like inlay and crown. This chapter mainly focuses on the materials that can be used for direct tooth filling.

With the development of science and technology, a variety of materials have been applied to restore the tooth defects, such as composite resins, glass ionomer cements, and silver amalgam. The ideal tooth filling materials should (1) be strong enough to bear the bite force, (2) have poor conductivity so as to not transmit temperature and current, (3) be operated easily, and (4) have color that closely resembles that of teeth. Despite better understanding of the materials and chemistry, and improvements in physical properties, no material has been found to be ideal for tooth filling so far [1].

For example, the silver amalgam, which is the traditional filling material, has better durability and longevity, lower incidence of cuspidian fracture occurrence and recurrence of caries compared with composite resins [2]. Hence, dental restorations have employed silver amalgam for more than a century. However, the toxicity of mercury within amalgam has posed a crucial drawback [3]. Another major concern is that the metal hue of amalgam is not suitable for esthetic tooth restoration. Due to the advances in tooth-colored restorations and adhesive technology, the use of amalgam has been increasingly replaced by alternative materials.

Currently, dental composite resins are the most commonly used material for tooth filling. Dental composite resins have been used to restore teeth since their first introduction about 50 years ago [4]. Compared with dental amalgams, they are safer and more esthetic. But the wear resistance of the composite resins is poorer than the silver amalgam. And it is easier to get food lodged between teeth due to the matrix bands. Based on the report in 2005, the composite resins were used in more than 95% of all anterior tooth direct restorations and about 50% of all posterior tooth direct restoration [4]. They are mainly composed of three chemically different components: the organic matrix (usually a resin or synthetic monomer), the inorganic matrix (fillers), and a coupling agent (usually silane) for bonding the filler onto the organic matrix. Composite resin can be classified either according to their composition or the filler particle size. A widely accepted classification based on filler particle size proposed by Lutz F et al. is: macro-filler composites (0.1–100 μm), micro-filler composites (0.04–0.1 μm), and hybrid composites (fillers of different

sizes) [5]. Later, nano-filler (1–100 nm) is developed and categorized as nano-modified composite resin. This new type of resin has the properties which the traditional one does not have, which will be discussed later.

Glass ionomer cements, which were introduced by Wilson and Kent in the 1970s as a dental filling material, are also commonly used in dentistry [6]. The milestone timeline in the development of glass ionomers cement in dentistry is described in Fig. 3.1. Currently, there is no universally accepted cement that fulfills all requirements. However, there are a variety of cements whose properties and manipulation lead them to be an appropriate choice for a specific application. Generally, glass ionomers require the mixing of the acidic powder and the basic liquid form, utilizing the principle of acid–base reactions. The powder form consists mainly of fluoroaluminosilicate (FAS) glass particles and ions such as calcium, lanthanum, and strontium, while the liquid form is a viscous fluid which consists of a copolymer of maleic acid or itaconic acid and acrylic acid. The reaction takes place after mixing these two forms, with the initial setting taking place within 3–4 min. Because of its remarkable qualities such as strong chemical bonding to tooth structures, good compatibility, and fluoride release, glass ionomers have become increasingly widespread in usage. However, the inferior mechanical properties of glass ionomers have limited their application. For traditional glass ionomers, the most intractable problem is the lack of strength and toughness. Because of its excellent performance, resins were added to the matrix to improve the clinical performance of glass ionomer cements. However, this did not significantly overcome the drawbacks. After immersing in water for 12 months, compared with traditional glass ionomers, the strength, toughness, and microhardness of the resin-modified glass ionomers were

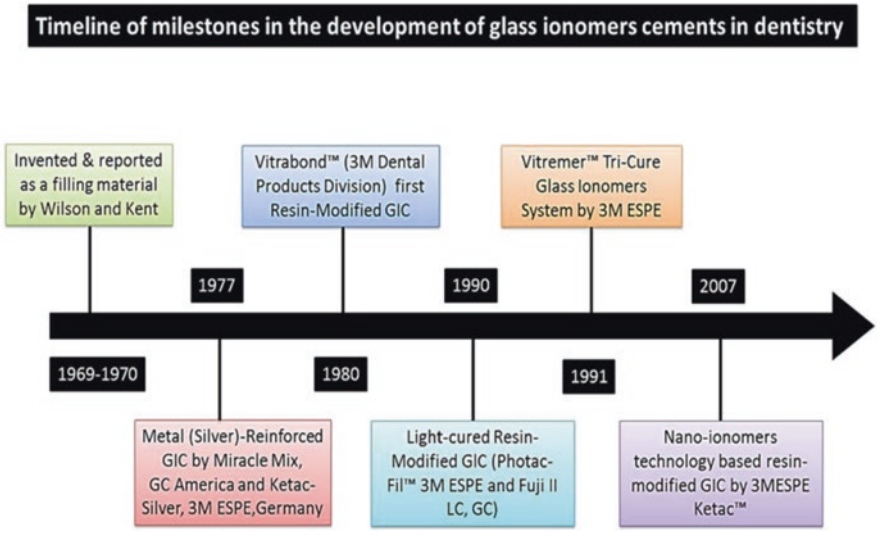

Fig. 3.1 Timeline of milestone in the development of glass ionomer cements and nano-ionomers for dental restorations [7]

not improved with the addition of resins. Alternatively, there have been extensive efforts to improve the glass ionomer cement by fluoride (F)-releasing properties. It is expected that the F ions can enrich tooth structure, promote remineralization, and inhibit demineralization. Glass ionomers, resin-modified glass ionomers, compomers, and composites have received much attention due to their release of F ions that can be incorporated into tooth mineral, resulting in fluoroapatite or F-enriched hydroxyapatite, both having reduced solubility. Moreover, nano-modified glass ionomers are developed to improve its mechanical properties.

The composite materials are promising in esthetic restoration. However, these materials are very technique sensitive, and the mechanical properties are not as good as amalgams [8]. A favorable method to increase the properties and performance of the final restorative material is by increasing the percentage and reducing the size of the filler in the matrix. By adding fillers, the mechanical properties of composites are enhanced, polymerization shrinkage is reduced, thermal expansion coefficient of the composite is modified to match that of the tooth, handling is improved, and radio-opacity as well as translucency and wear resistance are endowed in the composites [9].

Most of the current conventional composites typically range between 0.04 and 0.7 μm in filler particle size. Terry DA found that the size of these particles hinders the interaction with the nanoscale (1–10 nm) structural components of enamel and dentine, such as the enamel rods, dentinal tubules, HA crystals, and collagen fibers [10]. This results in a compromise between the restorative material and the surrounding tissue. After the introduction of nano-fillers (particle size 1–100 nm), the possibility of creating more ideal advanced composites became a reality. Ideal composite materials should have two important properties: strength and esthetics. Both the properties depend upon the size of the filler particle. The small particle size can decrease curing shrinkage, offer more uniform particle distribution, allow a higher filler load, reduce viscosity, offer better handling properties, and endow stronger mechanical properties [10].

New materials introduced in recent years, especially nano-fillers, demonstrate noteworthy advances in composites [11]. Bowen et al. generated the resins using silane couplers and bisphenol A-glycidyl dimethacrylate (bis-GMA). Circa the same era, words such as nano were conceived by the noble laureate Sir Feynman R in 1959 [12]. This breakthrough was a key milestone for the advancement of dental composites. Henceforth, composite fillings have been added as a valuable treatment in the restorative armamentarium. Rapid advances have occurred in the past decade in dental restorative materials, including the resin-based composites. Likewise, the discovery of nano-filler particles arose from the introduction of nanotechnology. Research has continuously strived to improve the physical properties of materials as well as tackle obstacles such as wear resistance, polymerization shrinkage, micro hardness, and patient dissatisfaction due to poor esthetic appearance [8, 9].

Titanium dioxide nanoparticles (TiO_2 NPs) has many excellent properties to be an inorganic filler: it is chemically stable and nontoxic and evinces a highly efficient photocatalytic effect. Furthermore, research on TiO_2 NPs as a filler in epoxy found that the addition overcomes the shortcomings of traditional toughers, such as

rubber beads and glass, by improving the strength, stiffness, and toughness of the epoxy without undermining its thermo-mechanical properties. Moreover, they are granular and can easily agglomerate, which makes it useful for practical applications. However, homogenous dispersion in organic solvent remains a challenging obstacle. To overcome this, suitable modification to the surface is needed. Coating inorganic fillers in resin-based composites with organosilane strengthens their bond to the resin matrix and prolongs the operational life of the composite. Additionally, the substance advantageously promotes the dispersal of silanated filler particles within the matrix. So, TiO_2 NPs were coated with an organosilane (namely allyltriethoxysilane, hereafter ATES) and blended into dental resin-based composites. The result showed that surface modification by the organosilane ATES improved the dispersion and linkage of TiO_2 NPs within a resin matrix, and thus improved the microhardness and flexural strength of dental composite resins [13].

In another study, the mechanical behavior of the TiO_2 nanoparticle-reinforced resin-based dental composites was predicted by using a nanoscale representative volume element (RVE): these results were then compared against those obtained the non-interaction approximation (NIA). The results illustrated that the nanoparticle-reinforced composites have a mechanical advantage over those reinforced with glass fibers. Further assessment was conducted to analyze the effects of nanoparticle aspect ratio, volume fraction, and stiffness under the criteria of yield strength of the composite and effective Young's modulus. And the result showed a mechanical advantage of nanocomposites over microcomposites. Compared with glass fiber, the reinforce effect of the nanoparticle with 3% volume fraction contains the same stiffness as at only half the volume fraction. Additionally, an approximately linear increase in effective Young's modulus and yield strength of the composite was found at volume fractions from 1% to 5%, considering perfect nanoparticle dispersion. These results provide better understanding of the mechanical behavior of nanoparticle-based resin-based dental composites and can provide insight into further optimization of the composition of dental composites [14].

Nanocomposite resins have excellent properties. However, what is the best concentration of the NPs? Can adding more nanoparticles achieve better performance? According to many in vitro studies, nano-filled materials have greater wear resistance than micro-filled materials. When 40 nm filler particles were added to a composite with 3 μm particles, wear resistance was improved until a concentration of 15% vol [15]. But determining the most appropriate concentration still needs further scientific investigation. Three experimental nanocomposites were formulated with different weight percent filler loads (25, 50, and 65%) by Lawson NC [16]. The elastic modulus, flexural strength, and hardness of the composites and the unfilled resin were measured. Notably, the wear resistance exhibited by the composites decreased as the filler content was increased beyond 25%. Comparatively, flexural strength and hardness and modulus increased with increasing filler content up to 50%. Scanning electron microscope (SEM) inspection of the worn specimens revealed that while the resin and 25% filled materials cracked and failed because of fatigue, the 50 and 65% filled materials expressed microcutting and failed as a result of abrasive wear. So, concentrations between 25 and 50% may be the best choice.

Thus, it seems that there does appear to be an optimal maximum of the percentage of nano-filler in the nanocomposite, beyond which the mechanical properties do not improve further, or even deteriorate [17]. Similar result was obtained in another study. Nanocomposite dental resins comprised mainly of polyhedral oligomeric silsesquioxane nanocomposite matrix with 0, 0.5, 1, 1.5 and 2 wt% nano SiO_2 as filler were created by light curing. Characterization of these resins were performed by compressive, three-point flexure, nanoscratch, and nanoidentation tests as well as SEM analysis and optical microscopy in order to study how different SiO_2 contents affect the resin. It was found that nano SiO_2 effectively enhanced the mechanical properties of the composite resins at low content (below 1.5 wt% SiO_2 addition). But with the increase of the nano SiO_2 content (above 2.0 wt% SiO_2 addition), the mechanical properties decreased [17]. The reason may lie in that increasing filler load beyond a high concentration led to the agglomeration of filler particles which acted as crack-initiating flaws [18].

In order to improve the properties and overcome these shortcomings of the glass ionomer cement, active research is in progress, such as in the addition of cellulose fibers, hydroxyapatite, fluoroapatite, and nanotechnologies. The application of nanotechnology is the focus here.

Novel CaF_2 NPs (56 nm) were synthesized via spray-drying and incorporated into resin. F release increased with increasing the nano-CaF_2 content and decreasing pH. Nearly threefold flexural strength was found as compared to that of resin-modified glass and also exceeded that of composite resin while exhibiting little F release. To summarize, the modified CaF_2 NPs exhibited high F release at low filler levels, making space in the resin for the reinforcement glass [19]. This relatively high F release arising from the nanocomposite was presumably due to the small size and consequently the high surface area of the NPs. Ball milling or grinding can reduce the size of these traditional particles; however, it is difficult to achieve a median particle size of 1 μm or smaller. The CaF_2 in Xu's study had a surface area of 35.5 m^2/g [20]. While Anusavice KJ's study used a traditional commercial CaF_2 powder which had a surface area of 1.9 m^2/g [21]. This is likely attributed to the high and sustained release of F ions from the nanocomposite.

Another exciting development is the Equia system, a new nano filling material. This material uses liquid dispersed inorganic silica nano-fillers (40 nm in size) to reinforce the produced polymer matrix. Shrinking the initial setting time and improving the wear resistance is accomplished by adding 15 wt% silica nano-fillers. Since these materials can better resist against dissolution, wear, and disintegration, the maintenance of a polished surface was prolonged. The translucency, optical properties, and esthetic appearance of nano glass ionomers compared with conventional ones are also improved remarkably, being claimed as good as that of natural teeth [22]. Friedl K et al. presented a retrospective study on new glass ionomer cements to evaluate their performance. They found that 151 restorations were placed in permanent molars (n = 94) and premolars (n = 57) in 43 patients. Restorations were evaluated at 4.5× magnification using modified USPHS criteria. They concluded that Equia system is good for posterior filling materials [23].

The effects of additives such as nanoscale hydroxyapatite (HA) and fluoroapatite (FA) on the properties of glass ionomers is a hot topic [24]. HA crystals are a key contributor to the chemical structure of natural enamel and dentin and are recognized for their biocompatibility. Resin-modified glass ionomers have an 8–12 MPa bond strength to tooth structures [25]. Increases in bond strength occurred after adding micro-HA (5–10 μm) and improved even further if nano-HA (100–150 nm) was added. This suggests that tooth structures with higher surface area for bonding, as with the case of nano-HA, might result in improved bond strength. Additionally, high surface area of nano-HA also improves the surface finish, while the solubility of the material helps to fill the demineralized micropores in the tooth structure. A similar research was conducted by adding ethanol-based sol-gel prepared nano-HA and nano-FA to Fuji II glass ionomers [26]. Improved mechanical properties were exhibited by these modified nano-filled glass ionomers, such as compressive strength, diametral tensile strength and biaxial flexural strength. It is a remarkable fact that different mixing methods make different results. A new nanocomposite, glass ionomer cement (GIC)-nanoSiO_2-HA-ZrO_2 was fabricated by adding zirconia to increase the hardness. Synthesis of this nanocomposite was performed using two methods, one pot and spatulation method. Within the study limitations, the one pot method produced better GIC-nanoSiO_2-HA-ZrO_2 composite [27]. The probable cause is one pot method was able to produce less agglomerated and more uniform dispersion of nanozirconia. This type of dispersion from nanozirconia improves the strength mechanism of the resulting composite. If the nanopowder is highly agglomerated, the matrix of GIC may be altered, resulting in the decreases in hardness. In comparison, less agglomerated nanopowder promotes uniform distribution of nanopowder and may strengthen the matrix by filling up the empty gap within the GIC, which results in the improvement of the GIC strength.

Modification of the existing glass ionomers using nanomaterials is an active area of current research, with many having lofty expectations. Consequentially, there is an expectation that new nano-modified GIC and the properties of existing glass ionomers will be improved significantly [28].

Recent years have produced remarkable researches on nanomaterials, which has propelled it from theoretical foundation to clinical practice. A variety of nano-products for dental applications have emerged due to all the active researches. Here is the list of available materials: Ketac™ (3M ESPE, St. Paul, MN, USA), Ketac N100; Filtek Supreme XT (3M ESPE), Nano-ionomers (3M ESPE), Nano-primer, Fuji IX GP (GC, Leuven, Belgium), Premise™ (Kerr/Sybron, Orange, CA, USA), and Ceram X™ (DENTSPLY International, Milford, CT, USA). For example, Premise is a universal nano-filled restorative composite that contains 20 nm filler. The main nano-filler very likely is silica. But this is trade secret, so we are not clear. They are all available globally. They are easy to use and are alternatives to expensive compomers and composites and in many cases amalgam. And they cure extremely hard and are very wear resistant. They have strong wear resistance, high compressive strength, and more excellent mechanical properties compared with traditional materials. Though of course, this adds a premium to the price.

True glass ionomers, such as Fuji IX GP, bonds chemically to tooth structures, has similar values for coefficient of thermal expansion as compared to tooth, and releases substantial levels of rechargeable fluoride. It is provided either in hand-mix powder-liquid form or premeasured capsules. It is the ideal product for pediatric and geriatric restorations, non-stress area final restorations, intermediate restorations (IRM), core material, and either long-term or temporary restoration.

Although there have been many types of nano products, the new nano products are pursuing better performance, more proper appearance, and lower prices.

Nano-fillers have revolutionized the field of dentistry over the past 30 years. Despite these developmental advances, some problems still limit the use of composites in dental restoration. Most improvements are focused on the reduction of polymerization shrinkage, as well as the improvement of wear resistance, mechanical properties, biocompatibility, and processing properties.

For those with hypersensitive teeth, a new nanotechnology treatment proposed by researchers in Taiwan might one day bring pain relief. Researchers have found that dentinal tubules are increased (35.6% as compared to 9.3%) and the diameter widened (0.83 μm as compared to 0.43 μm) in sensitive teeth as compared to those in non-sensitive dentine. The Chinese researchers have demonstrated that gold NPs can be used to block these tubules: viewed as the world's smallest gold fillings. One such method to close sub-micron-sized dentinal tubules was explored by Dr. Chris Wang and his team, involving the sintering the highly concentrated gold NPs which were brushed into the exposed openings of dentinal tubules. The photofusion of these gold NPs was induced by laser irradiation via photothermal conversion. Clinically, modification of the material and improvement of the techniques to achieve a better fill in-depth may be required, especially considering the normal wear rate of dentine and the filling material [29]. It may be a good decision to develop a nano gold-modified resin to solve this issue.

Although spherical nano-fillers are popular, partly because they distribute stress more uniformly across the bulk volume of the composite resin and inhibit crack formation, tube-like fillers such as carbon nanotubes (CNT) have also been tried. For example, Zhang F et al. used single-walled carbon nanotubules (SWCNTs) to fabricate a composite [30], resulting in a nanocomposite with enhanced mechanical performance. While SWCNTs are noted to have superior strength, they also have the advantage of being accepted at higher filler concentrations by resin systems owing to their unique dimensional distribution (aspect ratio > 1000). Therefore, resins accommodating CNTs should be investigated further. However, there is a problem in the application of CNTs in dentistry. In contrast with the natural color of teeth, the resin specimen prepared through the method previously described is characterized as gray black, therefore rendering it incompatible for direct oral use. Applying other inorganic additives, for example, chromorphic xerogel pigment particles or sol gel-based opalescent fillers would help in fulfilling the esthetic requirements. However, further research on this subject is still required.

3.1.2 Nano-Modified Adhesive Material

The basics of the adhesive are discussed in this section. One of the most revolutionary developments among recent advances in dentistry has been the utilization of adhesive dentistry toward treatment. As compared to traditional mechanical factors for retention, "adhesive dentistry" depends only on techniques and procedures for "adherence" to tooth structures. Such examples include the placement of composite resin restoration, resin-bonded bridgework, and porcelain and composite veneers.

The success of adhesive dental techniques hinges on the establishment of an "adhesion" or "bond" between the underlying tooth and restorative materials. A comparison between traditional and adhesive techniques can be examined through the filling of composite resin in a cavity after the removal of caries. "Traditional" restorative materials, like amalgam, require the dentist to remove substantial healthy tooth to create undercuts for maintaining mechanical retention. In contrast, "adhesive" restoration renders this unnecessary as mechanical undercuts are not necessary for retention. Since the removal of healthy tooth substance is reduced, the tooth can be maintained for a longer period [31].

Adhesives play an important role in tooth defect, dentition defect, and orthodontics. These can all be divided into two categories according to which parts of the teeth the adhesive sticks to.

Therefore, it is essential to understand the outer parts of the teeth where the adhesive "sticks" to. The external surface of the tooth, enamel, is mainly inorganic. At the microscopic level, enamel is composed of millions of prisms that adhere to each other spreading from the dentin–enamel junction (DEJ) to the tooth's exterior (imagine millions of "Toblerone" bars spreading from the DEJ to the tooth's surface). By contrast, dentine has far more organic material, such as the protein collagen. The dentine is composed of millions of dentinal tubules which each contains odontoblast extensions (tissue cell extensions from the pulp) and tissue fluid. To better visualize dentine, one can picture a block of Swiss cheese. In the Swiss cheese, the "holes" resemble the dentinal tubules, while the material in between is the dentine.

Adhesive restorative materials as well as luting cements "adhere" to a tooth via two ways. They can be described as:

- Micromechanical retention, by which the prepared tooth surface is roughened and the restorative material meshes with the created pits and crevices via retentive tags.
- Chemical adhesion, by which the restorative material or luting cement chemically bonds with the tooth substance.

3.1.2.1 Enamel Bonding

During the advent of adhesive dentistry techniques, bonding to enamel (enamel bonding) achieved greater success than bonding to dentine (dentine bonding). Enamel bonding can be achieved by the following method:

1. First, etching the cut enamel surface by 37% phosphoric acid (applied as either a liquid or a gel) for 30–40 s. This serves to dissolve the underlying enamel prisms at different rates. Since some prisms dissolve quicker than others, a roughened surface is created, with deep pits denoting where the enamel has been dissolved. Afterward, the surface is washed to remove the phosphoric acid, and then dried. The above steps result in the enamel having a familiar frosted appearance.
2. Then, a primer is etched onto the enamel surface. This allows the bonding resin (applied subsequently) to more easily seep into the roughened enamel pits.
3. Finally, application of the bonding resin is placed on the enamel. Restoration of the cavity is performed using this dilute (or runny) state of the composite resin. Then resin seeps into the etched enamel surface and set when exposed to curing light (this is due to the linking of millions of tiny molecules, called monomers, into polymer chains when shone by the curing light). These chains then fabricate the millions of minute resin tags, which eventually fill the cavity at the micromechanical level.

3.1.2.2 Dentine Bonding

Dentine bonding was difficult to achieve during the early years of adhesive dentistry. More recently, denting bonding technology has been improved. The steps required for dentine bonding are similar to enamel bonding.

- As compared to enamel etching, the etching of cut dentine surface requires less time because it contains less inorganic material. Dentine etching mainly serves to remove debris from the entrances of the dentinal tubules.
- Afterward, a primer is applied, serving to increase the flow of the bonding resin as well as encouraging the proteins appearing in dentine to chemically link with the bonding resin.
- Finally, a bonding resin is applied, entering the dentinal tubules and forming resign tags (micromechanical retention). The resin is also believed to chemically link with the dentinal proteins (chemical linkage).

The component of new adhesives is different from the traditional ones. Twenty years ago, the etch, primer, and bonding agent all existed as three separate parts. Combined primer and bonding agents were produced later, which reduced the number of steps and time needed to place composite resin restorations. Further improvements in these areas have been seen in the last 15 years due to the introduction of combined etch, prime, and bonding systems (or self-etching systems) [31].

The chemical composition of adhesives aims to fulfill all above-mentioned processes. Although dental adhesives can be classified into either the etch and rinse (E&Rs) or the self-etch adhesives (SEAs) (Table 3.1) groups, the ingredients are similar and completely independent of the number of bottles required for the adhesive. Nevertheless, these different classes differ in their proportional composition. Typically, adhesives consist of acrylic resin monomers, organic solvents, initiators and inhibitors, and occasionally filler particles. Obviously, every component has its unique function. Knowledge of the chemical properties of these components are paramount for understanding or possibly predicting their behavior.

Nano-modified adhesive material. At the resin dentin interface, the adhesive layer has the lowest elastic modulus among the components of the bonded complex and was reported to be weaker than the resin-infiltrated demineralized dentin layer (hybrid layer). When the complex encountered stresses, the adhesive layer, the component with the lowest elastic modulus, would suffer the greatest stain among all the components. If these stresses are concentrated while the polymerizing composite resin is undergoing shrinkage or during occlusal overloading of this layer, defects, cracks, or even abrupt catastrophic failure of the resin dentin bond may occur dependent on the amount of stress [32]: consequent failure may occur either in this layer or its adjacent layers [33].

The mechanical strength and viscosity of the adhesive layer might be improved with the addition of fillers [34]. These filled adhesives were predicted to serve as an intervening shock-absorbing elastic layer between the two stiff components, the composite resin and dentin, and increase the bond strength. However, when the nanoscaled fillers were added into the dentin adhesives, too much nano-filler addition increased the viscosity, hindered the penetration of the resin monomers into the interfibrillar spaces within the collagen network, and caused internal voids within the adhesives. NPs which are of the similar size to those of the polymer chain have led to good interaction between the chain/polymer due to the increased surface to volume ratio of the fillers. Thus, the method that reduces the particle size down to the nanoscale level has been widely used. Reports have also found that flexural and

Table 3.1 Etch and rinse adhesives and self-etch adhesives

Group	Sub-step	Conditioner	Primer	Adhesive resins
Etch and rinse adhesives	Two-step	Acid	Solvent mono-methacrylates + dimethacrylates HEMA + initiator + inhibitor + filler	
	Three-step	Acid	Solvent mono-methacrylates + initiator + inhibitor	Dimethacrylates HEMA + initiator + inhibitor + filler
Group	**Sub-step**	**Self-etch adhesives**		**Adhesive resins**
Self-etch adhesives	One-step	Solvent Mono-methacrylates + dimethacrylates HEMA + initiator + inhibitor + filler		–
	Two-step	Solvent acidic mono-methacrylates initiator + inhibitor		Dimethacrylates HEMA + initiator + inhibitor + filler

tensile strength can be significantly increased by incorporating 1–10 wt% silica nano-fillers into adhesive resins [35].

Recently, evidence has revealed that adding hydroxyapatite nanorods at 0.2–0.5 wt% to an experimental adhesive significantly increases its flexural strength and diametral tensile strength. Lohbauer et al. found that increasing the concentration of zirconia nano-fillers in either the primer or the adhesive greatly increased the microtensile bond strength [36]. However, different studies yield diverging conclusions regarding nanoparticle incorporation into adhesive resins, and dentin bond strength increases. Some studies found that adding filler content up to 20 wt% significantly increases the bond strength, whereas exceeding 50% results in bond strength deterioration [37]. If hydroxyapatite was used as the nano-filler, significant increases in bond strength occurred at 0.2 wt% incorporation, but decreased to the starting level with further packing [38]. Abdelaziz EM found that these exact systems also decreased viscosity while improving the microtensile bond strength [39]. On the other hand, if when these adhesive systems used 1 wt% HA-modified adhesive, the results were completely reversed. This can be explained by silane coupling having a stronger chemical-grafting on the surface of fillers at lower fill concentration, which improved stability and dispersion, and hence improved flowability while reducing the viscosity of the dental adhesives. In contrast, the particles were in closer proximity with each other at higher filler concentration, which result in greater collision probability under shear and creates aggregates. So, the mechanical strength will be the greatest at proper nanoparticle concentration.

Success of restorations are highly dependent on a durable and strong adhesion to dental hard tissues. Other challenges to dental restoration such as chewing forces and biofilm acids interact with the oral environment and limit the longevity of the restoration. Currently, the weak link in restorations can be attributed to the resin-bonded tooth interface, while secondary caries at the margins are the main impediment to longevity of restorations.

Adhesives containing CaP [$Ca_3(PO_4)_2$] particles could remineralize the remnants of tooth lesions in the cavity as well as the acid-etched dentin. Thus, they are promising to improve the longevity of the restorations. Due to the release by the adhesive of Ca and P ions, which serve as seed crystals, mineralization can be facilitated in the hybrid layer (HL) as well as the tooth-restoration margins. Furthermore, other advantages include the likely protection of the exposed collagen within the bonded interface by the CaP adhesive, which improves bonding stability and durability and the protection of the weak link of tooth restoration. However, since Ca and P ion release is only short term, lasting only weeks to a few months and then diminishing, this poses a major flaw for Ca–P containing resin. A previous study confirmed this short ion release duration for CaP resins, showing an ion release of only 1–2 months. Therefore, it would be advantageous to develop a rechargeable CaP adhesive to constantly refill the Ca and P ions to provide long-term ion release for remineralization and the ultimate inhibition of caries. Thus, nano-CaP (NACP) (116 nm) is synthesized which is rechargeable to provide long-term Ca and P ions. Because NACP has smaller particle size, it can more easily flow with the bonding agent into the dentinal tubules to result in resin tags. This adhesive can also be considered “smart”

because they released Ca and P ions, which are beneficial to combat caries, at low cariogenic pH. Among all the bonding agents tested (the NACP content from 0% to 30%), adhesive with 30% NACP appeared to be the best, considering its highest dentin bond strength, Ca and P ion release, and recharge and re-release. NACP filler levels ≥40% were not used due to a decrease in dentin bond strength in preliminary study. After recharge, the resins had continuous release of ions for at least 2–3 weeks, before another recharge would be needed [40].

Moreover, incorporating high atomic number containing particles may induce radiopacity in adhesive systems, compared to the typical radiolucent property of the adhesive layer. Regrettably, these radiolucent radiographic images might misguide dentists as they may resemble those of defective restoration and secondary caries, as a consequence, resulting in possible clinical misdiagnosis or needless replacement of restorations which cause increased cost, chair time, and patient discomfort.

To overcome these, oxides such as silicon dioxide (SiO_2), titanium dioxide, strontium oxide, zirconia dioxide, barium oxide, and barium sulfate have been incorporated as radiopacifiers. Additionally, radiopacity been satisfactorily shown by using ytterbium trifluoride (YbF_3) as a source [41], while using SiO_2 NPs improved the cohesive strength of adhesive resins.

Unfortunately, unwanted problems could arise from the additives. For instance, incorporating a large concentration of fillers into dental resin significantly reduced the inter-particle spacing, increased the number of particle collisions, and increased the suspension viscosity, thereby possibly adversely affecting the mechanical properties of the material. Beyond that, excessive fillers could also cause the composite materials to lose dimensional stability, deteriorate in bond strength, weaken in flexural strength and diametral tensile strength, and decrease elastic modulus as well as fracture toughness.

To address this problem, YbF_3 (40–80 nm average particle size, nanostructured and amorphous materials) is added into the adhesive. For nano-based materials, it is of utmost importance that NPs do not jeopardize the chemo-mechanical stability of the modified material. Compared with nanoparticle-free adhesive, the YbF_3-modified adhesive improved radiopacity and maintained the bond strength to dentin stable over time. Considering the different results of the nanoparticle-modified adhesives, gaining radiopacity without sacrificing beneficial characteristics of dental bonding agents may depend on the type of radiopacifiers added into the material. As such, YbF_3 could be considered a promising candidate to develop novel radiopaque dental adhesives [35].

Contemporary adhesives are limited by their inability to successfully infiltrate into the acid-etched or self-etched exposed collagen fibril network of the demineralized dentin [42]. Denuded dentin collagen exposure along the dentin–resin interface is the result of this incomplete infiltration of resin and the hydrolysis of the polymerized resin. Bacterial acids and enzymes, as well as activated host-derived proteases, further degrade this already defective bonded interface, thereby eroding the lifespan of the resin–dentin bond [43]. Although several methods aimed to optimize the infiltration of resin into the collagen matrix of the demineralized dentin, such as

the usage of catalysts, various solvents, and hydrophilic resin monomers, they all failed.

To solve the limited resin penetration problem, a novel solution is to develop a novel adhesive that can be actively manipulated and forced to infiltrate and penetrate the interfibrillar spaces within acid-etched dentin and the dentinal tubules. This adhesive is bis-GMA-based and doped with spherical nanoparticles. The nanoparticles are composed of an iron core, a whitening coat shell of zirconium oxide (10–20 nm), and silanized by a monolayer of vinyl groups grafted on the surface of particles to covalently bond to the resin matrix. This nanoparticle-doped resin can be actively pulled into dentin interfibrillar spaces by magnetic force (~60 s), using off-the-shelf magnets (1.2 T). We hypothesize that this magnetic nano-adhesive system can enhance resin infiltration and penetration of dentinal tissues and that tubules thus enhance the adherence and seal. Magnetic forces, unlike the passive self-diffusion, might be able to actively enhance the adhesive penetration. Under the guidance of this external force, magnetic nanoparticles (MNP) can deliver more drugs to a target than either iontophoresis or diffusion. This system should also increase the surface area of dentin available for bonding and may help counteract adhesive lift due to polymerization shrinkage of composite restorations, result in an improved bonded interface with dentin, and ultimately enhance the longevity of composite resin restorations. Furthermore, this system should not adversely affect pulpal health [44]. However, it did not have bactericidal and remineralization abilities. So, another study developed a novel magnetic nanoparticle-containing adhesive system with antibacterial and remineralization functions for the first time [43]. MNPs, dimethylaminohexadecyl methacrylate (DMAHDM), and amorphous calcium phosphate nanoparticles (NACP) were mixed into a commercially available adhesive resin (Adper ScotchBond, Multi-Purpose Adhesive, SBMP) at 2%, 5%, and 20%, respectively, by mass. The two types of magnetic nanoparticles, iron oxide nanoparticles and acrylate functionalized iron oxide nanoparticles (AINPs), each were added to the resin at 1% by mass. A commercial cube-shaped magnet was used to apply a magnetic force for 3 min for inducing dentin bonding, subsequently measurements were taken of dentin shear bond strengths. Furthermore, resins were used to grow *Streptococcus mutans* biofilms, and colony-forming units (CFU), metabolic activity, and lactic acid were measured. The phosphate (P) ion concentrations, calcium (Ca) ion concentrations, and pH of the biofilm culture were also determined. Results showed that it had greater dentin bond strength and antibacterial and remineralizing capabilities. The reason is that teeth restored using the nanoparticle-doped adhesive and magnetic pull displayed an extensive network of resin tags penetrating dentin, both vertically and horizontally, compared to control. Additionally, teeth restored by nanoparticle-doped adhesives displayed a significantly greater number of resin tags per field of view and average length of resin tags as compared to controls (Fig. 3.2).

Adhesives have been revolutionized by developments and improvements in material compositions and placement technology [40]. With the development of the nanotechnology, nano-scale adhesive is extensively studied. Here are the main nano-scale adhesives currently available: Prime&Bond NT (DENTSPLY); Adper™

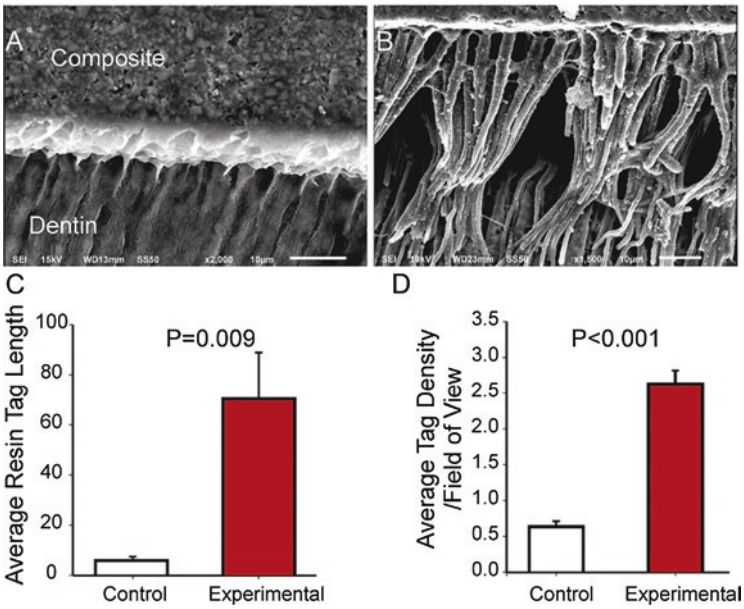

Fig. 3.2 Representative examples of SEM images from teeth restored using (**a**) control adhesive, (**b**) nano-adhesive with magnetic pull. (**c**) Quantification of average resin tag length and (**d**) density [44]

Single Bond 2 (3M ESPE); SE-BOND (kuraray). But the ingredients included are unclear. Due to its excellent adhesion strength, sealing property, desensitization effect, antibacterial property, and no significant markup in prices, nano-scale adhesive reaches a broader global market.

Above all, we know magnetic forces have a great effect on adhesive. But as yet, reports that have tested magnetic nanoparticle-doped adhesives with magnetic nanoparticle-doped adhesives have been scarce. There are still many aspects which need to be improved. For example, when the adhesive is applied, a magnetic field force should be exerted for 3 min. But the 3 min of magnet application is too long for clinical applications [45]. Therefore, further efforts are needed to investigate the incorporation of drug-loaded magnetic nanoparticles into bonding agents to help establish a new and effective strategy in conservative dentistry.

3.1.3 Nano Root Canal Filling Materials

In the human oral cavity, pulpitis and periapical periodontitis are the most common bacterial infections, with the major symptoms being pain, tooth defect, and dysfunction. The most effective treatment for pulpitis is root canal therapy (RCT) [46]. RCT, also known as endodontic treatment, an operation to treat pulp necrosis, root

infection, apical periodontitis, and endodontic retreatment in dentistry, is used to treat the infected pulp tissue that is composed of blood vessels and nerve tissue within the tooth. The aim is to eliminate inflammation and pain, while protecting the tooth against future reinfection as well as preserving its function. Conventional RCT usually applies dedicated instruments and methods to clean and restructure the root canal and appropriate drugs to disinfect and sterilize the root canal. Finally, it uses special materials to fill the root canal firmly (Fig. 3.3), thus eliminating the inflamed pulpal tissue, helping the healing of apical tissue, and preventing the development of periapical periodontitis [47]. The process requires the placement of a root canal filling with high sealing properties.

To clear the infections, a perfect root canal preparation and root canal filling are necessary. The chief criteria for the successful treatment of periapical periodontitis is the reduction or elimination of bacteria. To accomplish this, a combination of mechanical instruments, various irrigation solutions, and antibacterial dressings or medicants placed into the canal are required [48]. Before root canal preparation, the root canal wall has a dentine smear layer attached. This could negatively affect the root-filling material adhesion to the root canal wall and result in apical leakage [49, 50]. Three main factors are involved, namely the complexity of the anatomical structure of the root canal, the biofilm lifestyle, and the low permeability of the lavatory fluid into the dentinal tube. Thus, the conventional procedure rarely achieves complete roots canal disinfection and closure [51, 52]. The complexity of the anatomic root canal system enables bacteria to hide and multiply [53], limiting the elimination of microorganisms in specific areas of the root canal despite the use of high-quality instrumentation and various irrigating solutions [54]. Thus, the major problems in RCT are the inability to completely clear and restructure the canal using standard cleaning and shaping procedures. Eliminating bacterial biofilms surviving within the anatomic complexities and uninstrumented portions of the root

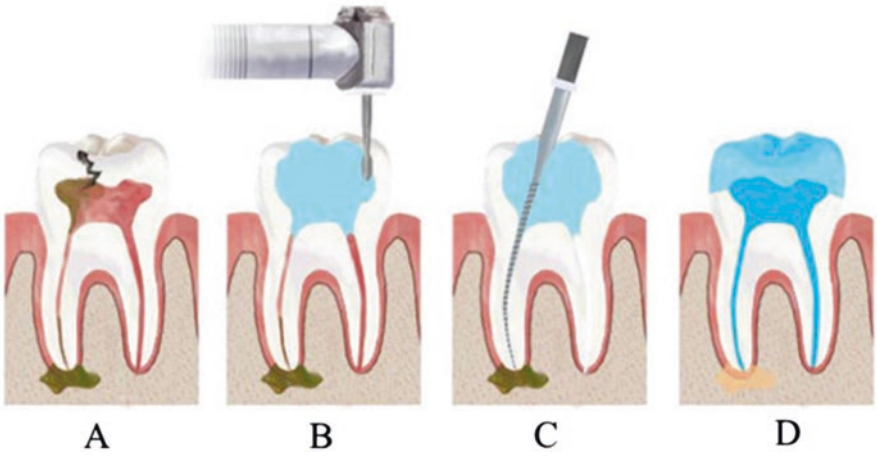

Fig. 3.3 The condition of the tooth before and after root canal therapy: (**a**) pulpitis caused by dental caries; (**b**, **c**) root canal preparation by appropriate equipment; (**d**) perfect root canal filling

canal system continue to pose a challenge [55]. Therefore, the basic root canal preparation greatly affects the success rate of RCT.

High-quality instruments are vital to root canal preparation. Some of the critical features of the instruments are now measured using the latest nano-indentation technology. Nano-indentation, also known as depth-sensing indentation and ultra-low-load indentation, is a relatively new form of sensitive mechanical testing that is appropriate to capture the incidence of localized phase changes [56]. A study by Jamleh et al. demonstrates that the nano-indentation technique can be applied to determine the performance of NiTi instrument [57]. This application has an important influence on the development of RCT.

Hermetic sealing is the primary factor associated with the success of RCT. Ingle et al. pointed out that 58% treatment failures were due to incomplete obturation [58]. Microorganisms and their byproducts are the main etiologic factors in the initiation, propagation, and persistence of pulpal and periapical infections [59]. Minimizing gap and void formation during root canal obturation is clinically relevant because as little as 1% shrinkage of the sealers may result in filling deficiencies due to the penetration of bacteria and their byproducts [60]. RCT prevents bacteria in an oral environment from entering and re-infecting the root canal. It also prevents the tissue fluid from going into the root canal, which otherwise would become the culture media for the residual bacteria and result in periapical periodontitis. The apical third is the most complex and critical area in the root canal system, not only for root canal instrumentation but also for root canal filling [61]. Thus, the apical sealing of the root canal is one of the most important indicators for a successful RCT. Therefore, complete root canal filling is another important factor in RCT.

The traditional root canal filling material refers to the material that is used to fill the root canal in RCT treatment, eliminating the dead cavity and preventing reinfection in the root canal. It includes three categories: a solid, a paste, and a liquid. The solid material includes gutta-percha points, silver cones, and plastic points. The paste material is often used in conjunction with solid materials, which is mainly used to bond the solid material with the root canal wall, while filling the gap between the solid material and the root canal wall. It includes zinc oxide lilac oil root canal filling material, root canal paste (AH-Plus), calcium hydroxide pastes, thymol paste, and iodoform paste. The liquid material is mainly phenolic resin.

The obturation of root canal system is an essential part in endodontics to close the leakage pathways from the coronal and apical directions [62]. Many types of root canal sealers have been introduced to seal the canal. But an ideal root canal sealer must have the following properties: sufficient setting time to allow enough working time, an excellent sealing and perfect dimensional stability after setting, adequate adhesion with canal walls, tissue tolerance and high biocompatibility, and insolubility to tissue fluids [63]. Decontamination and 3D obturation are essential in RCT. However, most of the obturating materials cannot provide an effective seal [64]. As a result, root canal filling materials are continuously improved, and bioactive materials are becoming increasingly popular.

Currently, commercially available sealers can be broadly categorized into the following types: zinc oxide eugenol-based, calcium hydroxide-based, glass

ionomer-based, resin-based, silicone-based, and calcium silicate-based sealers. However, none of the existing sealers fit all the criteria required to be ideal. Dissolution when contacting periapical tissues are a common drawback for zinc oxide eugenol-based [65], calcium hydroxide-based [66] and glass ionomer-based sealers. Moreover, slight shrinkage occurs during settings with zinc oxide eugenol-based sealers [60, 67].

Different materials and techniques have been investigated in order to establish the ideal root canal seal for filling the root canal space. Among these, the cold lateral condensation of gutta-percha (GP) technique in conjunction with a sealer remains one of the most popular techniques that has both been extensively investigated and employed [68]: some authors refer to it as the gold standard [69, 70]. Currently, RCT obturation uses the current standard materials: GP, an inert thermoplastic polymer consisting of the GP latex, zinc oxide, a radiopacifier which allows clinical X-ray imaging to monitor the treatment, and a plasticizer [71]. Filling the root canal space with GP is the standard of care for endodontic therapies, and it has a long history of application. It offers numerous advantages, including good biocompatibility, low cost efficiency, and easy removal. However, it also has several limitations. Conventional GP obturation may still lead to endodontic failures due to reinfection of root canals, which are in part associated with microleakage. These poorly obturated root canals create possible passages for bacteria, fluid, and chemical substance to penetrate [72, 73]. The phenomenon occurs because the sealers that are currently available, such as the widely adopted root filling material (thermoplastic GP), reveals volume shrinkage after cooling [74]. Conventional GP may be conducive to bacterial regrowth. If there are bacterial remnants in the root canal space, the tissue fluid would reestablish contact. While being frequently used, it has been previously reported to be associated with microleakage which would allow oral fluids and bacteria to access the treated root canal and suboptimal mechanical properties with respect to handling the material, potentially resulting in buckling during obturation [75]. Due to the dimensional changes and lack of adhesion from GP, it is not easy to achieve a complete filling with the current root-filling materials. Therefore, the adaptability of a sealer to the dentin is the primary method to prevent the microleakage and reinfection of the root canal.

Epoxy resin-based sealers demonstrate good dimensional stability [76] and good adhesive properties [77]. Therefore, they are good candidates to provide an adequate root canal seal. The intricate nature of their adhesion to dentin [78] and their ability to undergo slight expansion [79] may partly explain their thorough sealing properties. AH-Plus is used frequently in clinic and is commonly chosen as the control in studies of the new sealer's properties because of its good flowability, proper film thickness, and viscosity [80]. It is an epoxy resin-based sealer with good physicochemical properties [81] and antibacterial effect [82]. Previous studies suggested that AH-Plus can be considered the gold standard for root canal sealants. The combination of AH-Plus and GP is commonly used in RCT. It has many advantages in clinical practice, including permanent seal, excellent X-ray resistance, easy mixing, unapparent shrinkage after solidification, good long-term space stability and

sealing characteristics, no irritation, and non-interference with the bond strength of fiberglass posts cemented by adhesive resin cements [83].

However, the contemporary dental root-end filling materials still do not possess all desirable features, such as biocompatibility, adhesion to tooth structures, and antibacterial activity [84]. The good biocompatibility of the sealing material is needed when it is in contact with surrounding tissues to achieve a successful long-term outcome [85]. Mineral trioxide aggregate (MTA) is a kind of trioxide agglomerate and gray powder, including calcium silicate, calcium oxide, calcium phosphate, and other components. Several studies have investigated the properties of MTA, including composition, radiopacity, setting time, and biocompatibility. Since the introduction of MTA to the market, it has gained popularity due to its excellent biocompatibility [86]. Previous reports have recognized that MTA acts as a bioactive material [87] which promotes mineralization [88]. MTA serves many purposes, such as internal and external root resorption repair [89, 90], furcal perforations sealing [91], apexification [92], as a direct pulp capping agent [93], and also as a retrograde filling material [94]. MTA can also be used as root canal filling material, which is usually used for pulp capping, perforation reparation, root tip formation, and root pour filling materials. However, MTA exhibits certain disadvantages such as handling difficulty, low acidic resistance, and long setting time [95]. Long setting time increases the probability of the contamination of MTA by oral fluids before the material is completely solidified.

Hence, the ideal material for root canal filling should have favorable biocompatibility, bioactivity, antibacterial property, flowability, small particle size, no setting shrinkage, no setting dissolution, complete coverage of root canal space, adhesion to tooth structures, etc. Nanotechnology is applied to improve the performance of present dental materials.

Due to the imperfection of the existing material, the field of nanomedicine has been applied to make important clinical advances in recent years. Nanomaterials are natural, incidental, or manufactured materials containing unbound, aggregate, or agglomerate state particles in which 50% or more of the particles based on number, size, distribution, or one or more of the external dimensions is within the 1–100 nm range [71]. Nanomaterial offers enhanced physicochemical properties, such as increased chemical reactivity, ultra-small sizes, and large surface area/mass ratio, as compared to their bulk counterparts [96, 97]. Nanoparticles (NPs) have been the focus of attention in the past few decades owing to their innovative and functional properties. Particles with dimensions of 1–1000 nm made from any type of biocompatible substance can be defined as NPs (but commonly defined as 5–350 nm in diameter) [98]. The use of nanotechnology has allowed many other developments in dentistry and advances in oral-health-related nanomaterial and therapeutic methods [99]. Nanotechnology has been developed rapidly and created a myriad of biomedical applications such as drug delivery, tissue regeneration, antimicrobial application, gene transfection, and imaging [100, 101]. Moreover, nanotechnology is used to produce many dental materials, including light-cured restorative composite resins and their bonding systems, impression materials, ceramics, the covering layers of dental implants, fluoride mouthwashes, and so on.

In nanoscale, a decrease in dimensions to the atomic level leads to a considerable increase in the surface area of the agent. Therefore, the contact of NPs with dentin can effectively achieve good sealing effect. Some studies show that bacterial biofilms are considered the major cause of both primary and secondary root canal infections [102, 103]. Silver nanoparticles (AgNPs) as a medicament and not as an irrigant showed potential to eliminate residual bacterial biofilms during root canal disinfection.

The quality of the filling material affects its ability to prevent reinfection of the root canal and the healing of apical lesion. Nanotechnology makes improvements in root canal filling materials. The nanotechnology is used to modify the current materials by adding nanostructured materials to enhance its properties. It enables the material to be antibacterial and enhances the obturation in RCT. Furthermore, the application of nanomaterials in clinic is more and more extensive. In the field of endodontics, nanomaterials is mainly used to improve antimicrobial efficacy, mechanical integrity of diseased dentin matrix, and tissue regeneration [104, 105]. The research and development of nanomaterials with excellent apical sealing ability is a significant contribution to increase the success rate of RCT. The advantages of using nanoparticles in endodontic sealers include improving their physicochemical characteristics, enhancing the antibacterial property, decreasing microleakage, and increasing biocompatibility [7, 106, 107].

Nano-modified cements can help to produce ideal root-end filling materials. Attempts have been made to improve the properties of MTA by incorporating nanomaterials. For example, incorporation of silicon dioxide (SiO_2) nanoparticles has improved the microstructure of MTA and accelerated the hydration process. Alternatively, antibacterial activity can be improved by adding chitosan or silver (Ag) nanoparticles [85]. Iron disulfide (FeS_2) nano or microparticles, originating naturally from hydrothermal sources and clay in the form of iron sulfide particles, have been extensively studied [108]. Results show that the FeS_2 nanocrystals are biocompatible, indicating their potential applications in biomedicine. These nanostructures can also effectively modulate MTA's mechanical properties. At small concentrations (0.2–0.6 wt%), these nanostructures prevent volume changes, resulting in a stiffer material and reduced initial setting time than conventional MTA [109]. Thus, the addition of FeS_2 nanostructures to MTA can improve its physical and biological properties [110]. The particle size of MTA is important for its clinical applications in dentistry due to its influence in creating an adequate seal between the material and the dentin [111]. It is necessary that the particle size of the MTA should be smaller than the size of the dentinal tubules. It allows the MTA particles to penetrate the tubules and provide an adequate seal.

A nano-modification of white mineral trioxide aggregate (WMTA), mainly changes in the surface porosity, microhardness, and setting time, is a new root-end filling cement with similar composition to WMTA. However, the former was more resistance to an acidic environment and set ten times faster than WMTA [112]. The initial setting time of WMTA was approximately 40 min, while that of nano-WMTA (nano-modified with white mineral trioxide aggregate) was only about 6 min [112]. Therefore, a shorter setting time prevented the bacterial reinfection in the root canal

to a certain extent and reduced the chance of tissue fluid infiltrating into the root canal. An acidic environment had an adverse effect on the microhardness of WMTA cement, which was consistent with previous studies [113]. However, WMTA was affected much greater than nano-WMTA, which might be attributed to the greater porosity of WMTA compared with nano-WMTA. The greater porosity might accelerate the acid penetration into the surface texture and decrease the surface microhardness. Nano-WMTA revealed low surface porosity and high acid resistance. Greater porosity can also increase crack propagation. The push-out bond strength is recommended in further investigation, because WMTA has been widely applied to perforation repair materials, root canal retrograde filling materials, and the materials of the formation of the top barrier in clinical practice. The push-out bond strength of nano-WMTA is significantly higher than WMTA [114]. It is indicated that the strong adhesion between materials and the dentin tubules, which can reduce the microleakage and improve the maneuverability of root canal filling materials. The smaller particle size and uniform distribution of constituents in nano-WMTA are the two influencing factors.

Recent literatures lay strong emphasis on the prospects of nanotechnology to improve mechanical strength (e.g. nanofibers incorporation) and provide antibacterial protective effects (e.g. silver nanoparticles and chlorhexidine diacetate) to resin-based dental sealants [115]. Chitosan (CH), chitin's deacetylated form, is a nontoxic biopolymer which has garnered much attention based on its biocompatibility, antioxidant, anti-inflammatory, and antibacterial properties. While nylon is a polyamide having excellent strength, flexibility, and abrasion resistance [116]. Due to these properties, reinforcement of dental composites has used electrospun nylon-6 (N6) nanofibers [117]. Hamilton et al. successfully prepared CH and N6 nanofibers via electrospinning to improve the mechanical properties and provide an antibacterial protective effect to resin-based dental sealants [118] (Fig. 3.4). The overall results indicated that the CH groups exhibited significantly higher flexural strength (FS) and hardness than any other group. Molecular weight (MW) and degree of deacetylation (DA) vary among the different types of chitosan; these factors that independently influence the antimicrobial activity of chitosan. It has been reported that lower MW CHs have greater antimicrobial activity than high MW CHs. However, with the limitations of above in vitro study, further investigation is needed to evaluate whether chitosan with different molecular weight and degree of deacetylation may enhance the physicomechanical and antibacterial properties of the materials tested.

We have earlier discussed the merits and drawbacks of GP in RCT. While GP offers numerous advantages, it cannot provide adequate seal to prevent bacterial percolation which is a challenge in endodontic therapy. To address these challenges, clinicians have explored other root canal filling materials than GP. Detonation of nano-diamonds (NDs), which are carbon nanoparticles approximately 4–6 nm in diameter, are very attractive. They are the waste byproducts that are readily processed for biomedical applications [119–127]. NDs may offer unique advantages due to their favorable properties, particularly for dental applications. These include versatile faceted surface chemistry, biocompatibility, and their role in improving

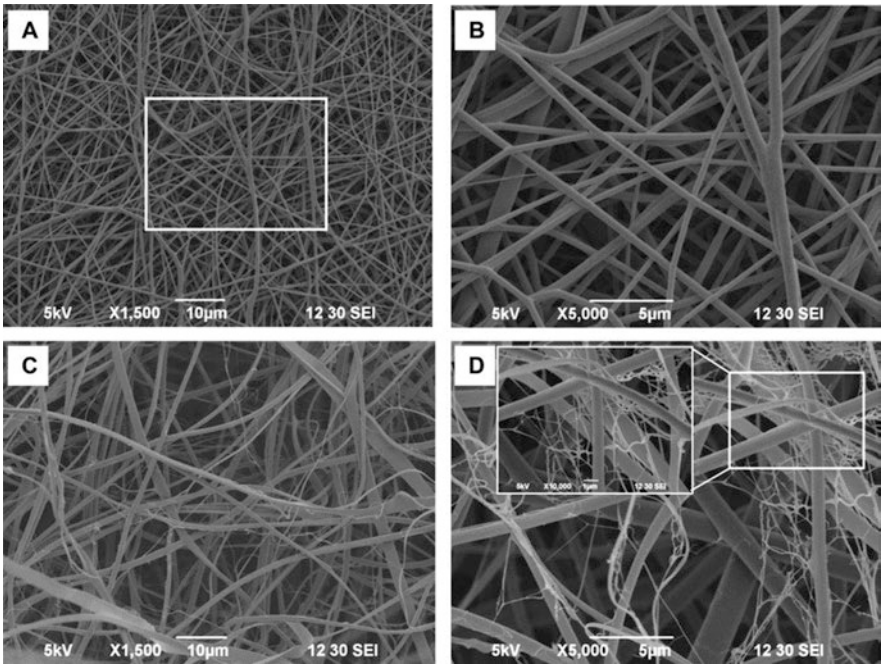

Fig. 3.4 Electrospun nylon-6 (**A**, **B**) and chitosan (**C**, **D**) nanofibers shown using representative SEM images at various magnifications. (**D**) The selected area as shown under higher magnification (5000×). (**D**) Chitosan fiber branching shown under higher magnification images (5000× and 10,000×) [119]

mechanical properties. Importantly, NDs with consistent particle size and surface chemistry properties can be largely synthesized, supporting their clinical transition [128–130]. Thus, researchers developed a nano-diamond gutta-percha composite (NDGP) embedded with nano-diamond amoxicillin (ND-AMC) conjugates (Fig. 3.5a), which can reduce the likelihood of root canal reinfection and enhance the treatment prognosis. While multiple nanoparticles have been evaluated in human studies, there remains a need to accelerate novel, nanotechnology-enabled strategies which can enhance the efficacy and safety of therapy [132, 133]. ND-containing composite materials have also been shown to exhibit superior mechanical properties compared with unmodified materials (Fig. 3.5b) [125, 131, 134]. Examination of the clinical potential of these key ND attributes [131] finds that the thermoplastic biomaterial, ND-embedded gutta-percha, could be utilized as a nonsurgical RCT filler material in preventing reinfection and enabling lesion healing. While the administration of NDGP also confers simultaneously the beneficial properties of GP with the increased mechanical strength and ND-mediated antimicrobial and/or pharmacological antimicrobial activity. Therefore, these capabilities may reduce the risk of root canal reinfection and improve the long-term treatment outcomes.

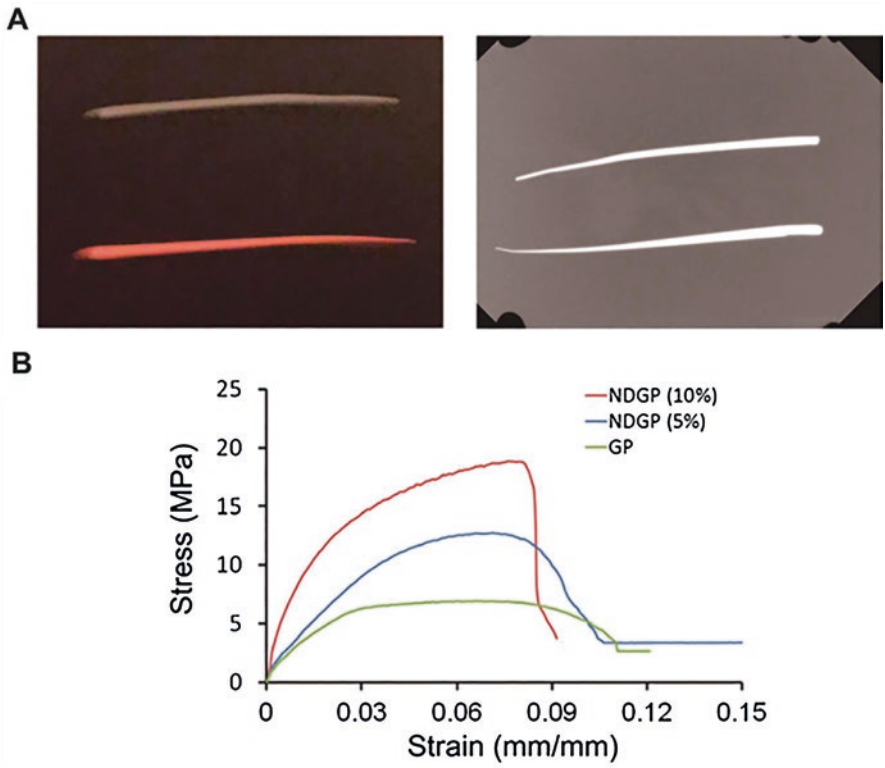

Fig. 3.5 Radiopaque and mechanical properties of NDGP. (**A**) Left: NGDP photographs (top) and unmodified GP (bottom). Right: Digital X-ray imaging showing the radiopaque property of NDGP (top) was similar to that of the unmodified GP (bottom). (**B**) Tensile test results expressed as stress–strain curves of G, and NDGP (5 wt% ND, 10 wt% ND). The tests were conducted at 0.3 cm/min strain rate with the sample gauge lengths set to 0.89 cm. Results clearly indicated the areas under the NGDPs curves (5% and 10%) were larger than the corresponding unmodified GP, suggesting the modified NGDPs were more mechanically robust [131]

Researchers fabricated bioactive mesoporous calcium–silicate (MCS) NPs (around 100 nm) with high specific surface area and pore volume to prepare the injectable materials for the filling of the root canal. The apatite mineralization ability, in vitro osteogenesis, drug delivery, and antibacterial properties of the material were evaluated. The prepared MCS was found to be easily injected. And it could fill the apical root canal successfully. MCS NPs induced apatite mineralization in DMEM solution without any cytotoxic effects [135]. Hence, it suggested that MCS NPs could be an advanced biomaterial with multiple functions due to their unique nanostructure, injectability, and antibacterial efficiency. Meanwhile, it also has the features of apatite mineralization, osteostimulation, and drug delivery [136].

Recently, a new group of sealers, bioceramic (BC) sealers, were introduced for dental practice [137]. BC materials, calcium silicate-based materials, are osteoinductive bioceramic products or components employed in medical and dental

application mainly as implants and replacements [138]. The calcium silicate-based material, which is a cement paste, has attracted considerable attention, because of its good penetration ability and flowability. The Total Fill BC sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland), one of the first commercially available sealers in Europe, contains the same chemical composition as the Endo Sequence BC sealer (Brasseler, Savannah, GA, USA), which is a nanomaterial. Endo Sequence BC sealer is one of the ideal bioactive sealants that contains nanoparticles facilitating its penetrating into dentinal tubules [139]. It is composed of calcium silicates, calcium phosphate monobasic, calcium hydroxide, zirconium oxide, other filler, and thickening agents. This is also marketed as the iRoot SP (Innovative. BioCeramix Inc., Vancouver, British Columbia, Canada) which has been provided as an ideal premixed, ready-to-use, and injectable biomaterial in dental clinic, exhibiting excellent radiopacity, insolubility, zero-shrinkage, and hydrophilic (due to using moisture from the dentinal tubules to start and complete the setting reaction) qualities [140]. The shrinkage during setting and dissolution when contacted with tissue fluid is the common problems for most of the currently available sealers [65]. Total Fill BC sealer is no exception. These shortcomings will result in the existence of gaps in the interface of sealers and root canal wall which lead to the microleakage in the root canal system. Such gaps ultimately affect the outcome of root canal treatment. Nanoparticles have improved the handling and physical properties of Total Fill BC sealer. BC sealer, due to its hydrophilic properties [108], uses the moisture within the root canal to finish the setting reaction. Hydration reactions of calcium silicates are facilitated by this moisture, which produces calcium hydroxide and silicate hydrogel. These products can partially react with the phosphate to form water and hydroxyapatite, along with the formation of a nanocomposites structure of calcium silicate and hydroxyapatite. The hydration reaction and setting time are affected by the availability of water [102]: setting time may be prolonged in overly dried canals. It sets and becomes hard in a few hours, providing excellent seal and dimensional stability. Upon setting, it forms the hydroxyapatite, providing excellent biocompatibility and bioactivity. Many in vitro studies have indicated its good biocompatibility [80], bioactivity [141, 142], antibacterial property [143, 144], and certain kinds of sealing ability [145, 146]. Moreover, it has favorable flowability, small particle size, and acceptable volume expansion [147, 148], which directly affect the root canal filling condition. The manufacturers recommend this sealer to be used together with the Total Fill BC gutta-percha (FKG Dentaire, La Chaux-de-Fonds, Switzerland), which when coated and impregnated with a nano-layer of BC particles improves the adaptation [149].

With its properties of almost no shrinkage when setting and no dissolution when in contact with tissue fluids, the new bioceramic sealer may be a promising filling material [150]. The iRoot SP penetration of sealers into dentinal tubules can form a physical barrier to prevent bacterial microleakage and recontamination of root canal system [151]. It will also generate micro-mechanical interlocking with dentine and strengthen the resistance to bacteria of the filling material, and the deep penetration into dentinal tubules can maintain their bactericidal effect [152, 153], which is favorable for the healing of the periapical lesion. In addition, the moisture

remaining in the dentinal tubules will trigger its setting reaction with the production of hydroxyapatite and create the chemical bond with root dentine [141]. A study [154] indicated that the depth of sealer penetration into dentinal tubules had no correlation with the sealability of nonbonded root fillings. However, it was of paramount clinical relevance, particularly for iRoot SP. Therefore, the sealing of the root canal is improved. It was found that bioceramic sealer iRoot SP penetrated dentinal tubules better than AH-Plus in the root tip 2 mm. The good penetration ability of iRoot SP may be one of the factors which are the reasons for the success of endodontic treatments.

Nowadays, MTA Fillapex is one of the recently developed endodontic sealers [155–157]. This endodontic sealer (MTA Fillapex; Angelus Solucoes Odontologicas, Londrina, PR, Brazil) consists of MTA, natural resin, salicylate resin, bismuth, nanoparticulated silica, and pigments. However, information about the physicochemical properties of MTA Fillapex is scarce. These properties must be further researched as they have an impact on the final quality of root filling [158]. For example, the effectiveness obturating the accessory canals and voids between master and accessory gum tips is dependent on the flow of endodontic sealers; adequate flow allows for proper filling of irregularities, whereas high could cause apical extrusion, leading to the cytotoxicity of the sealers causing periapical tissue injury [159]. Since proper fluidity has a great effect on the quality of the root canal filling, much attention has been paid to the flow properties of endodontics sealers. MTA Fillapex sealer was more flowable than the Endosequence BC sealer. An excessive flow rate increases the probability of extrusion into periodontal tissues. Moreover, the pH change of sealers, which is associated with antimicrobial effects and deposition of mineralized tissue [66, 160–162], may play a role in healing: neutralization of lactic acid produced by osteoclasts as well as the prevention of mineralized tooth dissolution can be associated with the effects of the alkaline pH of root canal sealers. Therefore, by activating alkaline phosphatases, the root canal sealers, especially bioceramic-based sealers, can assist in hard tissue formation [163]. Comparing the two novel root sealers, MTA Fillapex and the Endo sequence BC, the former although showing higher flowability and thicker film also displays shorter working time, setting time, and solubility than the latter. On the other hand, both sealers produce an alkaline pH when immersed in distilled water and even after setting, which may contribute to their osteogenic potential, biocompatibility, and antibacterial ability. All sealers satisfied the criteria for flowability, solubility, film thickness, and dimensional change. The new endodontic sealers, Endo sequence BC and MTA Fillapex, both possessed acceptable flowability and dimensional stability. But they had higher solubility, film thickness, and better sealing capability than AH Plus.

Zinc oxide-eugenol (ZOE)-based sealers have been widely used in endodontic for many years. Nanotechnology has been applied to overcome their limitations. A study showed that the incorporating zinc oxide nanoparticles enhanced the physicochemical characteristics (setting time, flowability, solubility, dimensional stability, and radiopacity) of Grossman sealer [107]. The new nano zinc oxide-eugenol (NZOE) sealer produced less microleakage than Pulpdent and AH-26 root canal sealers. NZOE has less cytotoxicity than Pulpdent sealer, suggesting that the

incorporation of zinc oxide nanoparticles can decrease the cytotoxicity of ZOE-based sealers [164].

Hydroxyl apatite nanoparticles (HAp NPs) have been used widely in medicine and dentistry. Typically, they are used as implant coatings for better biocompatibility and wear resistance [165]. They are also used for the preparation of bone graft [166]. The HAp NPs can easily integrate into the dental tubules, which is beneficial to root canal sealing. HAp NPs have greater surface area, resulting in strong binding with proteins as well as with bacterial and plaque fragments [167]. Biocompatibility studies indicated that hydroxyapatite can bind to bone, and it will not lead to any local and systemic inflammatory response. It has great significance in treatments such as root canal filling, tooth filling, and dental implant.

Another example is adding silver nanoparticles and GP powder to the silicon-based sealer (Gutta-Flow Sealer). Available in uni-dose capsule form, this material can be mixed and injected [108]. This nano-sealer has multiple advantages such as good biocompatibility, stable dimension, and short setting time. The material has also been reported to have improved sealing capability and better resistance to bacterial penetration.

Endodontic sealers are noted for their highly beneficial antibacterial activity. Recently, antibacterial quaternary ammonium polyethyleneimine (QPEI) nanoparticles have been incorporated into the existing sealers such as AH plus, Epiphany, and Gutta-flow [168]. Resin composites containing QPEI nanoparticles resulted in prolonged antibacterial activity without compromising the mechanical properties [169]. The addition of QPEI nanoparticles is very stable, leaching no byproducts in the surrounding and showing no influence on the biocompatibility.

The novel injectable self-curing polyurethane (PU)-based antibacterial root canal sealer by incorporating silver phosphate (Ag_3PO_4) particles is being developed. Ag_3PO_4 can be evenly distributed within the material. The release of Ag^+ can provide satisfactory antibacterial effects. Physical properties such as setting time, film thickness, and solubility were important indexes which were used to evaluate the properties of endodontic sealers [170]. The study showed that the setting time shortened with the increased Ag_3PO_4 content in the PU sealers. Film thickness and solubility increased with the increased Ag_3PO_4 concentration. In a study, PU was set with different concentration of Ag_3PO_4 as the amount of research such as 0 wt% (PU0), 1 wt% (PU1), 3 wt% (PU3), and 5 wt% (PU5) concentrations of Ag_3PO_4 [170]. The setting times of the PU sealers with different proportions of Ag_3PO_4 are just shorter than the setting times of AH Plus and can meet the requirement of ISO standard. These properties make fabricated PU-based sealers better than AH-Plus. As Ag_3PO_4 content is increasingly incorporated into the PU sealer, the curing time shortens, inferring that Ag_3PO_4 (or Ag+) might be a driving force for polymerization [170]. And PU0, PU1, and PU3 sealers have good flowability. Another important requirement for dental material, especially when exposed to a host environment for a prolonged period, is its resistance to solubility and degradation. The solubility of the fabricated PU-based sealers increases with the content of incorporated Ag_3PO_4. This correlation may be attributed to more Ag_3PO_4 on the surface of the material

dissolved in water [170]. More importantly, root fracture often happens after RCT. Root fracture risk is related to tangential strain, which is affected by the expansion of the material as well as the elastic modulus of the root canal sealer and dentin. Thus, sealers with low elastic modulus pose lower risk for dentin fracture compared to those with higher modulus. The low moduli of PU-based sealers can reduce the amount of damage to root dentin that is generated by volume expansion. The cytocompatibility evaluation revealed that the PU1 and PU3 sealers possess good cytocompatibility and low cytotoxicity. PU5 exhibited the highest cytotoxicity. PU3 sealer offers good physicochemical and antimicrobial properties along with acceptable cytocompatibility, which may hold great application potential in the field of root canal fillings. Therefore, the properties of PU reveal that it is an excellent candidate for root sealers.

Ideal root canal filling materials should possess good biocompatibility, mobility, X-ray radiopaque ability, antibacterial ability, and stability and can seal the root canal cavity completely. An ideal root canal sealer should be non-cytotoxic, non-mutagenic, and immunologically compatible with periapical tissues [171, 172]. Therefore, further research is required to improve the materials. Within the limit of our knowledge, we put forward several points here.

First, GP surfaces can be coated with nanoparticles. For example, optical fiber glasses tips can be covered with AgNPs, which is a polymeric material with light transmission properties and bacteriostatic qualities. Therefore, they can be a substitutive material to GP. Nanoindentation results demonstrated that the optical fibers covered by AgNPs achieved excellent nano-hardness and elastic modulus considerably. It made the material more rigid and more resistant to corrosion. And they ensure the compatibility with the glass fiber posts and resin cement materials. The main reason optical fiber-silver nanoparticles are suitable for dental root canals is due to their superior bacteriostatic properties. Another advantage is it contains optical fiber, the light transmission properties of which allows the use of light curing liquid resins in the root canal.

Second, novel new type of root canal filling materials can be designed and developed, such as nanocomposite hydrogels. Hydrogels, due to its excellent biocompatibility, swelling property, and stability, can prevent apical or coronal microleakage. Hydrogels also have the potential as root canal sealers, but still need to have certain degree of X-ray radiopaque and antibacterial ability. Nowadays, the current commercialized zirconia nanoparticles lack X-ray radiopaque property and long-lasting antibacterial function. Based on the dispersion and stability issues of water-soluble AgNPs, ZrO_2 can be diffused in the hydrogels of AgNPs to prepare ZrO_2, Ag, and ZrO_2/Ag nanocomposite hydrogels.

Third, the cold flowable GP can be improved. Gutta-flow is a novel cold flowable root canal filling system. Future work is required to improve its flowability, adjust its setting time, and deepen its penetration depth.

3.1.4 Nano Enhanced Resin-Based Materials for Dentition Restoration

The importance of healthy teeth goes beyond the practical use of teeth to chew and break down food. It also has a significant impact on a person's attractiveness, confidence, and ultimately their quality of life. The loss of dentition can have a great impact on an individual's local and systemic health including alveolar bone resorption, periodontal tilt, temporomandibular joint disorder, and digestive system dysfunction.

Therefore, the field of dentistry has put forth a significant amount of effort to restore the lost teeth. The removable partial denture (RPD) is one of the most commonly used resolutions. RPD is a type of restoration that patients can remove by themselves, which uses the natural teeth, the mucous membrane, and base tissue to underpin for support and retention. The artificial teeth are used to restore the missing teeth including the morphology and function. And the base materials are used to restore the missing alveolar bone, jaw, and its surrounding soft tissue.

RPD is composed of a denture base, artificial teeth, and retainers [173]. Artificial teeth assist in the restoration of masticatory efficiency, pronunciation, and improvement of esthetic appeal. The retainer is used to stabilize the base and improve masticatory function. The denture base is a major component of RPD, which covers the alveolar ridge in the missing tooth area and the palate. Its main function is to arrange and attach artificial teeth, conduct and disperse bite force, and connect all parts of the denture into a whole. Additionally, the denture base materials should also have good biocompatibility, good chemical and size stability, as well as rigorously tested mechanical properties such as the flexural, compressive strength, impact strength, suitable hardness, and wear resistance. The dentures also need to be easy to fix, nontoxic, and non-irritating and have low solubility in saliva. Furthermore, they should also be esthetically pleasing, cheap, and contain anti-microbial properties.

Dental acrylic resin has been widely used as denture materials since it was developed by Walter Bauer in 1936. These resins commonly consist of methyl methacrylate (MMA) and polymethyl methacrylate (PMMA) [174, 175]. MMA resin is a commonly used dental material, because it is quick to cure at low temperatures and is naturally balanced in terms of hardness and toughness. It has suitable wear resistance and maintains good transparency. PMMA is one of the most commonly used dental materials in prosthodontics due to its other desirable characteristics, such as accurate reproduction of surface details, lack of toxicity, better transparency, outstanding aging resistance, good insulation, corrosion resistance, and cost-effectiveness [174, 175].

Denture base resin can be classified according to the polymerization and curing mode into four kinds: (1) heat-curing denture base resin, (2) self-curing denture base resin, (3) light-curing denture base resin, and (4) thermoplastic injection molding denture base resin. The heat-curing resin is composed of MMA mixed with its homopolymer powder or copolymer powder. Different copolymerized powders result in different performance of the final resin. The impact strength and flexural

strength of denture base bracket are improved by MMA and butyl acrylate (BA) block copolymer. The copolymerized powder of MMA and methyl acrylate require less water for plastic filling, which improves the wear and abrasion resistance of the base support. MMA, ethyl acrylate (EA), and methyl acrylate (MA) ternary copolymerized powder improve the mechanical properties of the base resin. The graft copolymerized powder of MMA and rubber improve the impact strength of the base resin and obviously enhance its toughness. The self-curing resin refers to the base material that is polymerized by REDOX system at room temperature. It is composed of methyl methacrylate and PMMA homopolymer powder or copolymer powder, which can shorten the time that distension denture base powder is dissolved in the solution of the denture base. The light-curing denture base resin is a single component and dough-shaped plasticizer, and the resin matrix is mainly bis-GMA and isocyanate-modified bis-GMA. The material has an excellent working time before curing. Commonly used thermoplastic-injection-molding materials include polyamide (nylon), which is soft and elastic. It can be used to fabricate teeth or gum color resin clasp, which is known as the invisible denture. They are widely used in provisional prosthesis and orthodontic removable appliances, as temporary prosthetic base materials, and to repair dentures. In addition, because they are cheap and easy to make and repair, the materials are perfect for molding material [176, 177].

Full and removable partial dentures have been used widely in oral clinical applications such as traditional prosthetic methods [178]. However, current resin-based materials have some drawbacks including insufficient surface hardness, low flexural strength [179], and poor antibacterial activity [180]. It is also a common cause of abutment caries and denture stomatitis [181]. Current dentures also have relatively poor mechanical properties which can lead to the denture base fracture and affect the longevity of the dentures [175, 182, 183]. Due to these properties, these fractures are commonplace in prosthodontics and cause problems for both prosthodontists and patients. Fracture can also occur due to fatigue arising from the bending forces caused by inordinate masticatory forces or from denture deformation [184]. Another factor that determines the esthetics of denture is the transparency or transparency rate of base material. These deficiencies require special technique and material for improvements.

Recent advancement in materials science and the introduction of nanotechnology in dentistry leads to new materials, with improved esthetic, antimicrobial property, and durability. Nanotechnology [185] was coined by Japanese scientist Dr. Nori Taniguchi in 1974 and defined as the processing of separation, consolidation, and deformation of materials by one atom or one molecule [186]. Nanotechnology allows the manufacturing of products of less than 100 nm to create designs and perform functions which was impossible previously [187].

The global market for dental materials is expected to increase rapidly due to multiple factors such as improved awareness, healthier lifestyles, expanding populations and life expectancy. This demand stimulates the development of novel materials to remedy the inadequacies of current dental materials. Dentures will deteriorate over time, may fracture due to external stresses [183, 188]. The application of nanomaterials in dentistry will be helpful to improve the quality of life of the patients.

Various attempts have been undertaken to enhance the physical properties of the denture base material, such as chemically modifying poly(methyl methacrylate) (PMMA), developing alternatives to PMMA, and reinforcing PMMA with other materials, e.g., fibers and nano-fillers [189, 190]. To facilitate clinical applications, various micro- or nano-sized fillers have been incorporated into PMMA [191–194], with the aim to develop new materials or significantly improve the properties of existing materials.

Due to unique properties, nanomaterials are always the research focus of biomaterials scientists. Nanomaterials have been developing in four categories (metals, polymers, ceramics, and composites) for practical applications in health care [188]. They combine the advantages from various nanomaterials to improve the quality of life. Some studies have investigated the effect of incorporating inorganic NPs into PMMA [190, 195, 196]. The shape, size, as well as the concentration and interaction of these nanoparticles with a polymer matrix determine the properties of a polymer nanocomposite mixture [195]. The properties of the reinforced resin by nanoparticles depend on the size, shape, type, and concentration of the added nanoparticles [197]. The properties of the resin base material can be improved by dispersing nano-silica particles evenly into the resin material, including (1) improves the strength and elongation, (2) improve the abrasion resistance and the surface finish of the material, and (3) anti-aging performance.

Clinical handling has improved after nanoparticles are added to materials. As compared to amalgam, the dental resin 2,2-bis-[4-(methacryloxypropoxy)-phenyl]-propane (bis-GMA) has been commonly employed for decades as a restorative material. Since bis-GMA resin has relatively low mechanical properties, this material has been further researched through multiple studies [198]. Nanofibers are believed to have the potential to substantially improve the mechanical properties of bis-GMA resin for their ultrahigh interfacial area. Some researchers consider its self-tailoring ability to meet the requirement for the demanding mechanical properties [199]. Research has shown that the flexural properties of the post-drawn nanofibers reinforced composites were further improved by increasing the fiber fraction. Because of their superb interface adhesiveness, polyacrylonitrile (PAN)–poly (methyl methacrylate) (PMMA), a core–shell nanofiber reinforced dental composites, has been studied. Due to this property, it has the potential to be used for crown bridge material and denture base resin in the clinic. Tensile properties and flexural properties of both nanofiber membranes and nanofiber reinforced bis-GMA/TEGDMA (tri-(ethyleneglycol)dimethacrylate) composites were also studied. Adding PAN–PMMA nanofibers into bis-GMA/TEGDMA clearly demonstrated the reinforcing effect. The flexural modulus (Ey), flexural strength (Fs), and work of fracture (WOF) increases as the nanofiber mass fraction increases from 0%, 0.6%, 0.8%, 1.0% to 1.2% [200]. Moreover, the addition of nanofibers could increase the storage modulus of the composite as a merit of high nanofiber strength, good nanofiber deposition, and strong interfacial bonding between the nanofiber and the matrix [201].

Clinically, the usage of PMMA denture is widespread. Generally, dentures suffer from tensile, compressive, and shear forces as well as the hazard of sudden drop,

which might cause denture base fracture [202]. Reinforcement of the PMMA denture base by adding inorganic filler is a strategy which aims to improve these properties [203]. To improve the base material itself, several materials have been used to repair fractured denture bases, including auto-polymerized, visible light polymerized, heat polymerized, or microwave polymerized acrylic resin [204, 205]. Most (86%) of denture base repairs are made by using auto-polymerized acrylic resin [206] because of its manageable properties; it is easily manipulated, fast setting, and therefore chair-side friendly [207]. Unfortunately, its strength compared to intact heat polymerized denture resin is only 18–81% [208, 209]. Metal oxide nanoparticles have recently been investigated. Additions of nano-zirconia (ZrO_2) to PMMA denture base have been reported to increase the transverse strength due to its small size and homogenous distribution [210]. Nano-ZrO_2 is a metal oxide and may be used as a reinforcement material to improve the transverse strength of denture base resin [211, 212]. Meanwhile, nano-ZrO_2 is regularly used as particle fillers, which possess excellent properties such as high strength, high fracture toughness, excellent wear resistance, high hardness, and excellent chemical resistance. ZrO_2, also being a typical bioceramic, shows great biocompatibility and bioactivity and possesses various desirable properties, such as excellent mechanical strength and toughness, resistance to corrosion and abrasion, and biocompatibility [203, 213, 214]. The nano form also has excellent mechanical properties, allowing it to endure against crack propagation. Moreover, it has the highest hardness among any oxide nanoparticles. Therefore, nano-ZrO_2 may be considered as a new approach for denture base repair. Meanwhile, reinforcement of acrylic denture base with nano-ZrO_2 significantly increases its transverse strength [215]. The nano-ZrO_2 resin resulted in significantly higher transverse strength as compared to unreinforced repaired resin [216]. But agglomerations of nano-scale particles often reach micrometer scales. The particles need surface modification to reduce clustering and improve their dispersion throughout the resin matrix. Left unchecked, this tendency can limit improvements to the mechanical properties and translucency of the composites. On the contrary, increases in the transverse strength is possibly attributed to the increased distribution of the nano-size particles, enabling better infiltration into the spaces between polymeric chains, thereby resulting in increased interfacial shear strength between the nanoparticles and the polymeric chains [215]. Moreover, the silane coupling agents used to treat the surface of nano-ZrO_2 could eliminate its aggregation and improve its compatibility with the polymer matrix [216]. The strong adhesion formed between the coupling agent on the surface of the nano-ZrO_2 and PMMA matrix improves the mechanical properties of the nanocomposites [203, 216]. Typically, it is used mechanically to reinforce polymers and improve the strength of the reinforced PMMA matrix [215, 217]. In regard to tensile strength, the group containing nano-ZrO_2 reinforced fillers showed a significant improvement over the control group (58.07 ± 3.14 MPa). Good adhesion and homogeneous dispersion of nanoparticles within a resin matrix improved the flexural properties of a polymer/nanoparticles composite [216]. Furthermore, the large interfacial area of the nanoparticles increases the contact area between the nano-ZrO_2 and PMMA, thereby improving mechanical interlocking and changing the properties of the ZrO_2/

PMMA nanocomposite, shown by an increase in the PMMA matrix ductility and plastic deformation [218–220]. A significant increase in the tensile strength was found with increase of nano-ZrO_2 levels (specifically 2.5%, 5%, and 7.5% of nano-ZrO_2). This outcome agreed with previous studies, which found that the addition of nano-ZrO_2 significantly increases the mechanical properties, and the two were positively correlated [221]. Therefore, the amount of filler used to reinforce the acrylic resin is another important factor in affecting the mechanical properties.

Determining how nanocomposites affect hardness and strength is also critical for potential clinical use. The result of a study showed that ZrO_2/PMMA nanocomposites achieved the highest surface hardness (19.97 ± 1.62 MPa) and flexural strength (87.37 ± 4.48 MPa) when 1.5 wt% nano-ZrO_2 was added, which achieved 23% and 12% increases, respectively, when compared to the pure PMMA. Another study shows that aluminum borate whiskers (ABWs) which were first developed in 1980s can offer superior mechanical properties at a low cost [196]. The ratio of nano-ZrO_2 and ABWs added to PMMA showed that it significantly influenced the surface hardness and flexural strength of ZrO_2-ABW/PMMA nanocomposites. Achieving maximum flexural strength (increased by 52% as compared to pure PMMA) required adding the optimum ratio of nano-ZrO_2 and ABWs to reinforce PMMA (adding 2% of nano-ZrO_2 at a ZrO_2 : ABW ratio of 1:2). Whereas, achieving maximum surface hardness (increased by 27% as compared to PMMA) required adding 3% of nano-ZrO_2 with the same ZrO_2/ABW ratio [222, 223].

Furthermore, patients require prosthesis that have good esthetics. The esthetics of a removable prosthesis is dependent on the appearance of the denture base on the patient's oral mucosa, which mainly depends on the translucency [224]. Therefore, good optical properties of PMMA reinforced with inorganic fillers are vital. The filler reinforcements permitted by PMMA (Fig. 3.6) [225] may influence its translucence and, consequently, the overall esthetics. Attempts to improve PMMA properties were explored through the incorporation of filler particles (zirconia, alumina, glass, silica, fiber, tin, and copper) [226]. The translucency property of the material results from the color difference between the thickness of the examined material over a white background and the same uniform thickness of that material over a black background. Nano-ZrO_2, a material possessing a white color, has received considerable consideration because it is less prone to alter the esthetics as compared with other metal oxide nanoparticles as well as maintain excellent biocompatibility [214, 216]. As the nano-ZrO_2 concentration increased, however, translucency was reduced and therefore adversely affected. Therefore, the selection of an appropriate concentration is critical in both establishing enhanced mechanical properties of the reinforced PMMA denture base and maintaining proper esthetics.

Previous reports indicated that the rough surfaces and hydrophobic properties of oral bacteria help it to readily adhere to the surfaces of dentures. More concerningly, *C. Albicans* shows resistance to conventional antifungal medications, rendering denture stomatitis to be a major challenge for oral treatment [227]. The development of denture stomatitis hinges on the adherence of *C. albicans* to denture base resin surfaces, followed by biofilm formation. Hence, increasing the antifungal

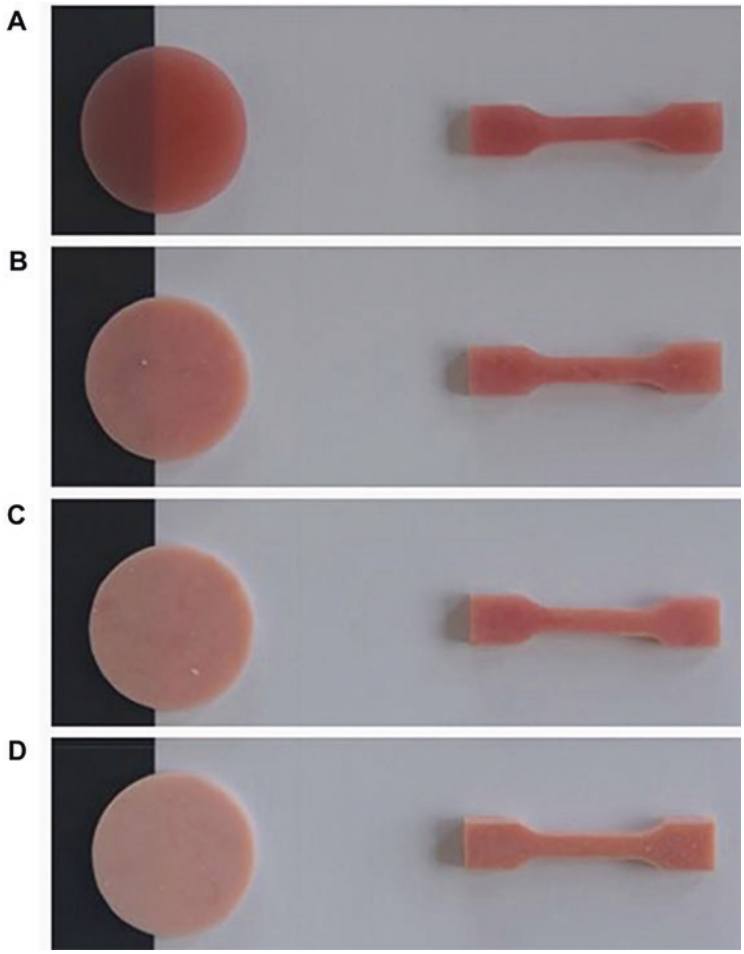

Fig. 3.6 The disc specimens are prepared for translucency tests while the dumbbell specimens are used for tensile strength test. Based on the reinforcement can be characterized as (**A**) unreinforced specimen ($n = 20$), reinforcement of the acrylic resin specimens with (**B**) 2.5% nano-ZrO_2 ($n = 20$), (**C**) 5% nano-ZrO_2 ($n = 20$), and (**D**) 7.5% nano-ZrO_2 ($n = 20$), nano-ZrO_2, zirconium oxide nanoparticles [225]

activity in denture base resins and controlling *C. albicans* adhesion and biofilm formation might efficiently control the occurrence of denture stomatitis [228, 229].

The colonization of oral fungi on denture soft lining material can result in infections and stomatitis of oral tissues. An in vitro study has confirmed that silver nanoparticles [230, 231] used as additives in denture base acrylic resins and tissue conditioners show antimicrobial effects. To incorporate these antimicrobial properties, a method was developed to integrate silver nanoparticles into silicone soft lining materials. Confirmation of fungicidal activity for these composites was established by Chladek et al. [232]. In another study, they showed that the

mechanical properties of nanocomposites reinforced by AgNPs decreased as the AgNP concentrations increased [233]. Levels from 0 to 200 ppm of AgNPs were incorporated as an antimicrobial agent into composites to reduce the microbial colonization of lining materials. Increases in the nano silver concentration resulted in increases in solubility and sorption, decreases in hardness and bond strength, and a change in the failure type for the samples. Ideally, AgNP concentrations from the 20 to 40 pm range achieve the best combination of bond strength, hardness, solubility, and sorption was achieved. Introducing AgNPs into Ufi Gel SC (UG) soft liner material also prolonged the sample cross-linking timing. As AgNP concentration increased in these composites, the cross-linking time required increased as well: at 40 ppm cross-linking took 15 min longer than that of UG, but at 80 ppm required approximately 40 min longer. Composites with AgNP concentrations up to 40 ppm presented properties that were no worse than those of the UG liner material. At these concentrations, the composites conform to the ISO standard requirements for soft lining materials (the bond strength after 24 h of soaking in distilled water, values over 1 MPa were observed in at least 8 out of the 10 samples). Starting at the 80-ppm concentration, the hardness and tensile bond strength of the composites were greatly reduced, while the absorption and solubility increased due to problems associated with the cross-linking of the composites. Although materials with a silver nanoparticle concentration of 80 ppm were classified as extra soft, they did not meet the ISO standard requirements.

Nano-ZrO_2 particles have received a great attention due to their attractive scientific, technological, and medical potentials [209]. They were also found to possess remarkable antimicrobial and antifungal effects. The antifungal effect of nano-ZrO_2 incorporated into PMMA removable prosthesis was investigated and found that it had the capability to reduce the candida counts. The antibacterial activity of nano-ZrO_2 might be attributed to active oxygen species, which in turn causes a disruption of the cell membrane of microorganisms [234]. The addition of nano-ZrO_2 to room temperature cured acrylic resin is an effective method for reducing *Candida* adhesion to polymethyl methacrylate (PMMA) denture bases and room temperature cured removable prosthesis. This is the reason why nano-ZrO_2 can actively inhibit the growth of fungal strains by interfering with cell function and causing deformation in fungal hyphae [235]. However, to fabricate a PMMA/nanocomposite that holds antimicrobial properties without affecting the physical and mechanical properties, adequate concentrations of nano-ZrO_2 should be used. The incorporation of nano-ZrO_2 in denture bases and PMMA removable prostheses is a possible way to prevent denture stomatitis [234].

Four potential inorganic antibacterial agents ((titanium dioxide (TiO_2), titanium dioxide supported by silver (Ag/TiO_2), zirconium phosphate supported by silver (Navaron), and tetrapod-like zinc oxide whiskers (T-ZnOw)) were investigated by Chen RR to determine their mechanical behaviors, antibacterial activities against oral microorganisms, and cytotoxicity [236]. The TiO_2, Ag/TiO_2, Novaron, and T-ZnOw of 3 wt% were mixed with the composites, respectively. The 3 wt% additions of various antibacterial agents had significant antibacterial activities compared to the control and blank group. Compared to the other groups, the T-ZnOW and

Novaron groups displayed higher antibacterial property against both bacteria. Adding 3% of TiO_2, Ag/TiO_2, Novaron, and T-ZnOw antibacterial agents showed a respective 30.26%, 67.82%, 61.69%, and 49.81% antibacterial rate against *S. mutans*, whereas the antibacterial rate was a respective 21.63%, 50.16%, 50.16%, and 37.62% against *C. albicans*.

The type of antibacterial agent is important to affect the composite properties. Quaternary ammonium salts are the most widely used organic antimicrobial agent before the application of nanomaterials. Recently, there are also many studies about the quaternary ammonium antimicrobial monomer usage in methyl methacrylate-based resin systems. Quaternary ammonium salts not only have desirable antimicrobial properties comparing to other antimicrobial agents but also have the added advantages of good permeability, low toxicity, minor skin irritation, stable performance, low light corrosion, and long-lasting biological effects. Therefore, they have been widespread in multiple industries and other fields [237, 238]. Researchers are expected to obtain durable antimicrobial properties by incorporating quaternary ammonium salts into a methyl methacrylate-based resin system to prevent denture stomatitis [239, 240]. A few studies have reported that denture base resins have antimicrobial activity when silver ions or quaternary ammonium antimicrobial monomers are added [241–244].

Nano poly(4-vinylpyridinium) (NPVP) is a nano-modified cationic surfactant, which can be used as antistatic agent, fungicide, etc. The poly(4-vinylpyridinium) (PVP) is used in synthetic resin coatings, which can improve the peel resistance and weather resistance. A study evaluated the antimicrobial activity of the quaternary ammonium grafted AgBr nanocomposite (AgBr/NPVP), a newly developed organic–inorganic composite antimicrobial agent, as compared to unpolymerized AgBr/NPVP and modified room temperature-cured denture base resins, against *C. albicans*. The research also confirmed that the silver ions which pass through the cell walls could interfere with cell membrane continuity (Fig. 3.7), increase cell permeability, and affect the metabolism and respiration of microbes [245–249]. Statistical analysis showed that the negative control and blank control groups had a large amount of fungal growth. The control group revealed no antifungal activity, but at the 0.1, 0.2, and 0.3 wt% dosage of AgBr/NPVP, the PMMA resin antifungal ratios were (78.22 + 1.90) %, (82.58 + 2.35) %, and (97.82 + 2.05) %, respectively, before aging (Fig. 3.8). Therefore, higher doses of AgBr/NPVP revealed significant increases in the antifungal activity of the PMMA resin. Alternatively, quaternary ammonium salts could inhibit the free movement of microbes and their breathing by attracting the negatively charged cell membrane in so-called contact killing. Quaternary ammonium salts and silver ions could exert strong antifungal effects, which have an important role in denture base.

Large-area mucosal supported denture (full denture, distal-extension absence of teeth denture, implant over-denture, etc.) continues to have issues such as insufficient retention, pain from biting, and general discomfort: all of which causes patient dissatisfaction. Traditionally, the soft lining material can only be a temporary or semi-permanent material because of the loss of softness and elasticity with time, and the failure of the bond and the difficulty of maintaining the cleanliness are due

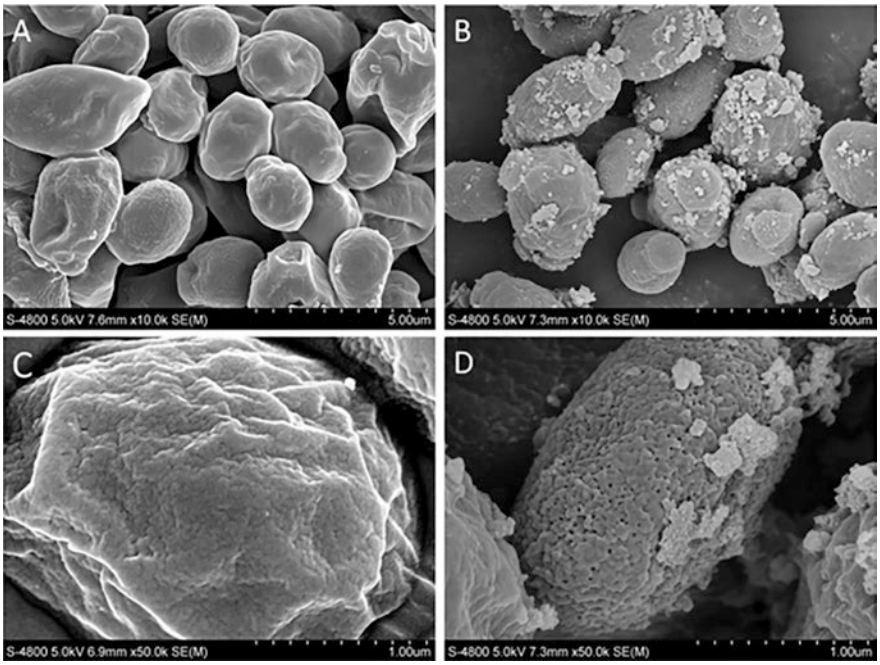

Fig. 3.7 The morphology of *C. albicans* ATCC90028 as displayed through field emission scanning electron microscopy (Fe-SEM) images after 24 h. Incubation (**A**, **C**) of *C. albicans* ATCC90028 normal morphology. (**B**, **D**) Morphology of *C. albicans* ATCC90028 after 24 h. Incubation with AgBr/NPVP suspensions (1 × MFC) [178]

to the easy adhesion of microbes. Therefore, the design and manufacture of rubber resin materials with different hardness values and rigid resin materials are urgently needed. The future trend is to explore the rubber-based resins with optimal oral mucosal tissue compatibility, the proportion of rubber-based resins to rigid resin materials which represents the thickness of "soft/hard" ratio. The "soft" rubber resin material is used as the base surface to contact the oral mucosa and the "hard" rigid resin material as the smooth surface which is exposed in the oral. The soft tissue surface has advantages in retention and can buffer and reduce local stress, which can greatly alleviate pain under masticatory force. The rigid surface has the advantages of increasing strength and stiffness, leading to a smoother and cleaner denture surface.

Therefore, the future trend is the application of nanotechnology to realize an ideal combination of soft and hard rubber resin material: (1) Nanotechnology to develop soft rubber resin material, (2) nanotechnology to develop hard rubber resin material, and (3) nanotechnology to combine soft with hard rubber resin material.

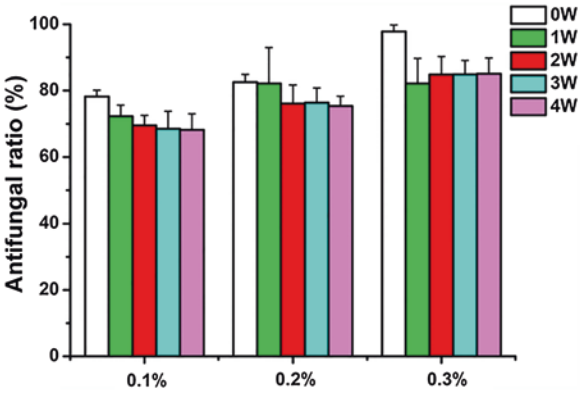

Fig. 3.8 Histogram of the antifungal ratio of PMMA resin. Each value is the mean ± SD ($n = 9$). The control group revealed no antifungal activity. As AgBr/NPVP increases, the antifungal activity of unaged PMMA resin also increases, with significant differences ($p < 0.017$) among the groups. Compared with corresponding unaged groups, the antifungal ratio at 1-week aging groups was less, especially at the 0.3 wt% group. However, the antifungal ratio for each group stabilized after 2 weeks of aging, with the 0.3 wt% group showing the best antifungal effect, achieving more than 80% [178]

3.2 Nanotechnology in Oral Tissue Regeneration

The oral and maxillofacial region refers to the complex area from the cranial base to the mandible, which includes oral organs (teeth, lip, tongue, etc.), facial soft tissue, maxillofacial bones (maxilla, mandible, etc.), temporomandibular joints, etc. Tissue defects of oral and maxillofacial regions are of high incidence due to inflammation, trauma, tumor, and atrophy after tooth extraction or a variety of genetic disorders such as cleft lip and palate, and hereditary dentine disorders (type 1). Reconstruction of these damaged tissues and organs is quite challenging due to their intricate structures, diverse functions, and highly esthetic requirements. Among many methods (e.g., autografts) to tackle these challenges, oral tissue engineering is a promising approach, which has been developed in the last three decades.

Tissue engineering focuses on the development of biological constructs to restore the function of damaged tissues and organs. The defects in oral and maxillofacial regions can also be treated by this method. There are three key factors in tissue engineering: scaffolds, seed cells, and growth factors. To date, numerous improvements have been performed to enhance tissue regeneration. Among them, nanotechnology has been applied to fabricate high-performance scaffolds with tunable physical, chemical, and biological properties, to label and track seed cells, and to deliver gene, drugs, and growth factors. Therefore, nanotechnologies and nanomaterials are of great potential to enhance oral tissue regeneration.

3.2.1 Nanotechnology to Improve Stem Cells

Stem cells are undifferentiated cells with the ability to self-replicate throughout life and to differentiate into multiple specialized cell types. Human stem cells can be found in various tissues of both epithelial and mesenchymal origin, including skin, adipose tissue, periosteum, cartilage, and teeth. Stem cells are of great importance to be applied in tissue/organ regeneration owing to their various potentials, such as (1) differentiation potential: stem cells can give rise to myogenic, adipogenic, osteogenic, and chondrogenic mesodermal lineages; (2) migration and homing capacity: stem cells can migrate to inflammation sites and injury sites under a variety of pathologic conditions; and (3) secretory ability and immunomodulatory functions: stem cells can secret a variety of paracrine and autocrine factors as well as extracellular vesicles, affect almost all cells involved in innate and adaptive immune reaction by secreting factors and cell–cell contact.

However, there are still limitations in widening the applications of stem cells. How to get isolated and purified stem cells quickly and effectively? How to achieve controllable and sustained release of bioactive molecules? How to obtain highly sensitive, real-time, noninvasive, in vivo stem cell tracking? And so forth. These problems are urgently needed to be solved. Fortunately, several techniques, such as genetic and epigenetic modifications, and nanotechnology have been performed to achieve wider application of stem cells in regenerative medicine.

Genetic modification has been generally regarded as one of the promising approaches to modify stem cells which will alter the secretion of some crucial proteins (such as cytokines and growth factors) by changing the expressions of related genes. It offers a promising potential for stem cell-based therapies. Therefore, numerous clinical benefits could be obtained. Currently, numerous viral and non-viral gene delivery techniques have been applied to deliver genes in an optimal manner for specific requirements. Just as its name implies, viral methods use virus as vectors (adenoviral vectors, retroviral vectors, and lentiviral vectors) for gene delivery. The advantages of viral methods are high transduction efficiency and long-term gene expression. However, their application is still limited due to some disadvantages, such as toxicity, immunogenicity, carcinogenicity, poor target cell specificity, high costs, and inability to transfer large size genes [250]. On the other hand, non-viral methods can be classified as physical, chemical, and inorganic nanoparticles techniques. The physical techniques include microinjection, electroporation, sonoporation, and so forth. The chemical techniques generally use cationic lipids or cationic polymers as vectors. The inorganic nanoparticle techniques involve the application of inorganic nanoparticles, which will be discussed here. The non-viral methods have been confirmed to be safe, easy to prepare, and are able to transfer large-sized genes. In addition, non-viral vectors can be modified with tissue- or cell-specific ligands for target gene delivery [251]. However, these vectors do not always lead to favorable transfection efficacy and transient transgene expression of non-viral gene delivery methods has limited their application. In addition to

genes, various micro RNAs (miRNAs) or small interfering RNAs (siRNAs) have recently been delivered into stem cells by viral or non-viral delivery systems.

Besides, epigenetic modification can also help adjusting the secretion of vital factors and proteins of stem cells. However, the gene sequence of stem cells stays unchanged after epigenetic modification. Epigenetic control is known to play a major role in transcriptional regulation of gene expression. DNA methylation and histone modification (acetylation/deacetylation) are classical control mechanism of epigenetics. It has been reported that inhibition of DNA methylation with DNA demethylating agent in human MSCs could improve their immunoregulatory capability and affect their differentiative capacity for therapeutic applications [252]. However, the epigenetic control mechanism for stem cells remains unclear. It is difficult to explain when controversial results were obtained using the same epigenetic control technique (e.g., DNA methylation) in different types of cells. This also limits the application of epigenetic modification in stem cells.

Compared with genetic and epigenetic modifications, nano modification in stem cells may provide a new sight. The application of nanotechnology on stem cells can be mainly classified into following fields: (1) separating and purifying stem cells; (2) labeling and tracking stem cells; (3) transferring biomolecules (gene, drug, growth factors, and so forth) into stem cells; (4) mimicking extracellular matrix (ECM) to promote the proliferation and differentiation of stem cells. The combination of nanotechnology with stem cells showed great potentials. For instance, stem cells can be easily separated via integrating with specific antibodies loaded on magnetic nanoparticles under magnetic field, which belongs to magnetic cell separation (MCS) techniques [253]. MCS techniques are effective and specific for stem cell separation and purification. Its advantage lies in that the application of magnetic nanoparticles (MNPs) can reduce the damage to stem cells due to their nano size compared with magnetic microparticles (such as neodymium and iron oxides). Besides, after human stem cells endocytose magnetic and/or fluorescence-labeled nanoparticles, noninvasive, and biosafety tracking stem cells can be realized both in vitro and in vivo. And it can provide important information for evaluating the efficacy of stem cell therapy. In addition, nanoparticles have shown potential as non-viral vectors for gene and drug delivery and growth factors release. Generally, nanoparticle vectors have several advantages, such as bio-safety, low immunogenicity, and good cell target specificity. And the released growth factors may influence the cell signaling pathway (such as MAPK/p38/AKT pathway and Wnt/â-catenin pathway) and regulate osteogenesis and angiogenesis in bone tissue engineering (Fig. 3.9). Furthermore, some nanomaterials, possessing similar nanostructure to natural extracellular matrix (ECM), showed the potential to enhance stem cells adhesion, migration, proliferation, and differentiation. In summary, it is of great potential to improve the stem cells using nanotechnology which can strongly promote the application of stem cells in tissue engineering.

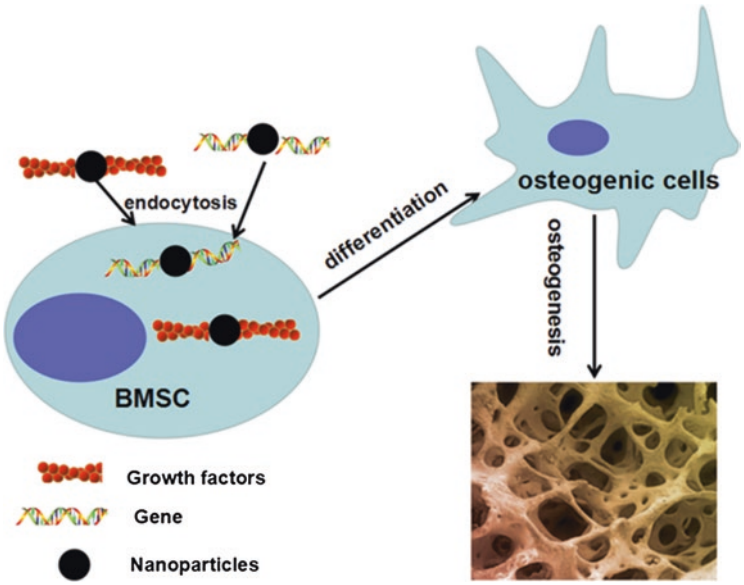

Fig. 3.9 The delivery of osteogenic gene and growth factors via nanoparticles for stem cells to promote osteogenesis

3.2.2 Nanotechnology and Dental Stem Cells

A tooth consists of the enamel, dentin, cementum, and dental pulp (composed of cells, fibers, nerves, blood vessels, and lymphatic vessels). After root development and cementum mineralization, the tooth fastens to the surrounding alveolar bone via the periodontal ligament (PDL), which contains a variety of cells such as fibroblasts, endothelial cells, and epithelial rests of Malassez (ERM) as well as extracellular matrix (ECM).

Dental mesenchymal stem cells (MSC) have been isolated from different locations within adult or post-natal dental tissues, such as the pulp of adult teeth (dental pulp stem cells, DPSCs) and deciduous teeth (stem cells from human exfoliated deciduous teeth, SHEDs), the apical part of dental papilla (stem cells from the apical papilla, SCAP) and the PDL (periodontal ligament stem cells, PDLSCs) (Fig. 3.10). These stem cells are often characterized by their ability to differentiate into the odontogenic, adipogenic, chondrogenic, or osteogenic lineages in vitro or to regenerate dental tissues in vivo. Currently, DPSCs are the most popular cells applied in oral tissue engineering among these dental stem cells due to their wide source and easy isolation. Besides, PDLSCs and SCAP also have been widely used. All these dental stem cells can be modified by nanotechnology.

DPSCs can be labeled by MNPs incorporated with growth factors to achieve patterned cell sheets and enhanced bone regeneration. For example, Zhang et al. designed new Fe_3O_4 MNPs coated with nanoscale graphene oxide (nGO-Fe_3O_4) to

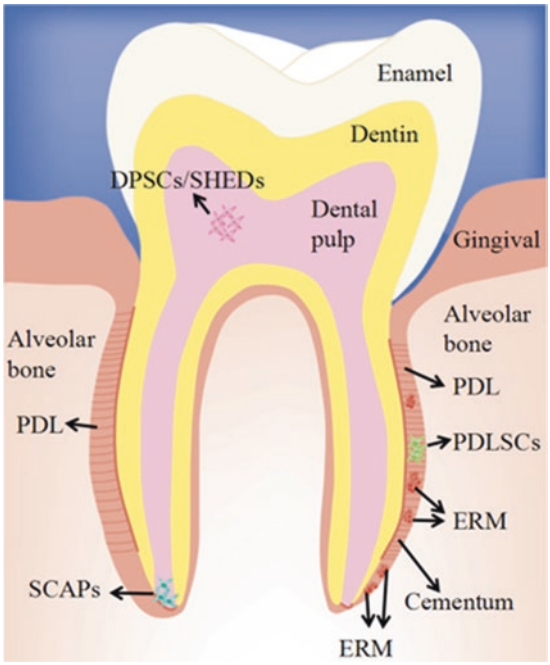

Fig. 3.10 The various dental stem cell populations within an adult human tooth. (*DPSCs* dental pulp stem cells, *ERM* epithelial cell rest of Malassez, *PDL* periodontal ligament, *PDLSCs* periodontal ligament stem cells, *SCAPs* stem cells from the apical papilla, *SHEDs* stem cells from human exfoliated deciduous teeth) [254]

label DPSCs and deliver BMP2 or TGF-â3 [255]. The nGO-Fe_3O_4 was easily swallowed by DPSCs, and these labeled DPSCs were cultured to form cell sheets. Cell sheet technology has been recently used in tissue engineering to repair tissue defect. The cell-sheet constructs, without restriction from the scaffolds, are largely maintained by the formation of cell-to-cell junctions and secretion of ECM proteins. Thus, these cell-sheet constructs are similar to the natural tissue compositions. To incorporate osteogenic growth factors into the cell sheets, the nGO-Fe_3O_4 were employed (Fig. 3.11). On the one hand, nGO-Fe_3O_4 MNP uptake into cells makes them controllable by magnetic force. On the other hand, the GO coating creates protein immobilization sites, allowing growth factors to be both incorporated into cell sheets and magnetically controlled. The arranged magnet can modulate the shape of the cell sheets with the help of nGO-Fe_3O_4 MNP. Therefore, the magnetic force can control the nGO-Fe_3O_4 MNP-labeled DPSCs precisely and prepare multilayered cell sheets with different patterns. Furthermore, the incorporation of BMP2 or TGF-â3 enhanced the osteogenic or chondrogenic differentiation of DPSCs. Given that the nGO-Fe_3O_4 nanocomposites provide a novel magnetically controlled vehicle to construct protein-immobilized DPSC sheets, other types of stem cells and growth factors can also be hired to form another kind of nanocomposite in tissue engineering. Thus, nanotechnology exhibits promising potential for future use in regenerative medicine.

Generally, there are two classical types of non-viral vectors, cationic lipids, and cationic polymers. However, sometimes, complex transferring protocols and

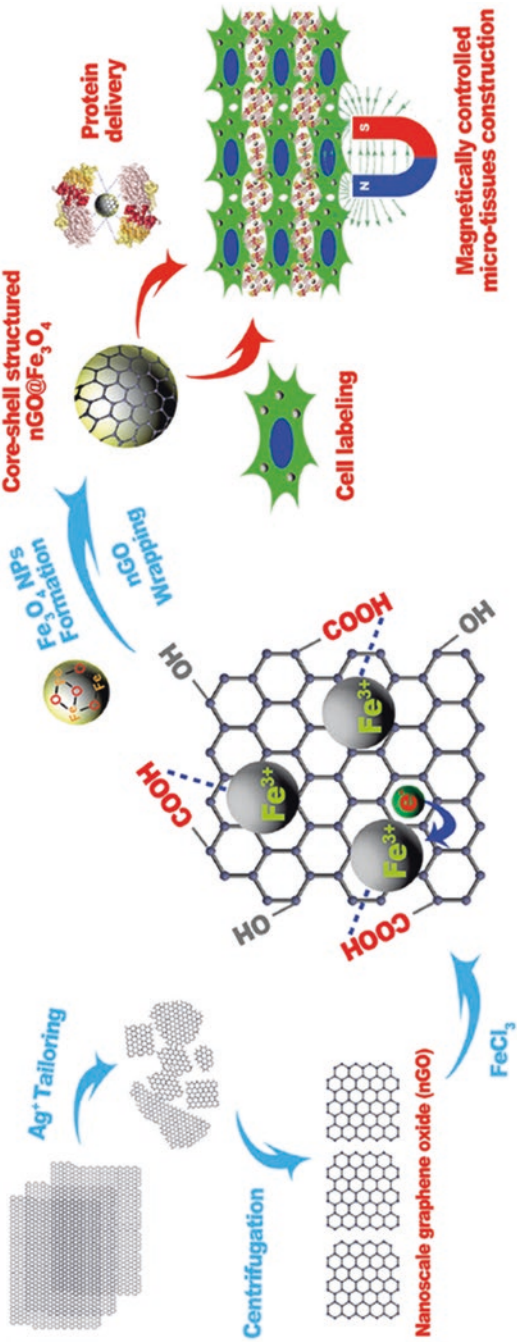

Fig. 3.11 Schematic illustration of the preparation of the nGO-Fe_3O_4 MNPs and application of these MNPs for magnetically controlled growth-factor-immobilized multilayer cell-sheet fabrication [255]

inefficient nuclear uptake of the two vectors may decrease the transfection efficacy. Comparatively, nanoparticles, which can be easily internalized by the cells, have been used as an alternative for gene delivery. For example, calcium phosphate nanoparticles, fabricated by Yang's et al. [256], were used for *hBMP2* gene delivery in rat DPSCs. These nanoparticles were less than 100 nm in diameter and can be easily taken up through endocytosis. And the DNA encapsulated in calcium phosphate nanoparticles was protected from the external DNase environment, which was benefit for DNA survival. Furthermore, it has been confirmed that calcium ions play an important role in endosomal escape, cytosolic stability and enhance nuclear uptake of DNA through nuclear pore complexes [257]. Although Lipofectamine (a kind of cationic lipid vectors used in Yang's study)-mediated cells were shown to produce higher *hBMP2* levels in the initial 4 days, the nanoparticles mediated cells reached higher levels at later time points. Furthermore, in Yang's study, the nanoparticles mediated *BMP2* gene transfected rat DPSCs (referred as NP-DPSC group), or untreated DPSCs were cultured on a fibrous 3D titanium mesh. The gene expression of dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) was increased in NP-DPSC group than that in untreated DPSC group. DSPP and DMP-1 proteins are two specific proteins which play a major role during dentin mineralization. These results revealed that the BMP2 secreted from the NPs-transfected rat DPSCs reached a functional level and helped to promote the odontogenic differentiation and matrix mineralization of cells. Therefore, nanoparticles were confirmed to be effective non-viral vectors for *BMP2* gene delivery and can be applied to improve the efficacy of DPSCs for odontogenic differentiation and hard tissue engineering.

Gene delivery via viral or non-viral vectors is not the only way for gene therapy. A novel type of three-dimensional nanomaterials, tetrahedral DNA nanostructures (TDNs), have garnered increasing interest and attention in biomedicine. Under certain conditions, self-assembly of the tetrahedral nanostructure by complementary base-pairing can be generated from four DNA single strands with preset programmable base sequences. Given that the original DNA is difficult to be taken up by cells without the assistance of relevant auxiliary agents, it is simple and direct for TDNs to be transported into cells by endocytosis. Therefore, the TDNs has been extensively applied in bioimaging, molecular transport, drug delivery, and molecular diagnosis due to its simple synthetic method, high yield, high stability, excellent biocompatibility, fast degradation, and low toxicity. Zhou et al. synthesized TDNs and co-cultured with DPSCs in vitro [258]. The results proved that TDNs can be successfully delivered into DPSCs without the assistance of other transfection reagents. Furthermore, TDNs can promote the proliferation and osteo/odontogenic differentiation of DPSCs by upregulating the expression of related genes and proteins. As the classical Notch signaling pathway has been confirmed to regulate the osteo/odontogenic differentiation of DPSCs by various studies, the authors also tried to explore its involvements. Their results demonstrated that proliferation and differentiation of DPSCs was driven by TDNs via activating the classical Notch signaling pathway. Therefore, the TDNs, as novel 3D DNA nanomaterials, have the

potential to be a promising and remarkable alternative approach for DPSCs-based tissue regeneration.

In addition to DPSCs, other dental stem cells like PDLSCs and SCAP also have been combined with nanomaterials and widely applied in recent decades. For example, Shrestha et al. synthesize dexamethasone (Dex)-loaded chitosan nanoparticles (CSnp) to obtain sustained release of Dex by encapsulation (Dex-CSnpI) and adsorption (Dex-CSnpII) methods [259]. The SCAPs were then co-cultured with CSnp, Dex-CSnpI, or Dex-CSnpII, and the expression of odontogenic differentiation related gene (alkaline phosphatase (ALP), DSPP, DMP-1) was evaluated. Results showed that Dex-CSnpII had faster release of Dex compared with Dex-CSnpI, but both demonstrated sustained release of Dex for 4 weeks. The sustained release of Dex resulted in enhanced odontogenic differentiation of SCAPs.

Niu et al. explored the effect of gold nanoparticles (GNPs) on human PDLSCs [260]. GNPs are attractive in regenerative medicine due to their unique physical and chemical properties. They have many prominent advantages such as excellent biocompatibility, facile synthesis method, and easy functionalization with biomolecules including growth factors, DNA, and peptides. They can promote osteogenic differentiation and inhibit adipogenic differentiation of mouse MSCs via activating the p38 mitogen-activated protein kinase (MAPK) pathway [261] and inhibit osteoclast formation of bone marrow-derived macrophages via downregulating the receptor activator of the nuclear factor-êB (NF-êB) ligand pathway. Therefore, GNPs have been developed as a new generation of osteogenic agents for bone tissue regeneration. In Niu's study, they treated PDLSCs with GNPs, and the cellular effects on the osteogenic differentiation of PDLSCs and the associated signaling pathways in cell differentiation were investigated. The results suggested that GNPs enhanced the osteogenic differentiation of human PDLSCs partially via activation of the p38 MAPK signaling pathway. This was consistent with the results of Yi et al. [261], and the two studies demonstrated that GNPs can affect stem cells via regulating signaling pathway.

Therefore, nanotechnology exhibits promising potential in modifying stem cells for application in oral tissue engineering.

With the fast development of medical technologies, the implant-supported dentures have been widely used as a restoration method to treat patients with lost teeth. However, the quality and quantity of bone at the implant site is usually unfavorable to achieve an ideal position and esthetics due to excess alveolar bone loss caused by inflammation, trauma, and physiologic atrophy. There are various methods for alveolar bone augmentation, including guided bone regeneration (GBR), sinus floor elevation, bone splitting, onlay graft and distraction osteogenesis, and so forth. GBR is regarded as the most commonly used methods by the patients due to the minimal trauma and pain among these methods.

GBR is a surgical procedure involving the use of guided bone regeneration membranes with bone grafts or bone substitutes (Fig. 3.12). A GBR membrane is placed into the surgical site, acts as the barrier to prevent fibrous connective tissue infiltration, and provides a more suitable local environment for bone regeneration. Besides, bone substitutes with osteoinductivity and/or osteoconductivity are placed under the

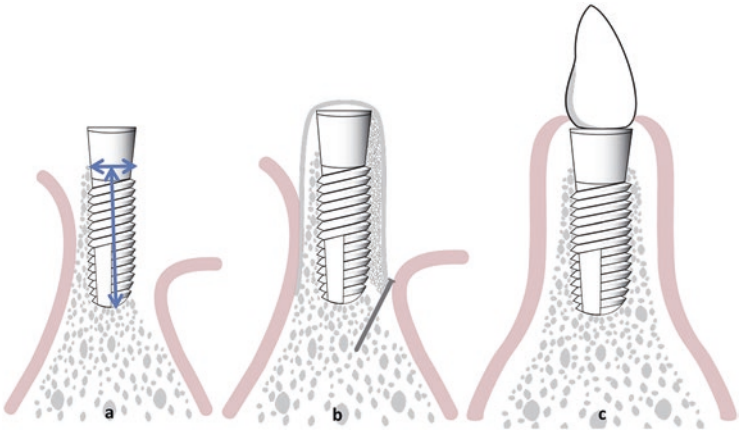

Fig. 3.12 (**a**) An adequate bone volume (height and width) is a prerequisite for successful implant treatment. (**b**) Barrier membrane and bone graft as bone substitute materials are placed to accelerate bone formation. (**c**) Final prosthesis is fabricated after the formation of new bone [262]

GBR membrane to maintain space and enhance osteogenesis. Generally, one face of the membrane is smooth to prevent the ingrowth of soft tissue, the opposite face is porous to allow the attachment and growth of osteogenic cells. And the integrity of the membrane also plays an important role in maintaining space in which the bone substitutes can be replaced by new bone and blood vessels. Both occlusive membranes (also called as GBR/GTR membranes) and bone substitutes are important for GBR and bone augmentation.

Recently, nanotechnology and nanomaterials have been applied to improving the structure and mechanical property, which is important for barrier membranes and bone substitutes.

GBR membranes are mainly divided into two types: resorbable and non-resorbable. Generally, clinicians and patients prefer the resorbable ones which do not need a second surgery. However, current resorbable membrane products still have some problems such as low osteoinduction and insufficient mechanical properties. Nanotechnology has been applied to overcome these limitations, such as insufficient mechanical strength, and poor osteoinduction.

The nanostructure of membrane surface mimic the architecture of a natural ECM and provide favorable micro-environments to improve the biological activity (such as adhesion, proliferation, odonto/osteogenic differentiation, and biomineralization) of dental stem cells and osteogenic cells.

Bachhuka et al. evaluated the role of surface nanotopography on the fate of human DPSCs (hDPSCs) [263]. They utilized density gradients of GNPs to examine, on a single substrate, the influence of nano-feature density and size on stem cell behavior. The density gradients of nanotopography were generated as follows: (1) 13 mm round coverslips were first coated with a 20-nm thin layer of plasma polymerized allylamine (AApp). (2) The modified coverslips were then immersed in a

rate-controlled fashion in a solution of GNPs of three different sizes (16, 38, or 68 nm) using a dip coating method [264]. Using this technique, the authors successfully controlled the contact time of every sector of the gold nanoparticle solution and the AApp-coated surface, resulting in nanoparticle density gradients along the surface. By using a predetermined size of the immobilized nanoparticles (16, 38 or 68 nm), the authors not only controlled spacing between nanoscale features but also generated surface nanotopography of controlled height. However, these substrates present not only variation in nanotopography but also difference in chemical composition. The underlying AApp coating is rich in amine/nitrogen while gold nanoparticles carry carboxyl acid groups on the surface. Previously, the authors demonstrated that AApp films of 5 nm are continuous and pinhole free. Thus, they provide uniform surface chemistry across the surface [265] and were able to uniquely tailor the outermost surface chemistry by overcoating the nanoparticle density gradients with a 5-nm AApp films. As a result, the GNPs were randomly distributed with an increase in nanoparticle density from region 0–12 mm (namely 0, 3, 5, 7, 10, 12 mm) and did not form aggregates. The hDPSCs cultured on GNP gradient coverslip were performed differently with changes in nanotopography. Results showed that the number of adhered DPSCs was higher, and cells proliferated faster on the sections of the gradients at certain density (e.g., 12 mm) of nanotopography features. Furthermore, greater surface nanotopography density (12 mm) could direct the differentiation of hDPSC to osteogenic lineages proving by the results of ALP staining and *ALP* gene expression. Besides, GNP diameters of 16 nm were most effective in promoting cell attachment, proliferation, and differentiation than those of 38 and 68 nm when the nanotopography density was the same among all groups. Though the mechanism was still unclear, this study provided new sight in designing the surface of biological membrane or scaffolds.

The incorporation of nano component, especially nano-hydroxyapatite (nHA), can improve the mechanical properties of GBR membranes. For example, Zeng et al. prepared new nano-hydroxyapatite/poly (vinyl alcohol) (nHA/PVA) composite membranes by solvent casting and evaporation technique. They found that the surface of composite membranes was biocompatibleand that nHA and PVA are distributed uniformly when the content of nHA is less than 20 wt%. Interestingly, the addition of nHA particles can increase the compressive strength, reduce the tensile strength and the elongation rate, and increase the Young's modulus of this composite membrane, which makes it easier for clinical practice in GBR [266].

In addition to GBR membrane, bone substitute material is another key point for GBR. Desired bone substitutes should have a 3D architecture and can promote osteogenesis and angiogenesis by allowing host osteogenic cells and vascular endothelial cells to adhere and migrate into the inner part. Furthermore, ideal bone substitutes should also possess suitable biodegradability, good biocompatibility, appropriate biomechanical strength, and beneficial osteoinduction. However, the integration of current bone substitutes with host bone suffers from long-term inefficiency due to their low osteoinductivity.

Generally, the bone substitutes are classified into three types: natural polymers, synthetic polymers, and inorganic ceramics. Natural polymers, including

fibrinogen, collagen, and polysaccharides such as alginates, chitosan and cellulose, hold similar structure as bone ECM, which are desirable to be free of immune reactions. Furthermore, as polysaccharides degrade rapidly, they could be used as porogens in composites. Synthetic polymers (such as poly(å-caprolactone) and poly(lactic-co-glycolic acid) (PLGA)) exhibit reasonable characteristics such as porosity, reproducible mechanical, physicochemical, and degradation properties, which can be modified for specific biomedical applications. Inorganic ceramics (like bioglass, tricalcium phosphate (TCP), HA, CPC, etc.) are utilized to provide a biochemical structure to enhance the osteoinductivity. However, insufficient mechanical strength, low supply, and high cost of natural polymers impede their use as bone substitutes. Besides, low osteointegration (which is due to the lack of endogenous vascular network) and lack of surface bioactivity are the main obstacle of applying synthetic polymers and ceramic scaffolds.

To design and fabricate functional bone substitutes, different nano-based strategies are employed. For instance, nanotopography can be achieved by nanoscale fabrication methods to produce nanometer features and patterns on the surface of current bone substitutes to mimic bone ECM which can guide and improve the osteointegration of bone substitutes with the native bone tissue. Besides, nanomaterials, incorporated into bone substitutes, can be used as nanocarriers to regulate the sustained release profile of biological macro-molecules and growth factors which are benefit for bone regeneration and vascularization. In addition to nanocarriers and nanocoatings, different fabrication methods such as electrospinning are used to provide 3D nanoporous structure for bone regeneration. Furthermore, the addition of MNPs, such as superparamagnetic iron oxides (SPIONs), has been proved to enhance bone regeneration by improving cell proliferation and osteogenic differentiation via activating MAPK/ERK signaling pathway [267]. There are several hypotheses to explain why the MNP-added biomaterials can stimulate cell proliferation and differentiation. One hypothesis proposes that every magnetic nanoparticle in the scaffold performs as its own nanoscale-level magnetic domain to induce micro-motions between the cell and scaffold interface, which in turn possibly affects the cell membrane ion channels, triggers the mechanotransduction pathway, and leads to enhanced cell proliferation and differentiation [268].

Surface topography of the bone substitutes is one of the major factors which can affect the osteointegration. To enhance the osteointegration, the surface of bone substitutes should trigger specific cellular responses. With the help of nanotechnology, the pore size and porosity of bone substitutes can be made more suitable to facilitate cell seeding, adhesion, migration of cells, vascular ingrowth, and tissue regeneration. Besides, the incorporation of bone substitutes with nanoparticles can lead to the increase of hydrophilicity and enable the sustainable release of growth factors. Nanoscale topographies on surfaces can be fabricated by different methods such as plasma-etching process, nanolithography, and nanoparticle/molecule grafting.

Calcium phosphate cement (CPC) is one of the promising bone substitutes due to their good injectability (owing to their self-setting and in situ hardening), acceptable mechanical properties, excellent biocompatibility, and osteoconductivity. Lee

et al. simply modulated the sizes of the initial CPC particles (the mixture of calcium hydrogen phosphate ($CaHPO_4$) and calcium carbonate ($CaCO_3$) and used two different protocols to obtain micro-CPCs and nano-CPCs samples [269]. A direct cell culture method was performed on the CPCs to assess the effect of micro or nano-CPCs in odontogenic media using human DPSCs. The ALP activity and the expression of marker genes such as *BSP*, *OPN*, *OCN*, *DSPP*, and *DMP-1* of DPSCs cultured on nano-CPCs was higher than those cells cultured on micro-CPCs with or without osteogenic media, which indicated that nano-CPCs substantially promoted odontogenic differentiation when compared to micro-CPCs. The integrin signaling pathway plays an important role in the cell–matrix interactions including cell adhesion, survival, proliferation, and differentiation. The á and â subunits of integrin are the major adhesive molecule-binding receptors in cells that form bone and dentin. The expression levels of integrin subunits á1, á2, and â1 increased significantly in nano-CPCs cultured as compared to micro-CPCs cultured cells, indicating that the hDPSCs more easily recognized the nanotopological substrate matrix receptors compared to the microtopological ones. The integrin downstream signaling might require the activation of several intracellular protein kinases, such as the focal adhesion kinase (FAK), Akt paxillin, MAPK, and NF-êB. The PCR results showed that the DPSCs on nano-CPCs expressed higher levels of *p-FAK*, *p-Akt*, *p-MAPK*, and *NF-êB*, which indicated that the nano-CPCs might act on the FAK, Akt, MAPK pathways to induce NF-êB activation in the DPSCs. Taken all these into consideration, Lee et al. claimed that nano-sized CPCs are superior to micro-sized CPCs in terms of their odontogenic differentiation in the hDPSCs through FAK, Akt, MAPK, and NF-êB signaling pathways.

Calcium phosphate cement can also be modified by other nanoparticles. Our groups designed a novel calcium phosphate cement (the mixture of tetracalcium phosphate ($Ca_4(PO_4)_2O$) and dicalcium phosphate anhydrous ($CaHPO_4$)) containing gold nanoparticles (GNP-CPC) [270]. The advantages of GNPs have been discussed in the previous paragraphs. In our study, GNPs were incorporated into CPCs via liquid to achieve a better dispersion and obtain homogenous particles. GNPs are proved to be able to enter cells through direct diffusion or endocytic pathway. Our results also showed that GNPs promoted the osteogenic differentiation of DPSCs even without the presence of CPCs. This may be due to the mechanical stresses on the cells from GNPs endocytosis through regulating the Yes-associated protein (YAP) activity [271]. Besides, we also found that the addition of GNPs improved the properties of CPCs such as wetting and protein adsorption. Furthermore, compared with CPCs, hDPSCs cultured on GNP-CPCs showed better cell attachment and larger spreading area. The osteogenic differentiation of hDPSCs on GNP-CPCs than those on CPCs were also greatly improved which was demonstrated by increases in ALP activity, osteogenic gene expressions and bone matrix mineral deposition. Interestingly, GNPs had no influence on cell adhesion and spreading without the presence of CPCs or GNP-CPCs, which may demonstrate that it is the nanosurface but not the nanoparticles themselves that played major role in cell attachment. Therefore, GNPs are promising to modify CPC with nanotopography by working as bioactive additives and enhance bone regeneration.

Instead of being used alone, the inorganic ceramics can also be utilized to fabricate composites with natural or synthetic polymers as bone substitutes to offer a nanostructured 3D network in human body. Samadikuchaksaraei et al. fabricated a nano-hydroxyapatite/gelatin (nHA/GEL) nanocomposite scaffold that mimic the natural ECM by the layer solvent casting combined with the freeze-drying and lamination techniques [272]. HA is one of the main mineral components of bone tissue, gelatin was added to mimic the organic component of the bone. The incorporation of nHA not only increased the mechanical properties of the gel scaffolds but also promoted the biocompatibility of rat BMSCs. Besides, the nHA/GEL nanocomposite scaffolds also showed better osteoinductivity in vivo than gel scaffolds alone when implanted in the calvarial critical size bone defect of SD rats. Therefore, this study indicates that nanocomposite can be a novel strategy for the development of bone tissue engineering scaffolds.

Interactions between the cells and their microenvironment determine the fate of grafted bone substitutes. Growth factors have been widely used to improve cellular bioactivity and increase stability and physiological activity of administered active compounds. Growth factors can be incorporated into bone substitutes or scaffolds for targeted, sustained, and controlled release of growth factors, to enhance osteogenesis, angiogenesis, and tissue regeneration. Growth factors which are commonly used as osteoinductive agents are bone morphogenetic proteins (BMP), platelet-derived growth factors (PDGF), and vascular endothelial growth factors (VEGF). Currently, multi-carrier structures (e.g., hierarchical structure, core–shell structure, and nanoparticle embedded structure) have been developed to incorporate growth factors within bone substitutes and scaffolds. However, the key challenge with the direct incorporation of growth factors within the scaffold is that they can be deactivated during the fabrication process (e.g., under high voltage or in contact with organic solvents [273]). Furthermore, another concern is the burst release. To address the issue, nanoparticles are used to encapsulate the proteins to preserve the bioactivity of the growth factor and provide a sustained release profile. Li et al. developed a new nanoparticle-embedded electrospun nanofiber scaffold for the controlled dual delivery of BMP-2 and DEX [274]. The preparation of this scaffold was shown in Fig. 3.13. Briefly, BMP-2 was loaded into bovine serum albumin (BSA) nanoparticles to maintain its bioactivity. Then, the BMP-2-loaded BSA nanoparticles were stabilized by a chitosan shell through electrostatic self-assembly (BNP). Encapsulation of both BNP and DEX were performed by electrospinning the blended solution of nanoparticles and PCL-poly(ethylene glycol) (PEG) copolymer (DEX was dissolved into this copolymer solution). Hydrophilic PEG was copolymerized with PCL to improve the water wet ability and degradation rate of PCL. Finally, a controlled dual delivery of BMP-2 and DEX can be acquired using this nanoparticle-derived fiber scaffold. Results showed that the bioactivity of DEX and BMP-2 was preserved in this scaffold. Besides, a controlled and sequential release pattern of the DEX and BMP-2 was achieved. Most of the DEX was released in the first 8 days, and the BMP-2 release lasted up to 35 days. Furthermore, the drug-loaded groups exhibited a strong ability to induce osteogenic differentiation both in vitro and in vivo and manifested the best repair efficacy due to a synergistic

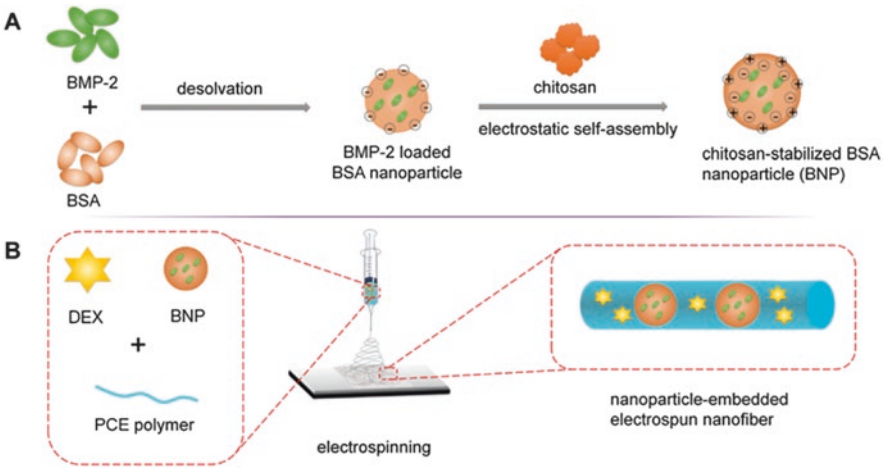

Fig. 3.13 Schematic illustration of BMP-2 and DEX-loaded electrospun nanofiber scaffold [274]

effect of BMP-2 and DEX. Therefore, the biomolecule-loaded nanofiber scaffold fabricated in this way is a strong potential candidate to repair bone defects.

Instead of biomolecules, stem cells have also been incorporated into bone substitutes in stem cell-based therapy. The incorporation of stem cells within bone substitutes have many advantages due to their differentiation potential, ability to secrete multiple bioactive molecules, and immunomodulatory functions. It is of great benefit to apply injectable scaffolds which could enable the seeding of stem cells into the deep internal space. Besides, using injectable scaffolds is advantageous when it comes to irregular bone defects.

He et al. designed a novel injectable, biodegradable, porous, and load-bearing nano-scale calcium sulfate/alginate (nCS/A) delivery system incorporated with *BMP2*-gene-modified rat MSCs in rat critical-sized craniofacial bone defects to evaluate the osteoconductivity of the system [275]. Calcium sulfate (CS) is a biocompatible, bioactive, and biodegradable material with crystalline structure whose compressive strength is greater than that of cancellous bone and has been applied to fill cysts, bone cavities, and segmental bone defects. Nano-calcium sulfate (nCS) particles (generally between 30 and 100 nm) may enhance physical properties, such as increasing the surface area to improve growth factor adsorption while potentially controlling the degradation rate, as well as mechanical strength for optimal osteoconductivity and fractures resistance. On the other hand, alginate is a natural anionic polysaccharide with good biocompatibility, mild requirement of gelation conditions, low immunogenicity, high hydrophilicity, and good biodegradability under physiological conditions and low cost (easily to be obtained from brown seaweed). It has been widely used in bone tissue engineering. Thus, they cross-linked alginate hydrogels with nCS to form the injectable nCS/A paste [275]. When the nCS/A paste was cultured in the medium, the degradation of ionically cross-linked alginate due to the loss of divalent cations (Ca^{2+}) will lead to the formation of nano pores

which enhances cell migration into the scaffold. Besides, the mechanical properties of the nCS/A paste were improved with an increased proportion of alginate. Then, a 25 μL cell suspension with 1×10^6 rat MSCs infected with *BMP2* gene was added to the nCS/A paste to form a rat MSC-based nCS/A composite as novel bone substitutes. Both rat MSCs and *BMP2* gene-modified rat MSCs in the injectable pastes remained viable, osteo-differentiated, and yielded high alkaline phosphatase activity after injection. In animal experiment, the authors found that *BMP2*-gene-modified MSCs in nCS/A (referred as nCS/A + M/B2 group) showed the best osteogenic activity and the amount of new bone formation, and vessel density was the highest in the group which grafted with nCS/A + M/B2 composite. Therefore, the overall results confirmed that the combination of injectable nCS/A paste and *BMP2*-gene-modified MSCs is a new and effective strategy to repair bone defects.

In summary, nano-modified membranes and bone substitutes have the potential to enhance osteogenesis and have been widely applied in oral bone regeneration.

3.2.3 Nanotechnology to Regenerate Keratinized Gingival

Good osteointegration is fundamental to a successful dental implant, while the connection between gingival and implant surface determines long-term success of a dental implant because bacterial invasion and infection can be prevented by a proper seal around a dental implant. It is of great importance to maintain enough keratinized gingival (KG, a part of oral mucosa covered with keratin or parakeratin) to obtain gingival seal mentioned above. It has been believed that 2 mm in width and depth of keratinized gingival is sufficient to maintain gingival health [276]. Generally, the absence of adequate keratinized mucosa around dental implants, especially in posterior area, was associated with higher plaque accumulation and gingival inflammation. Besides, wider and deeper zones of keratinized mucosa may lead to stronger resistance to the forces of mastication.

While it is practical to achieve adequate osteointegration between dental implants and host bone, the preservation of appropriate soft tissue architecture around an implant supported denture remains challenging. Several surgical techniques have been described to increase the amount of keratinized tissue around dental implants, including the free gingival graft (FGG), the connective tissue graft (CTG), apically positioned flap (APF) technique, and xenogeneic collagen matrix (XCM). However, the formation of a scar may occur occasionally while applying APF technique together with FGG or CTG, and higher postoperative morbidity, and the longer surgical time may limit the use of autogenous grafts and APF technique. Therefore, XCM has been investigated for the treatment of gingival recessions. As far as we know, the effect of available methods for keratinized gingival augmentation stays controversial, and the regenerative mechanism of keratinized gingival remains unclear. Given that nanostructure and nanomaterials are beneficial to bone regeneration, some researchers also focus on applying nanotechnology to keratinized gingival regeneration.

Nocini et al. investigated a newly designed bi-layered collagen nanostructured membrane (collagen matrix 10,826®, CM-1826) and evaluated its fundamental cell functions of human gingival keratinocytes, such as adhesion, proliferation, and interleukin 6 (IL-6) production, to assess the biocompatibility of this new membrane [277]. It has been proven that cell adhesion activates intracellular signaling pathways governing a multiplicity of cellular processes including the gene expression of cytokines, such as IL-6 and IL-6, and stimulates keratinocyte proliferation. Thus, the IL-6 production was selected to evaluate the effect of this membrane on keratinocytes. Results showed that the production of basal IL-6 doubles in the presence of CM-10826, strongly increases in the presence of collagen I, a major component of CM-10826, but appears almost unaffected in the presence of collagen IV. Besides, CM-10826 also demonstrated a positive biological activity on gingival keratinocytes growth and adhesion, without any toxic effect. However, there was no direct comparison between keratinocytes cultured with CM-10826 and collagen I. Therefore, it is hard to figure out whether the promotion of cell growth, adhesion and IL-6 production is due to the nanostructure or the major component (collagen I) of CM-10826. Further studies should be carried on.

However, Dorkhan et al. found that nanotopography of anodic oxidation-modified Ti showed no effect on adherence of keratinocytes [278]. They used titanium discs with three different surfaces. Commercially pure titanium discs (CpTi) were selected as controls (C) while the two anodically oxidized surfaces (N1 and N2) were selected as test surfaces. Preparation of N1 was performed by anoxic oxidation on CpTi, whereas the same method on titanium alloy N1 (TiAl6V4) was used to prepare N2. Results showed that the two anodically oxidized surfaces achieved nanostructuring with differing degrees of porosity. After cultured for 24 h, the keratinocytes adhered well to the nanostructured surfaces, although to a somewhat less degree than to CpTi. However, no differences in adhesion strength of keratinocyte could be observed among the two nanostructured surfaces and the CpTi. This study indicates that nanostructure may have no effect on the adhesion of keratinocytes. Thus, it is interesting to explore whether the nanostructure can influence the bioactivities of gingival keratinocytes.

3.2.4 Nanotechnology to Regenerate Tooth

As mentioned before, a tooth consists of multiple tissues. In humans, tooth loss can lead to physical and mental suffering that compromise an individual's self-esteem and quality of life. Dental caries and periodontal disease are the two major causes for the loss of teeth. In addition, facial trauma, resection of maxillofacial tumors, and some genetic disorders may also lead to tooth loss. Due to its complex structure and limited self-healing capability, it is challenging to achieve tooth regeneration.

The current restorations for tooth loss are dentures (including removable and fixed dentures) and dental implants. Poor retention and instability of a removable denture make it uncomfortable for the patient. Besides, it is inconvenient to wear,

remove, and clean the denture every day. The fixed dentures are more comfortable than the removable ones. However, it needs to grind the adjacent healthy teeth as abutments to make a fixed denture. Furthermore, the fixed denture has to be removed or refabricated once abutment teeth suffered severe damage. Dental implants have been favorite choices for both patients and dentists in recent decades. However, high price, long treatment period and the pain caused by surgery is the main concern which impede the application of dental implants. Besides, dental implants currently cannot perceive noxious stimulations such as excessive occlusal loading and trauma because the absence of neuronal innervation in the periodontal tissue. The nervous system of a natural tooth contributes to the regulation of tooth physiological functions and the perception of noxious stimulations. Therefore, tooth regeneration should be the best way to restore the lost teeth.

The focus in damaged tissue restoration has undergone significant changes, beginning as substitution, then evolving to restoration or replacement, and finally to tissue regeneration. Novel therapeutic strategies have arisen about two decades ago, sprouting from the emergence of tissue engineering and regenerative medicine, evaluated by their potential to replace, repair, maintain, and enhance tissue or organ function. The strategy encompasses numerous elements, including biomaterials, stem cells, tissue-inducing substances, or biomimetic regenerative environments. Tooth regeneration strives to (a) regenerate a structurally and functionally sound complete tooth and (b) regenerate individual tooth structures such as enamel, dentin, pulp, cementum, and periodontal ligament. Like natural teeth, bio-engineered teeth are also expected to properly contact adjacent teeth, transmit masticatory loads, provide proprioception, and restore esthetics. Generating these teeth and restoring proper morphology require precise and orderly orientation of the epithelial mesenchymal cell layers placed onto the scaffold as well as directing the interaction of cells with the extracellular matrix. To achieve this differential placement of cells and ensure proper interaction with the matrix, 3D imprinting scaffold fabrication, cell seeding techniques, and recent developments in nanotechnology can be implemented. Currently, two approaches are considered for tooth regeneration: (1) scaffold-based approach which involves using scaffolds on which cells can be seeded in vitro or by cell homing in vivo; (2) scaffold-free approach aims at directly inducing developmental processes of embryonic tooth formation by stem cells or cell sheets. These processes have to be guided by appropriate signals to produce tooth structures that mimic natural teeth in size and shape. Nanotechnology has been widely used to modify stem cells and scaffolds, and most of the researchers focused on the former approach to achieve tooth regeneration.

Enamel, the hardest tissue located in the vertebrate body, is formed through biomineralization by ameloblast cells. These cells synthesize and secrete a complex of tissue-specific proteins into the extracellular space, where a HA-patterned matrix is self-assembled by these proteins to form a tough, wear-resistant composite material. The dominant protein of mammalian enamel includes amelogenin and ameloblastin which play important role in forming enamel matrix. The mature enamel composite contains no cells, no blood vessels, and almost no proteins. Therefore, it is difficult to regenerate enamel once it is destroyed by caries or trauma. To

hopefully achieve the long-term goal of establishing cell-based strategies for tooth regeneration, Huang et al. used an in vitro cell and organ culture system to study the effect of artificial bioactive nanostructures have on ameloblasts [279]. Huang's study hired the branched peptide amphiphile molecules "BRGD-PA," which contain the peptide motif Arg-Gly-Asp, for their ability to self-assemble into nanofibers. Primary enamel organ epithelial (EOE) cells and ameloblast-like cells (line LS8) were cultured within BRGD-PA hydrogels, and then injected into the enamel organ epithelia of mouse embryonic incisors. Results of cell proliferation assay, quantitative real-time PCR, and Western blot showed that LS8 and EOE cells responded to the BRGD-PA nanostructures with enhanced proliferation and greater amelogenin, ameloblastin, and integrin expression levels compared with cells responded to culture plates. Besides, at the site of injection in the organ culture model, Huang et al. observed that EOE cell on BRGD-PA proliferated and differentiated into ameloblasts as evidenced by their expression of enamel specific proteins. The histological analysis, transmission electron microscopic examination, and immunohistochemistry results showed that the nanofibers were inside the formed ECM, contacted the EOE cells, and engaged in enamel formation and regeneration. Therefore, this research demonstrates when BRGD-PA nanofibers are presented with enamel proteins, they participate in integrin-mediated cell binding to the matrix and deliver instructive signals for enamel formation.

Dental pulp is a complex organized tissue with various types of cells and structures, providing nutrition, sensation, and defense against various pathogens. In addition, dental pulp produces dentin and maintains the biological and physiological vitality of the dentin. Pulpitis, usually caused by dental trauma and caries, is one of the most common diseases related to dental pulp. Due to the complex structure, small volume, and insufficient blood supply of dental pulp, self-repairing is difficult to initiate in pulpitis. Traditionally, pulpitis is treated by root canal therapy, whereby the inflamed pulp is removed and replaced with inorganic material; however, tooth fragility and ultimately tooth fracture can be unfortunate end results. Therefore, a better choice is if we could keep or regenerate vital tooth pulps. Recently, scaffold- and/or stem cell-based therapy has been one of the approaches to achieve pulp regeneration. Li et al. designed and synthesized a unique hierarchical growth factor-loaded nanofibrous microsphere scaffolding system to achieve successful regeneration of pulp tissues in a full-length human root with a one-end seal [280]. In this system, heparin binds to the vascular endothelial growth factor (VEGF), which is then encapsulated into heparin-conjugated gelatin (HG) nanospheres and finally immobilized in the nanofibers of an injectable poly-(L-lactic acid) (PLLA) microsphere (HG-MS) (Fig. 3.14). For comparison, gelatin without heparin was used during the fabrication process, and the hierarchical microsphere obtained was abbreviated as G-MS. The hierarchical microsphere fabricated without gelatin or heparin, referred as MS, was used as blank control. The amount and bioactivity of released VEGF was measured and evaluated, and the result indicated that microsphere system hierarchy not only protects the VEGF from degradation and denaturation but also provides superb control of its sustained release. Additionally, the HG-MS integrates the ECM-mimicking architecture with a highly porous injectable

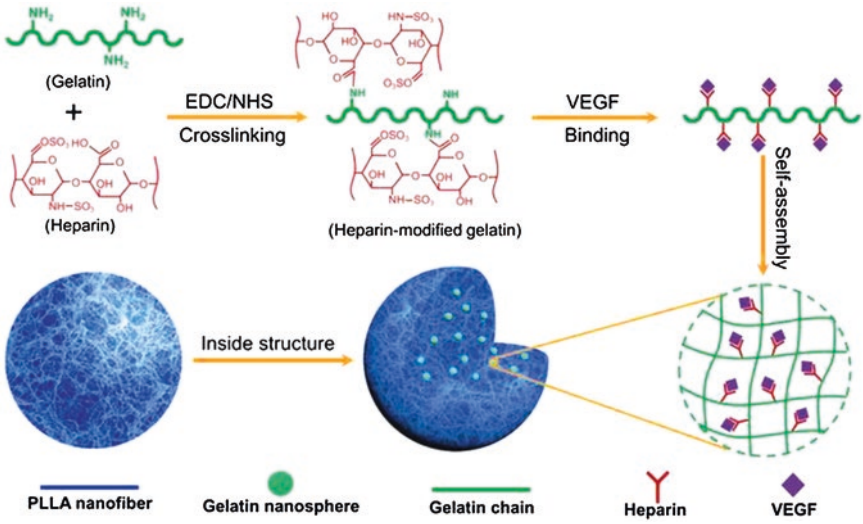

Fig. 3.14 Schematic illustration of the synthesis of heparin-conjugated gelatin (HG) and hierarchical VEGF-loaded heparin-conjugated gelatin microspheres (HG-MS) [280]

form, effectively accommodating DPSCs and assisting their proliferation and pulp tissue formation. Then freshly extracted human teeth from 18- to 30-year-old patients were collected for in vivo study. Briefly, the root for each tooth was standardized by cutting to a length of 13 mm. The root apex and coronal were processed to a length of 1.0 and 2.5 mm, respectively. During the in vivo experiment, mineral trioxide aggregate (MTA) at 2 mm into the canal space was used to seal the coronal end of the canal, leaving the remaining 11 mm of the root canal space for pulp tissue regeneration, which mimics the clinical operation. Then the pretreated roots were filled with DPSCs or HG-MS or HG-MS + DPSCs or left empty and implanted into immunocompromised nude mice. After 9 weeks, the histology and immunohistochemistry assay showed the successful regeneration of pulp-like tissues that fulfilled the entire apical and middle thirds and reached the coronal third of the full-length root canal in HG-MS + DPSCs group. In addition, many blood vessels were regenerated throughout the canal in this group. Therefore, nano-modified scaffold, together with dental stem cells, may provide new sight for dental pulp regeneration.

In regenerative endodontics, dentin and pulp are an inseparable unit because they are closely related embryologically, histologically, and functionally. Therefore, to achieve both dentin and pulp regeneration simultaneously is important to fully restore the biological and mechanical functions of the damaged tooth. Gronthos et al. combined human DPSCs with nano-hydroxyapatite/tricalcium phosphate (nHA-TCP) ceramic powder (worked as scaffolds) to explore the possibility that isolated ex vivo-expanded human DPSCs would also be capable of regenerating a dentin/pulp-like structure in vivo under similar conditions [281]. In addition, human

BMSCs were also used and compared with DPSCs to explore their potential to be applied in dentin/pulp regeneration. Immunohistochemical studies were performed to characterize the progeny of the DPSC and BMSC clonogenic populations. DPSCs and BMSCs share a similar immunophenotype in vitro according to their similar expression of markers associated with endothelium, smooth muscle, bone, and fibroblast. While functional studies revealed DPSCs produced only sporadic, densely calcified nodules without adipocyte formation, the BMSCs routinely were calcified amidst the entire adherent cell layer and had clusters of lipid-laden adipocytes. DPSCs or BMSCs were transplanted in conjunction with nHA-TCP powder into immunocompromised mice to see whether a dentin/pulp-like structure could be formed in vivo. The DPSCs produced a dentin-like structure, composed of a highly structured collagenous matrix, which lined nHA-TCP particle surfaces. Besides, a pulp-like interstitial tissue was surrounded by the odontoblast-like cells. Comparatively, BMSCs formed lamellar bone containing osteocytes and surface-lining osteoblasts, surrounding a fibrous vascular tissue with active hematopoiesis and adipocytes. This study confirmed that isolated postnatal human DPSCs combined with the nHA-TCP powder showed greater potential than BMSCs to form a dentin/pulp-like complex. It can be inferred that nanomaterials could be used as scaffolds in stem cell-based dentin/pulp regeneration.

In addition, the application of nanotechnology may provide more advantages in tooth regeneration. For example, a bio-root was regenerated by implanting pre-shaped 3D printing root-like scaffolds combined with mesenchymal stem cells into the alveolar bone. In this way, a functional root with root-like structure, periodontal ligament-like tissue, and dentin-like matrix structure was formed by Sonoyama et al. [282]. However, the bio-root showed lower compressive strength than that of natural swine root dentin, which may impede the long-time survival of the bio-root. Therefore, can we enhance its survival rate through improving the mechanical properties of the bio-root by nanotechnology? And as appropriate signals play the key role in regulating developmental processes of a tooth formation, we can try to use nanotechnology to modify stem cells and transfer growth factors in the future tooth regeneration research. Further studies are needed.

3.2.5 Shortcomings in the Current Application of Nanotechnology

Nanomaterials have unique biological activities to improve the physicochemical properties of oral stem cells, GBR membranes, and bone substitutes. Therefore, it helps with oral tissue regeneration. However, as one coin has two sides, the application of nanomaterials and nanotechnology may also have some potential problems.

One of the concerns for employing nanomaterials is their toxicity to oral tissue, which will mainly be discussed in the next part of this book. Besides, there are still other limitations when applying nanotechnology in oral tissue engineering.

When nanoparticles are used for gene delivery, lower concentration of nanoparticles is related with reduced gene loading capacity, while higher concentration is related with increased cytotoxicity. Therefore, it forms a dilemma between the two, and an optimal concentration is acquired [283]. Combining nanoparticles with other vectors, to form combo delivery system, may solve this challenge. In Zhang's study, (3-aminopropyl) triethoxysilane-modified iron oxide nanoparticles (APTES-IONPs) was designed and synthesized as gene vectors. Two other commercial vectors, Lipofectamine and TurboFect, were used as control. One gram of DNA was incubated with APTES-IONPs (weight range from 0 to 30 μg) or 1 μg Lipofectamine or 0.25 μL TurboFect for 30 min to evaluate the gene-loading capacity. Low level of APTES-IONPs failed to bind enough DNA, so did Lipofectamine or TurboFect. Comparatively, unbound DNA level was significantly decreased when high level of APTES-IONPs (30 μg) was added. However, the addition of Lipofectamine or TurboFect markedly decreased the unbound DNA level during complexation with low level of APTES-IONPs. Vice versa, 1 μg Lipofectamine or 0.25 μL TurboFect succeeded in binding enough DNA with the addition of low level of APTES-IONPs. Therefore, the commercial vectors could enhance DNA-binding capacity of the APTES-IONPs even at low concentration, and the nanoparticles could also increase the DNA-binding capacity of the commercial vectors. Besides, Zhang et al. also found that the combo gene delivery system, containing APTES-IONPs and Lipofectamine, was a time-saving method, could prevent DNA from degradation, improve gene delivery efficiencies in both adherent and suspension cells, and effectively mediate siRNA transfection. Therefore, the combination of nanoparticles with other vectors shows greater potential for gene transfection than using nanoparticles alone.

Scaffold, as one of three key factors in tissue engineering, has been modified via nanotechnology in regenerative dentistry. Magnetic nanoparticles (MNPs) have drawn great interest owing to their unique magnetic properties and have been utilized to obtain magnetomechanically functional scaffolds for bone regeneration. The simplest way to obtain magnetic scaffolds was to dip-coat the scaffolds into aqueous ferrofluids containing MNPs (such as superparamagnetic iron oxide nanoparticles, SPIONs) coated with various biopolymers. After dip-coating, the nanoparticles were integrated into the porous structure of the scaffolds. However, one concern of the magnetic scaffolds fabricated in this way is the uncontrolled and undesired release of MNPs. This concern may be addressed by designing and fabricating scaffolds with specific structure. Zeng et al. used HA powder to fabricate scaffold by the microwave-assisted foaming process [284]. This kind of HA scaffold had a specific structure with micro- and macroporosity and was shaped into disks of 12 mm diameter and 2 mm height. Then the HA disks were dip-coated with MNPs. The authors found a gradual release of magnetite over a long period in phosphate-buffered saline (PBS), simulated body fluids (SBF), and fetal bovine serum (FBS), which indicated that the integration of HA and MNPs involved an adsorption-desorption dynamic equilibrium process. At an extremely low percentage of released magnetite in the media, the release curves flattened out, suggesting that the large surface energy of HA and the specific porous structure helped for

MNPs retention in the scaffold, thereby delaying the desorption process. Therefore, specific structure of the magnetic scaffold may help to achieve desired release of MNPs.

There are many important growth factors and small molecules, such as BMPs, VEGF, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), which can promote oral tissue regeneration. Growth factors could be stabilized by conjugation to nano-constructed materials. Usually, growth factors immobilized to nanomaterials promoted a higher growth and differentiation of the cells compared with their counterparts due to the release of growth factors [255, 285]. However, it is still difficult to get controlled and sustained release of growth factors and keep their bioactivity during release process. It seems that the combination of nanomaterials with other biomolecules as vehicles for growth factors can improve the release of growth factors. As mentioned before, Li et al. designed and synthesized a unique hierarchical growth factor-loaded nanofibrous microsphere scaffolding system HG-MS (Fig. 3.14). In this system, heparin was used to further immobilize VEGF in the PLLA nanofibers. For comparison, gelatin without heparin was used during the fabrication process and referred as G-MS, and the hierarchical microsphere fabricated without gelatin or heparin was used as blank control, referred as MS. Results showed that encapsulating VEGF into gelatin (G-MS group) and HG microspheres (HG-MS group) significantly decreased the initial burst release (shown in MS group) of the protein VEGF. In addition, binding VEGF to heparin (HG-MS group) further reduced the burst release of VEGF than that of G-MS group. Besides, the release rates of the MS and G-MS groups reached a plateau after 1 week. In contrast, the HG-MS group released VEGF consistently at a rate of approximately 1–2% per day for the last 3 weeks of the experiment. As VEGF has binding domains with heparin, it can be inferred that heparin protects VEGF from denaturation and proteolytic degradation, which subsequently prolongs its sustained release. Therefore, it may be useful to utilize other biomolecules to improve nanomaterials better for the delivery and release of growth factors.

Though shortcomings are present, there is no doubt that nanomaterials and nanotechnology bring numerous benefits and amazing perspectives to stem cell-based regenerative medicine. Recent advances in nanomaterials increase the potential to control oral stem cell bioactivity, improve gene and growth factors delivery, create functional membranes, and improve the mechanical properties and osteoconductivity of bone substitutes for the treatment of oral and maxillofacial defect. Therefore, it is of great potential to implant nanomaterials labeled oral stem cells with nanotechnology-modified scaffolds into damaged dental tissue for regeneration. Besides, the application of nanomaterials in dentistry may act as another type of personalized medicine to achieve customized treatment for different patients and different oral and dental diseases. Furthermore, for dental implants, nanotechnology can be applied to form an “osteogenic coating” which may increase the bone–implant contact and improve the osteointegration. And this may be an alternative to bone augmentation in cases whose alveolar bone in implant site is compromised.

However, some problems are imperative to be solved before further application of nanotechnology in oral tissue engineering. Most importantly, nanomaterials

require improved testing and evaluation systems to assess their potential toxicity. As such, the consideration of dose–response studies is warranted. Moreover, the underlying mechanisms of nanomaterial–cell interactions are unclear, with many hypotheses remain to be proved. Third, the exact effects of nanomaterials on cells, tissues, and organs and their metabolic pathways in vivo remain unclear and require further research.

3.3 Application of Antibacterial Nanomaterials in Dentistry

The human oral cavity is the home to over 530 different species of microorganisms. However, only some of them are related to oral disease. In the past four decades, microbial ecologists have identified the suspected pathogens which cause human dental caries and periodontal disease. Many scientists concur that the microbes which are associated with oral disease are as follows: the principal pathogenic agents of dental caries are associated with the *Mutans streptococci*, especially *Streptococcus mutans* and *Streptococcus sobrinus* and the secondarily implicated are the *Lactobacillus species* and perhaps some non-*Mutans streptococci* in coronal caries, particularly the acid-tolerant strains: *Streptococcus sanguis* and *Streptococcus gordonii* [286, 287]. In addition, *Actinobacillus actinomycetemcomitans* are associated with juvenile periodontitis, while *Prevotella, Fusobacterium*, and *Actinomyces* species are associated with gingivitis [288].

Because the infections are closely associated with bacterial biofilms, the antimicrobial susceptibility of the important pathogenic bacteria in the biofilms has been extensively investigated for many years. Antibiotics are the most common way to fight against the bacteria. However, oral administration of antibiotics results in undesirable side effects, such as inadvertent destruction of beneficial bacterial flora and development of antibiotic resistance. Instead, local polymeric-based drug delivery systems which include fibers, strips, or nanoparticles can provide appropriate drug concentrations directly to the target site.

Recently, there has been remarkable attention in the field of antimicrobial nanomaterial and nanotechnology. Several nanoparticles (e.g., zinc oxide, silver, and magnesium oxide) had been proved to be effective in inhibiting the bacterial growth and applied in several areas of dentistry, such as endodontics, dental prostheses, periodontitis, and implantology. Abou et al. found that the antimicrobial mechanisms of nanoparticles include bacterial cell membrane disruption, reactive oxygen species (ROS) generation, active transport as well as sugar metabolism inhibition, electron transport disturbance across the bacterial membrane, DNA replication prevention, and ion displacement of those required for the enzymatic activity of oral biofilms [289].

Silver (Ag) ions have strong antimicrobial effects and have been used widely in biomedical field for many years. Being a potent antimicrobial due to sustained ion release, Ag has many advantages, such as no antibiotic resistance, low toxicity, and good biocompatibility. Likewise, silver nanoparticles (AgNPs) have shown potent

antimicrobial properties, also having non-acute toxic effects on human cells. They enhance the overall biological effectiveness through fast penetration and bioavailability while reducing potential cytotoxicity, drug dosage and production costs. AgNPs in different sizes have different effects on oral bacteria: 5 nm AgNPs seem to have the highest antibacterial activity [290]. The antimicrobial mechanism of AgNPs has been extensively investigated. Seemingly, the interaction of silver ions with the peptidoglycan cell wall causes structural changes, increased membrane permeability, and ultimately leads to cell death. Furthermore, the interaction of AgNPs with the exposed sulfhydroyl groups in bacterial proteins results in the hindering of DNA replication [291, 292].

ZnO nanoparticles (ZnO NPs) are believed to be nontoxic, biosafe, and biocompatible. They have been used as drug carriers, cosmetics, and filling materials in biomedical field. ZnO NPs have a wide range of antibacterial effects on various microorganisms, including gram-positive and gram-negative bacteria under normal lighting conditions. The antibacterial activity of ZnO NPs might involve the production of ROS and the accumulation of nanoparticles in the cytoplasm or on the outer membranes [293]. Another metal oxide nanomaterial, MgO has the advantages of being cheap, readily available, and biocompatible, making it a very promising antibacterial agent. The damage to the membrane of bacterial cell likely occurs due to the attachment of the nanoparticles to the membrane, in combination with the effects of pH change, Mg^{2+} release, and ultraviolet illumination. The attachment of particles may involve phosphate groups presenting on the surface of the cells, but there are likely other adherent mechanisms contributing to the antibacterial activity [294].

Subsequently, we discuss the incorporation of nanomaterials into dental materials, highlighting the aspects regarding microorganism inhibition and potential toxicity.

The survival of pathogenic bacteria in the oral cavity depends on their successful adhesion to the dental surfaces and their ability to develop into biofilms, known as dental plaque. Bacterial plaque is the unmineralized bacterial community in the mouth that cannot be washed away by water and adheres together or to the tooth surface. Because of the self-secreted extracellular polymeric matrix, it has high recalcitrance toward antibiotics. Bacteria from the dental plaque are responsible for the development of dental caries, gingivitis, periodontitis, stomatitis, and peri implantitis. The ability of antibiotic nanoparticles with sustained release capability to penetrate the biofilm has led to its emergence as one of the premier anti-biofilm formulations in the combat against biofilm infections. Moreover, biofilm resistance can be overcome by including drug delivery systems such as lipid or polymer nanoparticles. These particles can increase the treatment efficacy by improving antibiotic delivery to bacterial cells.

Some researchers examined the antibacterial efficacies and physical characteristics of the antibiotic-loaded polymeric nanoparticle formulations, and the result showed that most ideal formulation is ciprofloxacin-loaded PLGA nanoparticles, by virtue of their high drug encapsulation efficiency and high antibacterial efficacy while maintaining a low dose to combat against the biofilm cells and biofilm-derived planktonic cells of *Escherichia coli* [295]. Likewise, Forier et al. found that in many

cases antimicrobial efficacy can be improved by using lipid and polymer nanoparticles. To maximize the biofilm exposure to the antimicrobial agent and ensure delivery of the antimicrobial agent to the vicinity of the bacterial cells, strategies which include nanoparticle targeting, antimicrobial triggered release, and fusogenic liposomes are promising [296].

Other researchers fabricated two forms (sphere and wire) of chlorhexidine (CHX)-loaded mesoporous silica nanoparticles (MSNs) and investigated their releasing capacities and anti-biofilm efficiencies. The spherical MSNs with an average diameter of 265 nm exhibited a larger surface area and faster CHX-releasing rate than the MSN wires, the spherical nanoparticle-encapsulated CHX presented with a greater anti-biofilm capacity than the wire nanoparticle-encapsulated CHX, since the entanglement of wire nanoparticle-encapsulated CHX could restrict the drug release and interactions with the microorganisms. These findings revealed that the spherical nanoparticle-encapsulated CHX could preferably enhance its anti-biofilm efficiency through an effective releasing mode and close interactions with microbes [297]. Another researcher reported the synthesis of mesoporous silica nanoparticle-encapsulated pure CHX (nano-CHX), and its antimicrobial properties against oral biofilm. The nano-CHX elicited potent antibacterial effects against mono-species biofilms and planktonic bacteria such as *Streptococcus mutans, Streptococcus sobrinus, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, and *Enterococcus faecalis* at the 50–200 μg/mL concentrations. Moreover, nano-CHX successfully inhibited multi-species biofilms that include *Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, and *Porphyromonas gingivalis* until 72 h [298].

A traditional Chinese medicine, *Scutellaria baicalensis* (SB), has been used to treat infectious and inflammatory diseases. Baicalin, a flavonoid compound isolated from SB, possesses marked anti-inflammatory, antioxidative, and immunomodulating effects. It has potent antibacterial effects on oral pathogens, and it could indeed inhibit bacterial quorum sensing activity and exhibit protective effect on the development of experimental periodontitis and benefits for controlling periodontal disease. The combined use of another compound baicalein presented in SB with antibiotics has synergistic effects against oral bacteria [299–302]. It has been shown that nanoparticle-encapsulated SB enhanced the biological effectiveness via high bioavailability and fast penetration with less cytotoxicity, reduced dosage of the agents, and lowered the costs. This team also explored the synergistic effects of the combined usage of nano-MIX (nanoparticle-encapsulated SB and nano-CHX at 9:1 (w/w) ratio) with the aim to minimize the unexpected effects of CHX, against the mixed oral biofilms such as *Streptococcus mutans*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis*. The result showed enhanced synergistic antibacterial effects of the nano-MIX on common oral bacterial biofilms, which could be developed as a novel antimicrobial agent for clinical oral/periodontal treatment [303].

Besinis et al. found that the biofilm formation occurring on dentine surfaces and the bacterial growth occurring in surrounding media can be successfully controlled by applying silver nanocoating on dentine. This nanocoating was found to be

chemically and biologically stable and also can protect the teeth from dental plaque as well as secondary caries when applied as a dentine coating [304].

Dental caries is the most common and widespread oral disease, resulting from the acidic attack of the cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus* spp. Currently, composite resin is the most widely used restorative materials, due to their excellent esthetics and load-bearing properties. They are applied in core buildup, inlays/onlays restoration, cavity lining, and fissure sealing. However, one major drawback is that composites tend to accumulate biofilm and plaque in vivo [305, 306]. Such plaque accumulation with acid production by acidogenic bacteria could result in secondary caries. However, it has been shown that there is microleakage on the margins of the restoration. These gaps can be colonized by oral bacteria, resulting in secondary caries. To prevent or diminish biofilm accumulation, filling materials with antimicrobial properties have been developed.

In order to elicit acid neuralization ability as well as release calcium and phosphate ions, a dental composite including amorphous calcium phosphate nanoparticles (NACP) was created. Few studies have reported on antibacterial agents being incorporated into calcium phosphate composites. One study investigated the effect of AgNP mass fraction in NACP nanocomposite on mechanical properties and dental plaque microcosm biofilm. Five NACP nanocomposites were fabricated with AgNP mass fractions of 0, 0.028, 0.042, 0.088, and 0.175%, respectively. The results showed that the composites with AgNPs at 0–0.088% had the similar mechanical properties with those containing no AgNPs. The modulus of the sample containing 0.175% AgNPs was lower than the moduli of all other groups. Besides, counts of colony-forming units (CFU) containing 0.042% AgNPs for total *streptococci* were 75% less than the control group without AgNPs. A considerable antibacterial capability was imparted by the AgNPs in the NACP nanocomposite, which rose with AgNP concentration. However, increasing the mass fraction of the AgNPs to 0.175% revealed a brownish color and was accompanied by a precipitous strength drop. Therefore, to maintain both esthetics and mechanical strength, the AgNP mass fraction used must not exceed 0.042%. At 0.042%, the AgNP nanocomposites capably reduced biofilm metabolic activity greatly as well as decreased both lactic acid production and CFU counts, as compared to the commercial composite control [307].

AgNP-modified light-activated composites were evaluated by another study to determine the physical and antibacterial activity. Disks were produced with either the unmodified resin (control group) or the AGNP-modified resin at 0.3 wt% (MR03) and 0.6 wt% (MR06) concentrations. *Streptococcus mutans* and *Lactobacillus acidophilus* biofilms onto the disks were incubated and induced onto the disks in vitro. The result showed that the number of viable cells was statistically lower for MR03 and MR06 compared with control group. MR03 and MR06 showed no significant differences. MR03 was stronger in compression resistance than control group, and MR06 was statistically lower than control group and MR03. Both MR03 and MR06 inhibited the biofilm growth on their surfaces with no increase in surface roughness compared with the unmodified control resin [308].

Besides AgNPs, ZnO NPs also have antibacterial activity. It can inhibit the production of acid by the dental plaque through inhibiting *Lactobacillus* and *Streptococcus mutans*. In one study, ninety discoid tablets containing 0 wt%, 1 wt% AgNPs (20 nm), and 1 wt% ZnO NPs (50 nm) were prepared using flowable composite resin. Evaluation of the antibacterial properties of these discs were conducted using the direct contact test. 0.01 mL diluted solutions of the bacterial species *Streptococcus mutans* and *Lactobacillus* were each placed separately on the disks. The result showed that composites containing ZnO NPs and AgNPs both exhibited higher antibacterial activity against *Streptococcus mutans* and *Lactobacillus* compared with the control group. The effect of ZnO NPs on *Streptococcus mutans* was significantly higher than that of AgNPs. However, there were no significant differences in the antibacterial activity against *Lactobacillus* between composites containing AgNPs and those containing ZnO NPs [309].

Azarsina et al. added the 0.5 wt% and 1 wt% AgNPs into Z250 composite to evaluate the antibacterial properties against *Streptococcus mutans* and *Lactobacillus*, using those without AgNPs as control. And the result showed that addition of AgNPs into composite resin had a significant effect on the reduction of *Streptococcus mutans* and *Lactobacillus* colonies. The antibacterial properties of composite resins are different depending on the concentration of AgNPs. 1 wt% of AgNPs had stronger antibacterial properties than 0.5 wt% [310].

Aiming to enhance the mechanical properties and endow composite resin with high antibacterial activity, a novel composite resin was produced. Hydroxyapatite (HA) nanowires were synthesized and then coated with polydopamine (PDA) by submerging the nanowires in dopamine (DA) aqueous solution. A reduction reaction was then used to prepare AgNP-laden HA (HA-PDA-Ag) nanowires by adding glucose and silver nitrate into HA-PDA suspensions in deionized water. The loading amounts of AgNPs controlled by adjusting the feeding doses of silver nitrate and HA-PDA nanowires and then HA-PDA-Ag nanowires were readily obtained. Benefiting from the surface PDA layer, HA-PDA-Ag nanowires could successfully disperse in composite resin and form sound interfacial adhesion with the resin matrix. As compared to the neat resin, the addition of HA-PDA-Ag nanowires achieved significant increases both in modulus of cured composites and flexural strength. The distribution of AgNPs was homogeneous throughout the resin matrix in all designed groups, which endowed the composites with high antibacterial activity against *Streptococcus mutans* [311].

A lot of studies investigated the effects of AgNPs on the mechanical properties of dental resins since they are important for load-bearing dental restorative materials. Incorporation of small amounts of AgNPs (e.g., 0.02 wt%) did not significantly reduce the flexural strength [312]. However, greater amounts of AgNPs could decrease the mechanical properties of composites. The hardness of the light-cured resins containing 0.1 wt% Ag benzoate nanoparticles decreased significantly, compared with the control group [230].

Bonding agents help the adhesion of the composite restoration to the tooth structure in order to form a functional and stable construct. Generally, residual bacteria often exist in the prepared tooth cavity, and microleakage between the margins

could allow the invasion of new bacteria. Modern minimally invasive techniques are recommended for the treatment of deep caries to protect pulp vitality and preserve more tooth tissues. These techniques require to remove less-infected dentin to be the least-invasive surgical approach, possibly leaving behind the affected dentin in the cavity. Therefore, the antibacterial adhesives are needed to combat biofilms and reduce recurrent caries at the tooth-restoration margins.

Quaternary ammonium compounds which contain cationic monomers have been shown to be effective in reducing bacterial growth in a wide range of applications including the medical devices [313]. Due to the covalent bonding with the polymer network to exert "contact inhibition," the quaternary ammonium dimethacrylate (QADM) is immobilized to the resin and the adhesive [314].

Li et al. compared the antibacterial activity, contact-inhibition, and long-distance inhibition of a novel bonding agent containing QADM with that containing AgNPs. While the QADM-containing adhesive presented contact-inhibition exhibited by bacterial inhibition only on its surface, AgNP-containing adhesive had the added advantage of long-distance killing inhibiting away from its surface due to the releases of the silver ions. The novel antibacterial adhesives are promising for caries-inhibition restorations. QADM and AgNPs could cooperate and work together in inhibiting bacteria on resin surface as well as away from resin surface [315].

Since dentin primer directly contacts with the tooth structure, it would be beneficial to use antibacterial primers. QADM and AgNPs were incorporated into dentin primers (Scotchbond Multi-Purpose, "SBMP," 3M, St. Paul, USA) to investigate the effects on dentin bond strength and dental plaque microcosm biofilms. SBMP primer was the control group, and control + 10% QADM (mass), control + 0.05% AgNPs, and control + 10% QADM + 0.05% AgNPs were the modified primers. QADM + AgNP-containing primer increased the bacteria inhibition area by ninefold, compared with control primer. QADM-AgNP-containing primer reduced lactic acid production and CFU of total microorganisms (Fig. 3.15). In conclusion, novel QADM-AgNP-containing primers were strongly antibacterial. They are promising to inhibit biofilms and secondary caries [316].

Disinfection of the bacteria impregnating the dentin in vitro using extracted human teeth was researched using SBMP primer containing AgNPs and QADM. The interior of the dentin blockers was successfully impregnated by *Streptococcus mutans*. After impregnation, either an antibacterial primer or a control primer was applied to the dentin. Primer was applied to the dentin. Harvesting of the *Streptococcus mutans* CFU in dentin was then performed by sonicating from the dentin block for different groups. SBMP + 10% QADM + 0.1% AgNPs had bacteria inhibition zone eightfold that of control, and SBMP + 10% QADM + 0.1% AgNPs inhibited *Streptococcus mutans* in dentin blocks, decreasing the viable CFU in dentin by three orders of magnitude, as compared to the control dentin lacking primer. Therefore, it was more effective to use QADM + AgNPs than QADM alone. These results demonstrate that antibacterial primers were able to kill the bacteria residing inside the dentinal tubules of dentin blocks. Bonding agents containing AgNPs and QADM exerted a long-lasting effect against residual bacteria in the dentinal tubules

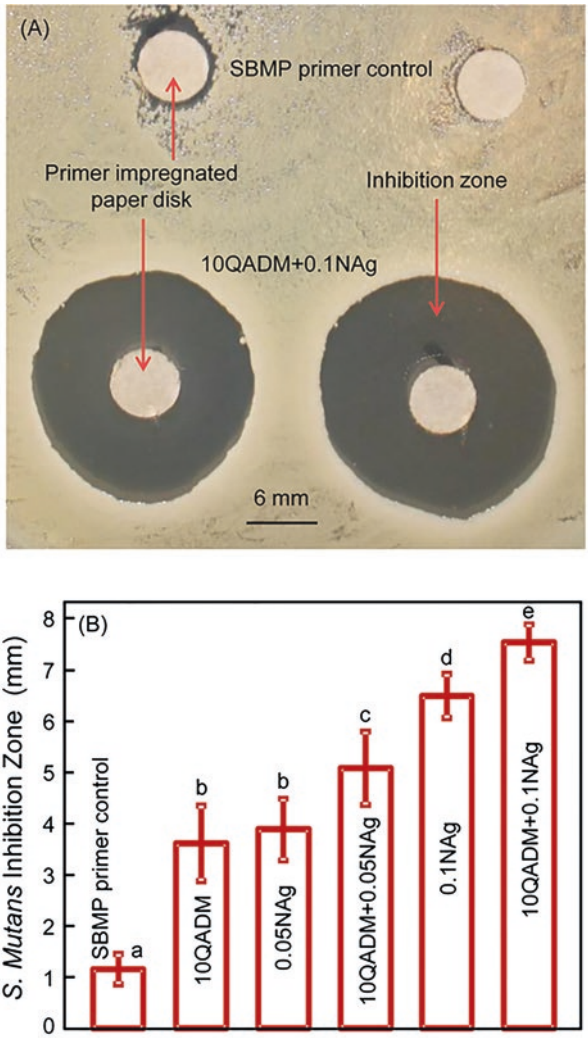

Fig. 3.15 Results of the agar disk diffusion for uncured primers against *S. mutans*: (**A**) representative specimens of SBMP control primer (top), and 10QADM + 0.1Nag added primers (bottom); (**B**) *S. mutans* inhibition zone sizes (mean ± SD; $n = 6$): dissimilar letters suggest the corresponding values are significantly different ($p < 0.05$) [316]

as well as new invading bacteria along the margins due to microleakage (Fig. 3.16) [317].

Unpolymerized 12-methacryloyloxydodecylpyridinium bromide (MDPB) has strong antibacterial activity, and the primer incorporating MDPB demonstrated bactericidal effect before cured, indicating the possible ability to kill residual bacteria in the prepared cavity [318]. A study was done to investigate the effects of dentin primer containing dual antibacterial agents, namely MDPB and AgNPs, on dentin

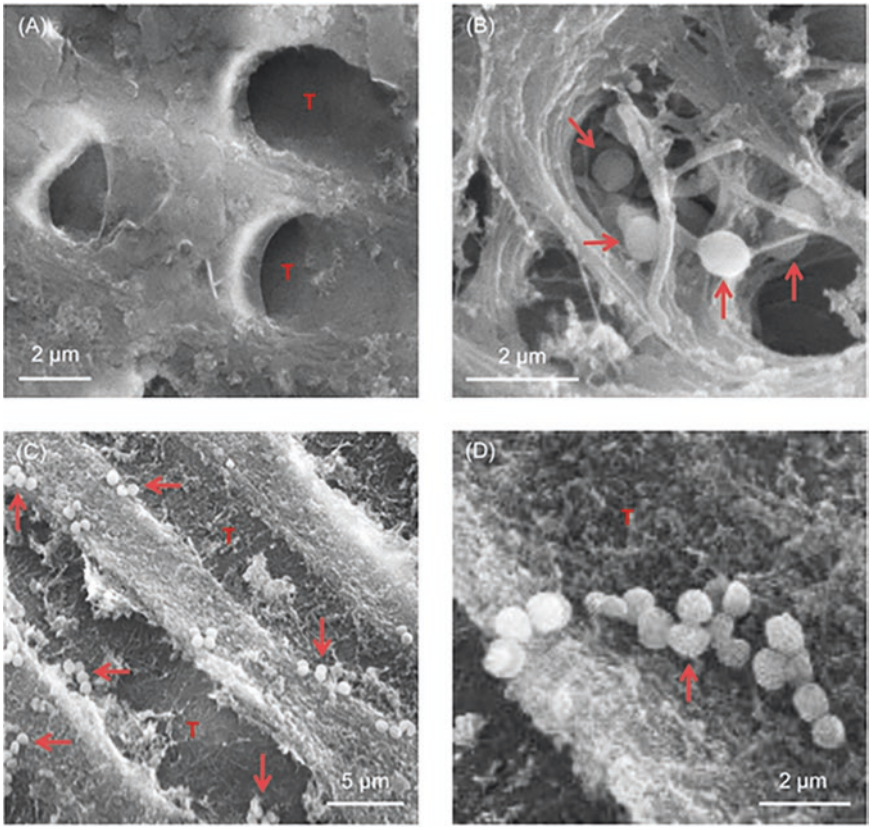

Fig. 3.16 (**A**) Dentinal tubules are shown before impregnation by *Streptococcus* and after impregnation (**B**). The dentin cross section is shown in (**C**), displaying *S. mutans* inside dentinal tubules. Shown under higher magnification (**D**). T: dentinal tubules [316]

bond strength and dental plaque microcosm biofilm response. SBMP was used as the parent bonding agent. Four primers were tested: SBMP primer control, control + 5% (mass fraction) MDPB, control + 0.05% AgNPs, and control + 5%MDPB + 0.05% AgNPs. Dentin shear bond strengths were tested utilizing extracted human teeth. The biofilms arising from the mixed saliva of ten donors were cultured to examine metabolic activity, lactic acid production, and CFU. The result showed that incorporating MDPB and AgNPs into primer did not reduce dentin bond strength, but they both greatly reduced acid production and biofilm viability was considerably diminished as measured against the control. Dual agents MDPB + AgNPs had far stronger effects than either separately, presenting as increased inhibition zone size and reduced metabolic activity, lactic acid, and CFU by an order of magnitude, as measured against the control [319].

To investigate the combined effects of antibacterial adhesive and primer on biofilm viability, metabolic activity, lactic acid, and dentin bond strength, MDPB and

AgNPs were added together into SBMP. MDPB and AgNPs were included in the SBMP primer and adhesive. Tests were conducted on five systems: SBMP adhesive, adhesive + MDPB, adhesive + AgNPs, adhesive + MDPB + AgNPs, primer + MDPB + AgNPs combined with adhesive + MDPB + AgNPs. The mixed saliva from ten donors were used to culture dental plaque microcosm biofilms. Investigations were then conducted on CFU, metabolic activity, and the lactic acid production of biofilms. The results showed that MDPB + AgNPs in the adhesive/primer did not undermine the dentin bond strength. Although MDPB or AgNPs each separately substantially decreased biofilm activities, the dual agents MDPB + AgNPs showed even more substantial decreases. But, when both the adhesive and primer incorporated MDPB + AgNPs, the inhibition of biofilms was the highest [320].

The nanoparticles of NACP were incorporated into the composites and endowed the composites with calcium ion and phosphate ion release properties. The release of calcium ions and phosphate ions were able to remineralize the tooth lesions and inhibit the secondary caries. Melo et al. incorporated NACP and AgNPs into bonding agents and explored the effects on dentin bond strength and plaque microcosm biofilms formation. AgNPs were added into primer and adhesive at 0.1% by mass. NACP were mixed into adhesive at 10%, 20%, 30%, and 40% (mass). It is found that the addition of AgNPs and NACP into adhesive did not decrease the bond strength. NACP had little antibacterial effect but could neutralize acids. SEM showed that numerous NACP infiltrated into the dentinal tubules. Dental plaque microcosm biofilm viability and acid production were greatly reduced by bonding agents containing AgNPs and NACP [321].

The antimicrobial mechanism is through silver ions that inactivate the vital enzymes of bacteria, preventing the bacteria from replicating its DNA which leads to cell death. AgNPs with the release of silver ions could generate ROS, damage the cell membrane, and interact directly with cell membranes. The NACP composite could release high levels of calcium ions and phosphate ions to neutralize cariogenic acid solutions and remineralize tooth lesions. The NACP composites with AgNPs which were able to hinder biofilm growth and acid production would be a promising method to combine three benefits, namely remineralization, acid neutralization, and antibacterial capabilities.

It has been known that the pulpal infection and periradicular lesion formation are often caused by Gram-negative anaerobes and residual bacteria in root canal. And they often lead to the treatment failure of the disease [322]. Ideally, endodontic materials should have some antimicrobial activity to improve the prognosis of endodontically treated teeth. Because the elimination of bacteria in root canal is the key to achieve the success of the treatment. Therefore, it is better that materials in root canal therapy, including root canal filling materials, root canal irrigation solution, and root canal sealing drugs, can be improved by nanomaterials to have the antibacterial properties.

One important step in the endodontic treatment is the chemomechanical debridement of pulpal tissue and pathogenic bacteria. As in every root canal system, there are spaces that cannot be cleaned mechanically. In such places, the cleaning is

dependent on thorough chemomechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. At this stage, irrigant solutions should be used, for dissolving tissue and disinfecting the root canal system. As an irrigant solution, sodium hypochlorite (NaClO) has been used for many years for dissolving tissue and disinfecting the root canal system due to its effective antimicrobial activity. Its working concentration varies from 0.5% to 5.25%; however, if NaClO went beyond the apex, it would damage the periapical tissues.

The liquid form of AgNPs with particle size 35 nm was prepared using a two-step procedure. Nanoparticles were produced using a catalytic chemical vapors deposition procedure and then added to distilled water. No surfactant was used in the liquid to prepare AgNP suspensions. The mixture was prepared using an ultrasonic homogenizer. Lotfi et al. used the AgNP solution and compared its antibacterial effect with NaClO against *Enterococcus faecalis*, which is a bacterium often detected in the failed endodontic treatments. No significant differences were found between the 5.25% NaClO and 0.005% AgNPs, suggesting that although the AgNP was used at remarkably lower concentrations, its bactericidal effect was equivalent to the 5.25% NaClO. Hence, it is a potential new candidate for intracanal irrigant [323].

Yet, questions remain on how effective AgNPs are in combating *Enterococcus faecalis* biofilm. Effectiveness against *Enterococcus faecalis* biofilm was evaluated and compared for the 1% and 5% NaClO, 2% CHX, 1% AgNP suspension, and 26% ZnO NP suspensions. Seventy-six human teeth prepared biomechanically were extracted, mounted onto a specific apparatus, and sterilized. Next, an *Enterococcus* faecalis suspension in 100 μL amounts were inoculated into the root canals, and replaced at 24 h intervals for 7 days. The teeth were randomly divided into six groups according to the irrigation solution: the control 0.85% saline, 1% NaClO, 5% NaClO, 2% CHX, 1% AgNP suspension, and 26% ZnO NP suspension. After the irrigation treatment, the biofilm susceptibility to disinfecting solutions was determined by CFU quantification. The result showed that the effectiveness of 5% NaClO and 1% AgNPs against *Enterococcus faecalis* biofilm was superior than 0.85% saline solution. When compared with the control, 5% NaClO was able to reduce 100% of CFU, followed by 1% AgNPs (97.6%), 26% ZnO NPs (96.1%), 1% NaClO (94.1%), and 2% CHX (93.1%). Based on the methodology used, 5% NaClO and 1% AgNPs was excellent and effective against intracanal *Enterococcus faecalis* biofilm [324]. As NaClO will damage the periapical tissues when it is beyond the apex, 1% AgNP suspension may be a more suitable irrigant solution to intracanal biofilm.

Typically, camphorated phenol (CP), a medicant categorized in the phenolic group, has been implemented either through a paper point submerged in the root canal or on a cotton wool pellet submerged into the pulp chamber, with the expectation that the vaporization properties can elicit antimicrobial activity [325]. However, the antibacterial action of this group of medicants may not be long-lasting. Thus, some bacteria may survive and have opportunity to multiply and persist in the root canal system.

An alternative, nanosilver gel (NSG), was assessed in comparison with CHX and CP against *Enterococcus faecalis* biofilm to determine its effectiveness. NSG was in three concentrations (0.05%, 0.1%, and 0.2%). Two percent CHX was used, and CP and normal saline were used as control. Among the groups (0.05% CP and NSG, 0.1% CP and NSG, 0.2% CP and NSG, 2% CHX and 0.1% NSG, and 2% CHX and 0.2% NSG), significant differences were found. Comparatively, there was no significant difference between CHX and 0.05% NSG. Thus, it can be concluded that 0.1% and 0.2% NSG is more effective on *Enterococcus faecalis* biofilm as compared with CHX and CP [326].

Other researchers synthesized and characterized the AgNPs with different surface charges to evaluate the antibacterial and cytotoxicity activity in the presence of dentine compared with NaClO and CHX. AgNPs with positive, negative, and neutral surface charges were synthesized and characterized. AgNPs having a positive surface charge were found to have the smallest minimal inhibitory concentration (MIC) against planktonic *Enterococcus* faecalis and also were active at notably lower concentrations compared with NaClO, CHX, and other evaluated AgNPs. At 5.7×10^{-10}mol/L, the positively charged AgNPs completely prevented *Enterococcus faecalis* growth after 5 min contact, a finding that was comparable to 0.025% NaOCl. And the most positively charged AgNP solution was the least toxic solution to L929 fibroblasts. So, they concluded that the surface charge of AgNPs was significant for bactericidal efficacy against *Enterococcus faecalis*. Antibacterial results against *Enterococcus faecalis* was promisingly displayed by the positively charged imidazolium-based ionic liquid protected AgNPs, which also showed a high degree of cytocompatibility to L929 cells [327].

A variety of materials have typically been employed for root canal fillings, especially gutta-percha [328]. The zinc oxide in this material has been proven to slightly provide it with antibacterial properties. However, effective bactericidal properties are still lacking. To counter this drawback, some researchers have developed a new kind of gutta-percha coated with AgNPs to improve the antibacterial effect. The new materials showed significant effect against some sorts of germs such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, and *Escherichia coli* [329]. In addition, to test its biocompatibility, the cytotoxicity of nanosilver-coated gutta-percha was tested using mouse fibroblasts compared with normal gutta-percha. And the results confirmed its good biocompatibility. They found that nanosilver-coated gutta-percha presented similar cytotoxicity to normal gutta-percha after 24 h. And it reached the lowest level of cytotoxicity compared with the gutta flow and normal gutta-percha after 1 week [330].

Ideal materials for sealing root-end cavities should prevent leakage, have dimensional stability, adhere to the cavity walls, and promote healing. They should also be nontoxic and biocompatible. However, most current root-end filling materials are unable to guarantee a hermetic seal. This results in a possible microscopic space that can exist between the interface of the filling material and the root-end cavity, providing a pathway for bacteria and cytotoxic produce to possibly penetrate. Thus, in addition to other properties, antimicrobial activity must also be addressed for ideal root-end-filling materials [331].

Due to low solubility, low cytotoxicity, good biocompatibility, and excellent ability to induce hard tissue formation, the mineral trioxide aggregate (MTA) had been used in many indications such as perforations sealing, external/internal root resorption repair, and apexification. But the antimicrobial properties of MTA are controversial and seem to be limited. Aiming to improve it, some researchers modified MTA by adding 1% by weight AgNPs [85] and then evaluated its effect against fungi species and oral bacteria. Compared to unmodified MTA, the AgNP-containing MTA possess enhanced antimicrobial effects against *Candida albicans, Enterococcus faecalis*, and *Pseudomonas aeruginosa* [85, 332]. Each gram of MTA powder was mixed with 350 μL of 25, 12.5, and 6.25 ppm preparations of AgNP solution. Both AgNPs and AgNP–MTA inhibited the growth of all four anaerobic endodontic–periodontal pathogens (*Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis,* and *Prevotella intermedia*) at 25 ppm concentration. AgNPs significantly improved the antimicrobial activity of MTA. And bacterial susceptibility to various concentrations of AgNPs was dependent on bacterial type. Overall, AgNP–MTA displayed a significant time- and dose-dependent inhibitory effect [333].

Antimicrobial effects against five microorganisms typically associated with dental infections were tested by adding two concentrations of AgNPs (100 and 200 ppm) to MTA (60 μg) and testing their effectiveness. The result showed that resultant MTA had antibacterial activities on all microorganism strains except *Enterococcus faecalis* and mixture group. These two cements in aqueous form could not inhibit the growth of *Enterococcus faecalis.* The main reason is that *Enterococcus faecalis* possibly has the ability to change its cell wall structure, whereby also increasing its resistance against temperature and high pH. Adding AgNPs, however, can significantly alter the antibacterial effects of MTA against *Enterococcus* faecalis and other assessed microorganisms. No statistically significant difference was found between two AgNP concentrations. The mechanism of the positive antimicrobial effects of AgNPs was that silver particle can decrease the attachment of microorganisms to the surface and also increase the antibacterial properties of endodontic sealer [332].

Evaluation of the inflammatory reaction of MTA and AGNPs was also performed by some researchers by comparing the subcutaneous inflammatory reaction of rat connective tissues to MTA with and without AgNPs (1 wt%). Polyethylene tubes (1.1 × 8 mm) containing experimental materials (MTA and MTA + AgNPs and empty control tubes) were implanted in subcutaneous tissues of male rats with different evaluation of time, 7 days, 15 days, 30 days, and 60 days. And it can be concluded that incorporation of 1% AgNPs into MTA does not induce the inflammatory reaction of subcutaneous tissue in rat models [334].

Endodontic treatment aims to eradicate microorganisms from the root canal space, or at least reduce microorganism levels to amounts suitable for periadicular tissue health [335]. After chemomechanical treatment of root canals, the population of microorganisms is significantly decreased. However, the microorganisms cannot be eliminated thoroughly. Therefore, endodontic sealers with high antimicrobial activity helps to decrease or prevent the growth of microorganisms and aid the repair process of apical and periapical tissues. However, fiber posts are generally

needed in endodontically treated teeth with great coronal destruction to obtain better retention of crowns or resin composite restorations. When implemented, it can help evenly distribute the stress generated by tooth function [336].

MicroMedica S.r.l. (a company in Italy) developed a new fiber post with AgNP incorporation. The purpose is to create a fiber post which combines the common elasticity, mechanical strength, adhesion, and esthetic characteristics, with the new antibacterial capacity provided by the incorporation of silver. Poggio et al. compared the antibacterial activity and the cytotoxicity effects of different fiber posts: glass fiber post, quartz fiber post, nanofiber post (glass fiber with zirconia nanoparticles), and silver fiber post (quartz fiber with AgNPs). Silver fiber post was the only one showing a fair antibacterial effect against all the three streptococcal strains, while the other posts tested did not present any antibacterial effects. The incorporation of AgNPs endows the fiber post with antibacterial activity, decreasing the development of recurrent caries and increasing the longevity of tooth restorations [337].

Dentures, mostly constituted by methyl methacrylate (MMA) and poly methyl methacrylate (PMMA) acrylic resin, have their inner surface considerably rough. The roughness, combined with other factors such as poor hygiene, xerostomia, and HIV infection, contributes to the emergence of denture stomatitis [338, 339]. This pathology mostly localized in palatal mucosa and presented in 50–70% of complete denture wearers. It is frequently associated with *Candida* species colonization. The biofilm formed by fungi is a key factor in the development of denture stomatitis [340]. The treatment of denture stomatitis is based on topical or systemic antifungal drugs, e.g., fluconazole and nystatin. However, antifungal resistance has been reported in *Candida* biofilms [341]. Therefore, this infection is often persistent. Another challenge associated with denture stomatitis is the difficulty for many geriatric prosthetic wearers in maintaining clean dentures, due to their reduced motor dexterity, cognitive impairment, and memory loss [342]. Considering the above factors, denture stomatitis represents a challenge in dentistry. And the prevention methods are urgently needed. Therefore, nanoparticles have been incorporated.

In a research, graphene-oxide nanosheets (nGO) were incorporated into PMMA to introduce sustained antimicrobial-adhesive effects by increasing the hydrophilicity of PMMA. nGO was added in quantities of 0.25, 0.5, 1.0, or 2.0% by weight relative to PMMA powder which coarsened its surface and enhanced its hydrophilicity without sacrificing surface hardness or flexural strength. The nGO-incorporated specimens showed increasing concentration-dependent levels of anti-adhesive effect after exposing 1 h to the microbials in artificial saliva, while neither producing significant cytotoxicity to oral keratinocytes nor requiring the loading of chemicals or drugs. Additionally, PMMA continuously expressed improved anti-adhesive effects against *Candida albican* for up to 28 days after nGO modification than expressed by pure PMMA as viewed through increased hydrophilicity [343].

Nano-chitosan particles, which have lasting antimicrobial activity, at concentrations of 0, 1%, 5%, and 10% (w/w), were added to the acrylic resins to study the inhibitory effect on the biofilm formation of *Candida* species. The results showed that there were significant differences between unmodified acrylic resin (control)

and acrylic resin with nano-chitosan particles in terms of biofilm formation. No significant difference was found in the formation of biofilm species on resins. With the increase in the concentration of nano-chitosan particles, the rate of biofilm formation is reduced [342].

AgNPs (100–120 nm) were incorporated into a commercial tissue conditioner, in the following concentrations: 0.1%, 0.5%, 1.0%, 2.0%, and 3.0% (vol/vol %: colloidal Ag/conditioner liquid). Their inhibitory effect was evaluated against *Staphylococcus aureus, Streptococcus mutans*, and *Candida albicans* after 24 h and 72 h. The authors reported that the modified tissue conditioner combined with AgNPs displayed antimicrobial properties against *Staphylococcus aureus*, *Streptococcus mutans* at 0.1% Ag incorporation and *Candida albicans* at 0.5% Ag incorporation after a 24 and 72 h incubation periods [344].

In another research, 2 wt% silanized zirconium dioxide nanoparticles (nano-ZrO_2) and 4 wt% silanized aluminum borate whiskers (ABWs) were mixed with PMMA powder to get ZrO_2-ABWs/PMMA composites. Titanium dioxide (TiO_2), silver-supported TiO_2 (Ag/TiO_2), Novaron, and tetrapod-like zinc oxide whiskers (T-ZnOw) antibacterial agents of 3 wt% were mixed with the composites respectively to fabricate standard specimens. Then, the composites were mixed with MMA monomer at a 2:1 powder-to-liquid ratio to test the antibacterial property against *Streptococcus mutans* and *Candida albicans*. Table 3.2 lists the groups of different composition prepared in this study. The 3 wt% addition of various antibacterial agents had significant antibacterial activities compared to the control and blank groups. The Ag/TiO_2 and Novaron groups had better antibacterial property than the other groups. As for Ag/TiO_2, it can consequently interact with O_2 and H_2O and form ROS, which can adhere to the membrane of the bacteria occurring lipid peroxidation reaction to cause the damage of cellular proteins and finally lead to cell death [236].

AgNPs have high antibactericidal activity and good biocompatibilities. They tend to aggregate spontaneously when their diameters are less than 200 nm. And their stability in air, water, or sunlight is not good enough for long-time storage. Novaron, a silver-supported inorganic antimicrobial agent, offers superb antimicrobial efficacy to a broad range of microorganisms. Presumably, the antimicrobial mechanism involves either one or both of the following steps: inhibition of the bacteria's vital metabolism by silver ions and/or the destruction of the bacteria cell membranes from the activated oxygen generated from water [181, 236].

Periodontitis is an inflammatory and infectious disease of the periodontium caused by pathogenic microorganisms. Left untreated, periodontitis destroys the

Table 3.2 PMMA composite components by group [236]

Group	Ingredients
Blank	MMA monomer, PMMA powder
Control	MMA monomer, PMMA powder, silane coupling agent: nano-ZrO_2, ABWs
TiO_2	MMA monomer, PMMA powder, silanized ABWs 3 wt% TiO_2, silanized nano-ZrO_2
Ag/TiO_2	MMA monomer, PMMA powder, silanized ABWs 3 wt% Ag/TiO_2, silanized nano-ZrO_2
Novaron	MMA monomer, PMMA powder, silanized ABWs 3 wt% Novaron, silanized nano-ZrO_2
T-ZnOw	MMA monomer, PMMA powder, silanized ABWs 3 wt% T-ZnOw, silanized nano-ZrO_2

alveolar bone of the teeth and supporting tissues. Current treatment of periodontal infections includes mechanical debridement, administration of antibiotics, and bone grafting.

The primary goal of periodontal treatment has always been the elimination of pathogen containing biofilms. Traditionally, the initial phase of treatment is using supra- and subgingival mechanical debridement. However, periodontal pathogens cannot be completely removed by this method. To augment mechanical debridement, adjunctive antimicrobial agents in forms of topical or systemic antibiotics or topical antiseptics have been employed [345, 346]. However, the systemic antibiotic therapies seem to constitute an inferior choice compared with the topical use of low-cost, broad spectrum antiseptic agents which is low in adverse reaction potentials.

To develop the high and safe antimicrobial subgingival irrigation, nanobubble water (NBW3) which is a form of gas nucleus less than 100 nm in diameter was developed by nanobubble-generating technology. The ozone concentration of NBW3 is 1.5 mg/L which is equivalent to the oxidation titer determined by electron spin resonance. In one research, mechanical therapy was completed in a single visit with an ultrasonic scaler running NBW3 or tap water as irrigant. The results showed that the NBW3 group posed significantly greater clinical attachment gain and reduction in the probing pocket depth as compared to the water group after 4 and 8 weeks. Moreover, the mean total number of bacteria in subgingival plaque showed significant reductions only in the NBW3 group during the investigation [347].

Yet, the mechanisms for how NB3 inactivates bacteria still require further research. However, one can postulate that the phenomenon might be similar to that of existing ozonated water. The ozone, as a potent oxidizing agent, in ozonated water could react with various organic substances and decompose them by free radical-mediated oxidation reactions. Ozone can be converted into oxygen when it reacts with organic substances. In this process, hydroxyl radicals are generated, which is an important ROS. These free radicals might play a role in the eradication of bacteria by NBW3 [347].

Nanoparticles have also been used in dentistry to locally deliver drugs in sufficient concentrations directly to the site of action. These delivery systems are usually injected into periodontal tissues or inserted into the periodontal pocket for enhancing the therapeutic effects while also reducing the drug effects due to systemic use and large dosage [348]. Because of their small size, nanoparticles can penetrate into areas, such as bacterial cells, alveolar bone trabeculae, and from the gingival sulcus inward to the underlying connective tissue and to the periodontal pocket areas below the gum line where may be inaccessible to other delivery systems [349].

The biogenic AgNPs, produced by *Escherichia coli* which served as a matrix preventing aggregation, were assessed for the possible inhibiting effect on a selected suspected periodontopathic bacterium, by bacterial colony counting assay. The result showed that lower concentrations of bio AgNPs (1, 3, 6, 12μM) failed to inhibit the growth of the selected bacterium, and the higher concentration (18, 30μM) showed significant bacterial inhibition effect. When the antibacterial activity of the biogenic silver, ionic silver, and chemically produced nanosilver is compared, the MIC of the former two was lower than that of the chemically produced nanosilver. It was the same in the minimal bactericidal concentration (MBC) results.

The existence of salts and organic matter reduced the antimicrobial activity of biosynthesized silver, thereby increasing the MBC and slowing the inactivation of the bacteria. Due to the high concentration of free silver ions and also the similarity in performance between both ionic silver and biogenic silver radical formation, the mechanism of biogenic silver action can mainly be attributed to silver ion release. (Fig. 3.17) [350].

Minocycline is a long-acting and bacteriostatic antibiotic, often acts against periodontal pathogens, and was chosen to be incorporated into PLGA nanoparticles by Kashi TS [351]. Novel minocycline-PEGylated PLGA nanoparticles, with an average particle size of 85–424 nm, was the best in drug loading, showing higher in vitro antibacterial activity than the free drug. The results of drug release test performed in phosphate buffer at pH 7.4 indicated slow release of minocycline lasting from 3 days to several weeks [351].

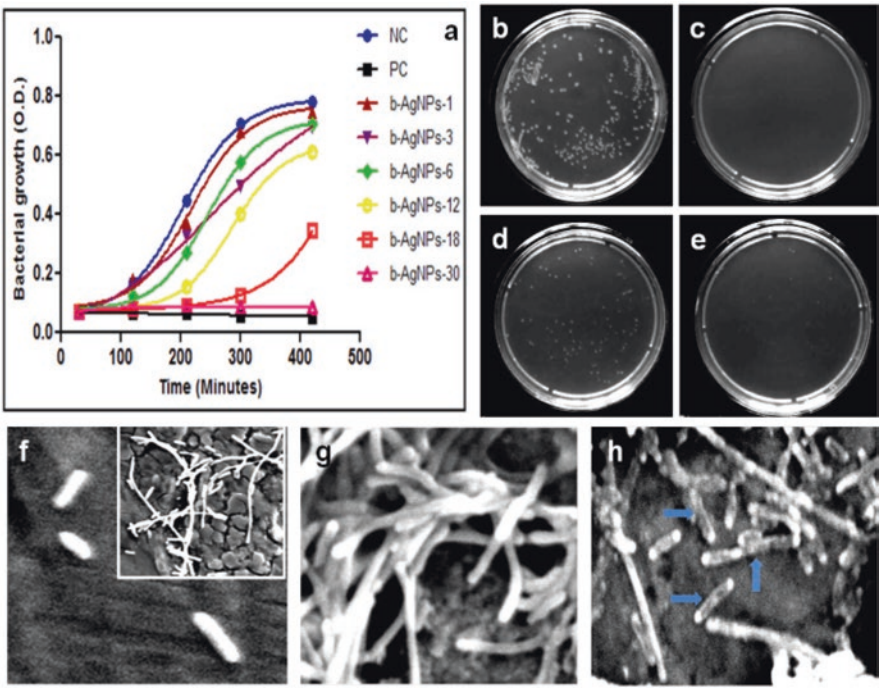

Fig. 3.17 Study of antibacterial activities: (**a**) liquid growth inhibition kinetics of *E. coli* using different concentrations of b-AgNPs. b-AgNP-30 (at 30 μM) shows almost 100% growth inhibition. Ampicillin has been used as a positive control (PC) and NC: negative control or untreated *E. coli*. The numbers indicate the concentration of b-AgNPs in μM. (**b**–**e**) optical images of bacterial colonies formed by *E. coli* cells, i.e., colony counting assay (after 24 h): (**b**) Control, (**c**) Ampicillin (100 μg/mL), (**d**) b-AgNPs (18 μM), (**e**) b-AgNPs (30 μM) and (**f**–**h**) SEM images of *E. coli* cells (**f**) without being treated (control), (**g**) treated with Olax for 1 h, (**h**) treated with b-AgNPs (30 μM) for 1 h. The SEM images show the silver nanoparticles damage the bacterial cell membrane (marked by blue arrows), whereas the bacterial membranes of untreated and treated *E. coli* with Olax are intact [350]

Some researchers developed an osteoconductive drug delivery system composed of apatite nanocarriers capable of providing sustained delivery of drugs in the periodontium. The antibacterial activity of calcium-deficient hydroxyapatite (CDHA) nanocarrier-loaded tetracycline with different Ca/P ratios was detected. CDHA nanocarriers of Ca/P = 1.61 was found to be an ideal carrier for local delivery of tetracycline with continuous release over a period of 120 h. Tetracycline showed a release profile having an initial burst release followed by a slowly prolonged releases over the next 5 days. The first stage is because of the desorption of loosely bound drug molecules on the surface while the second is from the slower dissolution of the CDHA crystal. It can be concluded that the CDHA nanocarriers are ideal drug delivery agents and have bone regenerative potential for local periodontal applications [352].

The concept of periodontal regeneration necessitates the exclusion of epithelial and connective tissue cells of the gingiva from the wound site. Therefore, it leads to the development and application of guided tissue regeneration (GTR) technique. GTR procedures using nonabsorbable and bioabsorbable membranes have been used successfully and predictably in treating various types of intrabony defects [353]. Because of the bacterial contamination of the wound site at the time of surgery or during the period of healing, the outcome of the GTR procedure could be significantly compromised. Numerous protocols, such as systemic antibiotic therapy, local application antibiotics in the form of antibiotic gel and antibiotic fiber, and irrigation with antibiotic solution, have been advocated in controlling or eliminating periodontal pathogens during GTR procedures, achieving improved outcomes during GTR therapy. Antibiotics such as amoxicillin and metronidazole have been loaded on GTR membranes for successful treatment outcomes [354–357].

AgNPs were impregnated into GTR membranes to evaluate the antibacterial activity in terms of bacterial adherence to the membranes and specific bacterial penetration through the membranes. Three sets of GTR commercial membranes used in this study were grouped as GTR-C: plain GTR membrane; GTR-NS: GTR membrane impregnated with 0.1 mg/mL 10 nm AgNPs; and GTR-DOX: GTR membrane impregnated with 25% (w/w) doxycycline hydrochloride acting. The bacterial strains used in this study were *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. The GTR-C group showed significantly higher mean bacterial adherence scores compared to the GTR-DOX and GTR-NS groups. Highly significant lower adherence scores were found for GTR-NS as compared to GTR-DOX among all four microorganisms [358].

To combine the beneficial properties of the bioactive-glass, the gelatin, and AgNPs together, a study added various concentrations of AgNPs (0, 5, 10, 20, and 40 mM) to a macroporous scaffold for bone tissue engineering which contained hybrid gelatin/bioactive glass. Incorporating AgNPs to the scaffolds may affect physicochemical properties of the scaffolds, such as gel fraction, porosity, swelling behavior, morphology, and the antimicrobial activity. The antimicrobial activity of the scaffolds was investigated against two types of bacteria *Staphylococcus aureus* and *Escherichia coli*, presenting significant inhibition of the growth of those bacteria and the reduction of the biofilm formation on the scaffolds. The antibacterial effect was increased with increased AgNP concentrations [359].

Due to being nontoxic and having high biocompatibility, biopolymers have been widely used for scaffolding in biomedical research. However, certain limitations still arise from using polymers (e.g., pure polymers have nonbactericidal nature) which can be overcome by bioinorganic hybrid nanocomposites [360]. Typical inorganic nanomaterials used for this purpose include metals (Au, Ag, etc.) and metal oxides (ZnO, TiO_2, MgO, CaO, NiO, CoO, etc.). When they are utilized in the synthesis of polymer nanocomposites, they act as reinforcement materials. Regenerated bacterial cellulose (RBC) nanocomposites with TiO_2 NPs were prepared with the aim to enhance the bactericidal activity and tissue regeneration. The results suggested that the bactericidal activities were due to ROS and membrane stress generated by the TiO_2 NPs present in the composite membranes.

Nanoparticle-mediated oxidative stress by nanoparticles might be caused by a variety of factors, which include the generation of ROS, e.g., H_2O_2, O_2^*, O_2^-, and OH. When the ROS generation becomes excessive, the mitochondrial membrane permeability increases and the cellular respiratory chain is damaged. So, the RBC-TiO_2 nanocomposites showed impressive adhesion and proliferation capabilities on animal fibroblast cells without any toxic effects in addition to antibacterial properties [361].

Titanium implants, which are widely used in dentistry, sometimes present infections around their surface, especially where the implant connects the gingiva soft tissue. This infection is still one of the major complications in orthopedics and implantology. Several methods have been presented to combat bacterial contamination, such as aseptic surgical protocols and implant disinfection. Nonetheless, bacterial invasion still occurs frequently after surgery. Thus, antibacterial coatings have been developed and tested to prevent biofilms from forming over the implant surface. However, most exhibit inadequate long-term antibacterial action and pose the problem of generating resistant strains after the possibility of generating resistant strains after extensive use [362]. Thus, some researchers incorporated AgNPs to implant surface and expected that it would be possible to produce coatings with long-term antibacterial properties by the controlled release of AgNPs [363].

In one research, pure titanium foils underwent electrochemical anodization to form a titania nanotubular (TiO_2-NT) layer, and then the TiO_2-NTs were soaked in $AgNO_3$ solutions with four different concentrations (0.5, 1, 1.5, and 2 mol/L) for 10 min, to introduce AgNPs to the wall of the TiO_2-NTs to get the Ag-loaded TiO_2-NTs (NT-Ag), and found that TiO_2-NT-coated Ag had inhibitory effect on the planktonic bacteria in the first 4 days. In addition, the high efficacy of Ag at very low concentrations and relatively large reservoir provided by the nanotubes can give rise to long-term antibacterial effects, which can last for 30 days, and guarantee normal wound healing in the early stage. Initially, a large amount of Ag is released. But it diminishes gradually with immersion time. For dental implants, the phase right after implantation poses the greatest risk and likelihood of infection. Hence, the robust ability of NT-Ag to eliminate surrounding planktonic bacteria assists in preventing postoperational infection and guaranteeing normal wound healing at the early stage. Afterward, primary healing of the surgery site will be completed and in time osseointegration as well. A low-level Ag release can be used in the later stage in order to inhibit bacterial adhesion and biofilm formation and thereby prevent later-stage

infection conjugated with the host defense. This strategy provides both the production of a long-term antibacterial surface while also decreasing the risk of cytotoxicity by controlling the Ag release rate. Furthermore, prevention of implant-associated infection in both early and medium stages is effectively controlled by the long-term bacterial resistance presented [363].

An attractive strategy for control of peri-implantitis is posed by titanium nanoscale modifications of antibacterial implant surfaces. Silica-based composite coating containing AgNPs (AgNP/NSC) produces a strong antibacterial effect on titanium surface by not only killing the adherent bacteria but also reducing the extent of biofilm formation by more than 70% on the coated surface compared to the control. Since relatively low levels of silver release in the aqueous solution was presented by the AgNP/NSC coating, bacterial contact or adjacent to the AgNP-doped nanoporous silica surface can explain the bactericidal mechanism. Nanometer-level erosion of the silica-coating matrix can appear in the aqueous medium, resulting in the metal nanoparticle exposure and release at the material/aqueous solution interface. AgNPs with the release of silver ion could generate the ROS and damage the cell membrane happened due to the interaction between nanoscale silver and cell membranes. The silanol functional groups on the surface of silica have shown stronger antimicrobial activity. Silica has silanol functional groups on its surface which have shown more potent antimicrobial activity than that of their analogous alcohols due to the physicochemical properties of silanol, particularly based on the higher H-bond acidity and hydrophobicity as compared to alcohols. Thus, the surface chemistry of the nanoporous silica matrix and the bactericidal effect of AgNPs in conjunction may explain the antibacterial activity of the AgNP/NSC nanocomposite coating [364].

Flores et al. modify Ti/TiO_2 surfaces with citrate-capped AgNPs by immersing the Ti substrates in the AgNP solutions (3.16×10^2 mg Ag/mL) in the dark for 24 h and evaluated the antibacterial activity of AgNPs against *Pseudomonas aeruginosa*. They tested the efficiency as antimicrobial coating of AgNP on Ti/TiO_2 surfaces by checking the ability of attached bacteria to form colonies in agar. The results from early stages of biofilm formation as detected by bacterial spreading on agar plates conducted on control Ti/TiO_2 substrates after incubating 24 h in nutrient agar were 0.28 and 1.24 cm for the AgNP-covered substrate and the control, respectively. These results support that there are less viable cells found attached on the AgNP-covered surface as compared to the control. Additionally, the halo area was reduced, indicating that growth of bacteria on the agar in the vicinity of the substrate was inhibited by the diffusion of silver ions from the AgNP-covered substrate. To quantitatively assess the effectiveness of AgNP-modified Ti/TiO_2 surfaces, viability assays were carried out with the LIVE/DEAD viability kit. The result showed that the total number of bacterial cells revealed on AgNP-modified implants was only 20% of those that were attached to unmodified surfaces. These results suggest that incorporating AgNPs on Ti implants can efficiently protect the implant surface against pathogen colonization [365].

AgNPs with various diameters and distributions were immobilized on stainless steel (SS) via silver-sourced plasma immersion ion implantation (Ag-PIII), for 0.5 and 1.5 h, and their antibacterial ability was investigated with four different

bacteria, including *Escherichia coli* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Staphylococcus epidermidis* (Gram-positive). Ag-PIII treatment is effective in inhibiting bacteria adhesion and biofilm formation, and the antibacterial activity of AgNPs immobilized on SS for 1.5 h was higher than that of 0.5 h. Moreover, Ag ions were minimally released by the Ag-PIII samples, and their antibacterial activity was maintained after multiple cycles of bacterial exposure, suggesting that antibacterial action of the Ag-PIII SS was independent of Ag release and more likely from Ag and SS substrate synergy. However, further studies are needed to fully elucidate the antibacterial mechanism. The study also found that new bone formation occurred on the surface of the Ag-PIII wires, and fibrous tissue occurred only surrounding the SS wire, suggesting that the Ag-PIII SS also had good osteogenic ability (Fig. 3.18) [366].

Silver ion-containing HA nanopowder coating was evaluated for their potential to reduce bacterial colonization on titanium rods in a methicillin-resistant *Staphylococcus aureus* (MRSA)-challenged rabbit femoral implant model. Compared to the uncoated and HA-only-coated rods, the coated rods exhibited significantly less bacterial growth at 10 weeks. Organ and tissue samples showed no detectable accumulation of silver, and likewise bone cells did not exhibit cellular inflammation or the toxic effect of silver. Silver ion-doped calcium phosphate-based ceramic nanopowder coating on orthopedic implants may prevent bacterial colonization and infection in open fractures compared with those without coatings [367].

ZnO NPs were deposited alone or in mixtures with nanohydroxyapatite onto the surface of glass substrates using an electrohydrodynamic atomization process. The coatings displayed significant antimicrobial activity against *Staphylococcus aureus* in in vitro test. As the concentration of ZnO NPs increased, an increased

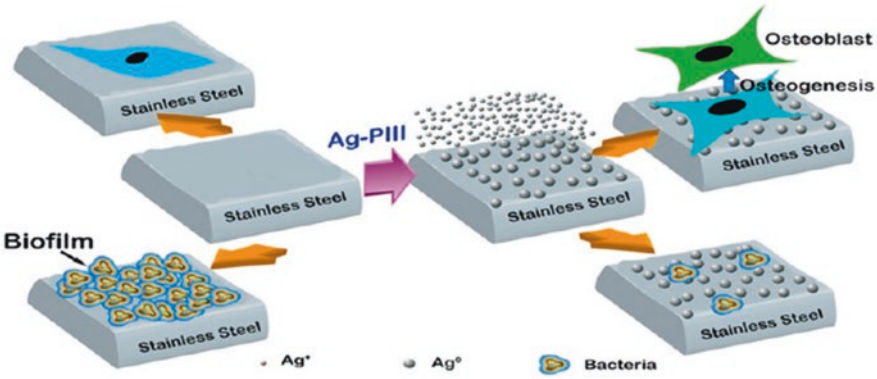

Fig. 3.18 Modification of the stainless steel surface (SS) was performed by silver-sourced plasma immersion ion implantation (Ag-PIII). Various distributions and diameters of the metallic silver nanoparticles were fabricated on the SS surfaces after treating with Ag-PIII for 0.5 and 1.5 h, respectively. In vitro and in vivo tests were used to evaluate the antimicrobial properties and osteogenic activity of SS before and after Ag-PIII treatment. The results showed that the Ag-PIII not only enhanced the antibacterial activity of SS but also promoted the osteogenic differentiation of human bone marrow stromal cells [366]

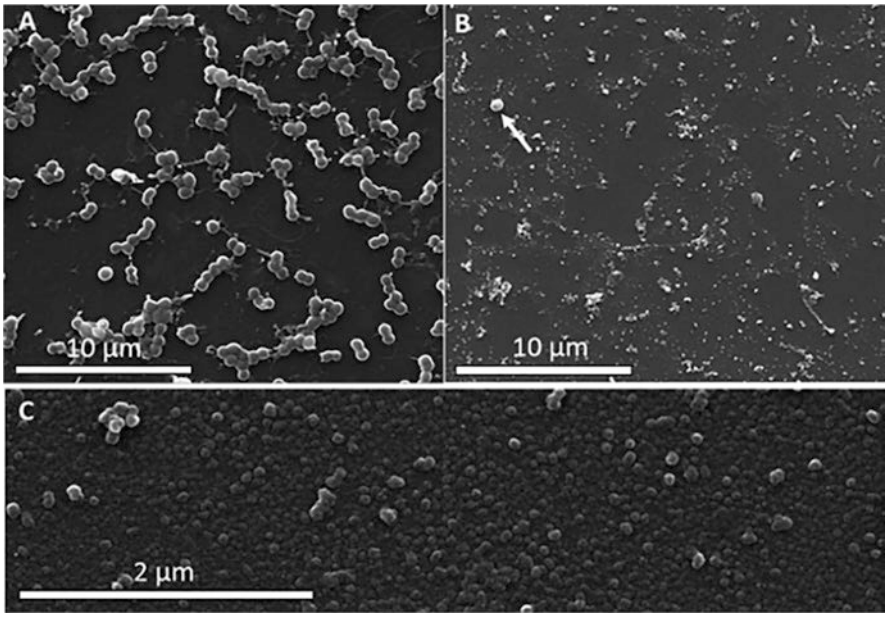

Fig. 3.19 The antimicrobial effect of nZnO-coated glass samples as compared to uncoated samples analyzed qualitatively. (**A**) Bacteria appearing on uncoated surface. (**B**) A single bacterium, as denoted by the white arrow, appearing on the coated surface. (**C**) Uniform characteristics of the coating as shown under high resolution [368]

antimicrobial activity was observed when submerged in a suspension of *Staphylococcus aureus* for a prolonged period. The coatings also demonstrated minimal toxicity to rat UMR-106, human MG-63 and human mesenchymal cells. And osteoblast cells UMR-106 showed an increase in proliferation and alkaline phosphatase activity on the coated surfaces (Fig. 3.19) [368].

To create titanium implants with potent antibacterial activity, a new "sandwich-type" structure of sulfhydrylated chitosan/gelatin polyelectrolyte multilayer films embedding AgNPs was coated onto titanium substrate through a spin-assisted layer-by-layer assembly technique. The coatings were shown to inhibit the growth and activity of *B subtilis* and *Escherichia coli* in vitro. It was indicated that Ag ions derived from AgNPs within multilayer films were released into peripheral region around titanium substrate and killed bacteria until AgNPs were completely consumed [369]. The cytotoxicity of AgNPs within multilayer films was attributed to some extent by the released Ag ion concentration [370]. To lower the cytotoxicity to osteoblasts, Ag ion release must be adjusted to a slow manner, possibly by using multilayer films, and the coordination between sulfhydrylated chitosan and Ag elements could adjust the Ag ions. On the other hand, the interaction mechanism between osteoblasts and Ag ions was different from that of bacteria and Ag ions due to their differences in size and structure. The proton-depleted region formed around AgNPs would disrupt the synthesis of adenosine triphosphate, leading to the death of bacteria [371].

Due to its small size, large surface area, and high probability of contact with pathogenic bacteria, nanomaterials have strong bacteriostatic properties to prevent and control oral infectious diseases. The nanotechnology has been exploited in dentistry for some time with significant success, particularly in the development of restorative materials. Researchers have been able to overcome the technical challenges in processing these materials and exploit the properties at nanoscale. Other areas which have significant promise to prevent and treat oral biofilms using nanotechnology are dental bonding materials, nanocoated implants, and so on. One point to note in the treatment of biofilms is that virtually all treatments have focused on killing the bacteria in the biofilm. The most commonly used dental microcosm biofilm model as the inoculum is the individual saliva. However, different individuals may have different biofilm compositions and dietary habits. Therefore, the antibacterial applications of nanomaterials in the treatment of oral disease need to be investigated in human in situ or in vivo models to get a better understanding of the underlying mechanisms.

The development of nanomaterials and nanotechnologies will become the core of the continuous progress of oral medicine and will make more remarkable contributions to maintaining oral health and improving the quality of life. We are also facing many challenges, such as the toxicity of nanomaterials to the mouth is low, but some nanoparticles may lead to the intestinal disorders and the organ lesions. So, the test of cytotoxicity and genetic aberration of nanoparticles and the biological safety of nanomaterials need the in-depth study. And these are the focus of future study.

3.3.1 The Toxicological Side Effects in Nano-Incorporated Dental Materials and Device

The benefits of nanotechnology are widely publicized. However, the discussion of the potential effects of their widespread use in consumer and industrial products is just beginning. And the knowledge on potential harmful effects of nanomaterials lags their increased usage in consumer products. Particularly, the toxicity data in the public domain on nanomaterials toward dental applications are sparse, with no information on oral toxicity from dental materials, e.g., biomaterials or implants containing nanomaterials, in animal models or patients. Therefore, the safety data on various nanomaterials applicable for risk assessment are urgently needed.

In principle, dental materials used in the oral environment must be chemically stable and inert. However, leaching of toxic compounds has always been a major concern, occurring as a result of either material instability or degradation, or clinician error due to inappropriate preparation or plication of the material [372].

Metal release such as amalgams and metal alloys from dental materials is common. Comparable to food or drink intake, reported elemental release from dental materials is usually similar or considered negligible. However, the leaching of

chemical substances released from endodontic sealers and resin composites has also been confirmed. This raises the concern that potentially toxic chemicals might be exposed to patients during and after treatment. Nanomaterials used in other fields can be discharged into the environment, which are then modified by pollutants, pH, temperature, and different biological conditions. In turn, nanomaterials also alter the atmosphere, water, and soil. These transformations and interactions can be detrimental to the environment and deleterious to human health [373, 374]. However, similar information for dental materials containing nanomaterials is lacking.

There is also a concern of toxicity to the cells and tissues of human body from the dental nanomaterials used in oral cavity. Some nanoparticles are not toxic but exhibit lack of activity. This lack of activity is presumably associated with an inability to modify the pharmacokinetics of the encapsulated drugs, as well as the rapid clearance of nanoparticles by the liver, spleen, and other organs [375]. Moreover, some nanoparticles have limited toxicity, such cationic nanoparticles. However, these nanoparticles can rapidly biodegrade, with both products and parents being able to deeply penetrate into cells and tissues, partly because of their small size, which finally results in the destabilization of the plasma membrane, cell death, tissue damage, and organ dysfunction [376].

The potential toxicities of nanomaterials on variety of target organs are attributed to various mechanisms, including oxidative stress, ROS generation, inflammatory reactions, DNA damage, protein structure alteration, and membrane integrity disruption [377]. Research has shown that several nanoparticles induce ROS and inhibit antioxidant function. Moreover, metal or metal oxide nanoparticles have consistently been revealed to induce oxidative stress in the liver, spleen, and kidneys. This can be attributed to the activation of specific stress-related cell signaling pathways, mitochondrial dysfunction, and DNA damage leading to cell cycle arrest and apoptosis [378].

Silver has raised the interest of many investigators because of its good antimicrobial activity and low toxicity. There are concerns regarding the possible toxicity of silver-related nanomedical devices. Research showed that free silver ions could affect the therapeutic and toxic properties. A study showed that impairment of hMSC vitality was detected at AgNP concentrations of 10 μg/mL and showed an inhibitory effect on tissue regeneration by repressing stem cell migration. These effects only occur at high concentrations. The migration is not influenced by AgNPs at concentrations of 0.01, 0.1, and 1 μg/mL [379]. There are also reports describing an inhibition of fibroblasts by silver-releasing wound dressings, the toxicity of AgNP-induced differentially expressed miRNAs regulated the expression of target genes and proteins, leading to fibroblasts toxicity through the destruction of cytoskeleton, reduction of intracellular ATP content, and induction of apoptosis [380]. The cytotoxic effects to cells seem to depend on dose. As reported by Tweden et al. [381], silver concentrations up to 1200 ppb showed no cytotoxic effect on fibroblasts in vitro. However, in vivo studies have not established a threshold concentration for silver that can lead to deterioration of the fibroblast. Furthermore, someone found that of 20 nm was more toxic than the larger nanoparticles in L929 fibroblasts. Altogether, these results suggest that AgNP effects on different toxic

endpoints may be due to its aptitude for inflicting cell damage. Additionally, the potency of nanoparticle-sized silver for inducing cell damage as compared to silver ions depends on both cell type and size [382].

Various works have shown that AgNPs have toxicological properties with a consensus on increased oxidative stress as the main mechanism of toxicity, but many focuses on acute, supernormal doses. Some researchers studied the toxicity of AgNPs (two different sizes 10 or 75 nm) with low dose (250 μM/kg) to key organs of rats for 4 weeks. The result showed that AgNPs were capable of entering mitochondria, which gives further strength to the fact that oxidative stress is most important in the toxicity of AgNPs. AgNPs diminished mitochondrial respiratory complex activities and affected the calcium-loading capacity of mitochondria. But the effects of AgNP exposure to cardiac and renal mitochondrial activity were hardly found. No evident changes in the typical hepatic injury serum markers were displayed, which might suggest that AgNP toxicity is a "silent" event, emphasizing that great care is required when using AgNPs for humans. Also, they found that from the two sizes of AgNPs utilized (10 and 75 nm), the smaller 10-nm ones were apparently more [383]. That was because the size of the nanoparticles determines the extent of endocytosis: internalization of smaller nanoparticles is more likely than larger ones. Also, smaller nanoparticles have a greater likelihood to produce cellular toxicities. After entering the cells, nanoparticles flow through the endosomal/lysosomal pathways and different cytoplasmic networks [383].

Studies have also found that nanoparticles can boost the quantity of several inflammatory cytokines. The intake of nanoparticles is usually performed by the macrophages in macrophage-rich organs, such as the spleen and liver, which induces the macrophages to release cytokines. The binding of nanoparticles can also be performed by specific macrophage receptors having collagenous structure. After binding, NPs are internalized and translocated inside the macrophages thru micropinocytosis or endocytosis [377].

TNF-α is known to stimulate several signaling pathways leading to inflammation, apoptosis, and tissue degradation: most importantly, AgNP phagocytosis stimulates the inflammatory signaling by generating ROS in macrophages cells, succeeded by induced secretion of TNF-α by activated macrophages cells, and resulting damage of the cell membrane ultimately leading to apoptosis [384].

To obtain information concerning the pathophysiologic effects of nanoparticles through systemic migration, acute pulmonary responses were examined after intraperitoneal administration of TiO_2 NPs (40 mg/kg) in mice, the exposure of TiO_2 NPs increased neutrophil influx, protein levels in bronchoalveolar lavage fluid, and reactive oxygen species (ROS) activity of bronchoalveolar lavage cells in 4 h. They could also activate inflammatory signaling pathways including the c-Src, p38 MAP kinase, and NF-κB pathways [385].

Iron oxide NPs (IONPs) has been widely used in the biomedical application, namely in magnetic resonance imaging, tissue repair, drug delivery, hyperthermia, transfection, and tissue soldering. However, the safety issues are still a matter of debate. Polyacrylic acid (PAA)-coated IONs and non-coated IONs were evaluated on the production of six cytokines (interleukin (IL)-6, IL-8, IL-10, IL-1β, tumor

necrosis factor (TNF)-α, and interferon (IFN)-γ) in human peripheral blood cells to establish the inflammatory pathways involved. The result indicated that PAA-coated and -uncoated IONPs induced all the evaluated cytokines and involved the activation of transforming growth factor beta (TGF-β)-activated kinase (TAK1), c-Jun N-terminal kinases, and p38 mitogen-activated protein kinases [386].

In summary, nanotechnology is one of the fastest growing fields of the last decade, with applications in the prevention, diagnosis, and treatment of oral disease. However, although there has been rapid development in the engineering and use of nanoparticles, the knowledge about its toxicity is still lacking. Therefore, further toxicological studies on nanoparticles is urgently required, especially prior clinical use. Particularly, the relationship between the structural and physio-chemical properties of nanoparticles as well as how it reacts within cells of various organs and tissues must be further explored.

Daily exposure must also be considered in the occupational health of the practitioner. For the health and safety in the workplace, safe systems should be applied with the aim to prevent the exposure, so that there is negligible risk. Potential exposure of the practitioner could arise from incidental ingestion or dermal contact. However, the clinical practice with protective device such as overalls, surgical gloves, and masks could minimize these exposure routes.

Exposure to aerosols of dental materials containing nanomaterials has not been quantified. There is an exposure from drilling or filing with nanomaterials [372]. In UK, there are limits for the exposure (10 mg m^{-3} for an 8 h exposure to dusts) to aerosol in the workplace. According to the reports from various health and safety agencies, the workers in nanomaterials manufacturing plants undergo an exposure of a few mg m^{-3} or less [387]. Most likely, by reducing the amount of dental material used at a time, the risk of aerosol exposure also reduces substantially. Intriguingly, findings on the abrasion/sanding of composites report releases of free nanomaterials that range from low to negligible; implying that the risk to the dentist by abrading/shaping a dental composite might also be low. However, distance from the exposure source is critical to the victim. In dentistry, the practitioner is inevitably very close to the patient. Therefore, further research is needed on workplace exposure to nanomaterials.

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