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NANOMATERIALS FOR THE REPAIR AND REGENERATION OF DENTAL TISSUES

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7.1 INTRODUCTION

Among all tissues of the human body, teeth are perhaps the most frequently exposed to the greatest variety of environmental stresses. Owing to the diversity of food items available in the present world, teeth must not only endure the wear created by the crushing and grinding of food but also resist considerable variances in temperature and acidity, as well as invasions by pathogenic bacteria that may compromise the integrity of the tooth structure through demineralization of the overlaying enamel. Constant exposure to these stresses, combined with the fact that adult human teeth cannot produce enamel and may only regenerate lost dentin (Nguyen et al., 2013), trigger the formation of regions conductive to the growth of caries. Once formed, dental cavities cannot be regenerated and may only be excised and replaced with a suitable filling material. More

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major tooth injuries, caused by trauma or periodontal disease, may likewise necessitate the artificial replacement of one or more teeth. As such, dental care is a major issue in the modern world and much effort has been spent in fluoridation and dental health campaigns to reduce the prevalence of tooth decay (Mullen, 2005).

Due to their unique function in masticating food, teeth exhibit a structure quite unlike other mineralized tissues. The outermost layer of the tooth consists of a heavily mineralized, wear-resistant substance called *enamel*, which is produced through the activity of specialized matrix-secreting cells called *ameloblasts* (Deutsch et al., 1995). In humans, ameloblasts are present only during the embryonic formation of teeth and undergo apoptosis following this process; as such, mature human teeth are incapable of regenerating enamel (although rodents retain a population of ameloblasts in their incisor teeth (Warshawsky and Smith, 1974), allowing their continuous growth throughout the life of the animal). Although no new enamel deposition occurs in the adult human tooth, demineralized enamel is nonetheless capable of accumulating minerals back into its structure, restoring its structural integrity over time (Lippert et al., 2004). Despite its hardness, however, enamel is brittle and requires structural support, which is provided through the underlying layer of *dentin*.

Unlike enamel, dentin is a softer and more elastic substance and is produced continuously in the human tooth, both to replace lost enamel and as a response mechanism against caries-forming bacteria. *Odontoblasts*, the cells responsible for dentin formation, reside within the inner periphery of the dentin layer and communicate with the pulp through *dental tubules*, which allow the transport of oxygen and nutrients to cells that otherwise have no access to blood flow (Pashley, 1989). The *dental pulp* itself is directly underneath the odontoblast layer and plays a crucial role in the maintenance of the dental cell population, as it is equipped with blood vessels and facilitates nutrient exchange. In addition, the pulp is innervated and thus allows the tooth to sense temperature changes and physical impacts. Lastly, the *cementum* is populated by *cementoblasts* and anchors the tooth to its socket through a fibrous *periodontal ligament* (Han, 2009). Cementoblasts are notable in that they (like bone and unlike the majority of the cells in the pulp) typically embed themselves completely within the matrix they produce; they are called *cementocytes* in this state (Nanci and Bosshardt, 2006).

The regenerative capacity (or lack thereof) of dental tissues renders it difficult to reverse the effects of caries, as enamel does not regenerate and dentin is soft and highly susceptible to decay. Nonetheless, efforts have been made to artificially induce the biomineralization of teeth to reverse

the process of tooth decay, to restore the function of enamel, or even to grow entire replacement teeth under *in vitro* conditions. These efforts often attempt to recapitulate the events that occur during embryonic tooth formation and incorporate a variety of stem and progenitor cells of both dental and alien origin, which are often implanted within a bioactive matrix to trigger their differentiation into the desired cell type. Likewise, the use of bioactive materials for the effective integration of artificial implants into the alveolar bone is an active area of research. Although such attempts are complicated by the layered structure of the tooth, considerable successes have been reported in the field of artificial tooth regeneration, and this chapter will detail the recent advances concerning the use of scaffolds and nanomaterials for this purpose.

7.2 FORMATION OF DENTAL AND OSSEOUS TISSUES

Biomineralization is the process responsible for the formation of hard tissues and involves the cell-mediated deposition of inorganic materials onto a specialized extracellular matrix. It occurs almost exclusively in teeth and the skeletal system and is directed by the enzymes and molecular scaffolds secreted by the native cells of these tissues (Goldberg et al., 2011). Collagen is often a major player in the formation of such scaffolds: The organic matrices of bone, dentin, and cementum are composed of collagens, other fibrous proteins, and lesser amounts of nonfibrous, noncollagenous material, while enamel is exceptional in that it largely lacks collagen. No matter its exact composition, the soft, proteinaceous scaffold must also be reinforced through a mineral component to create the rigid, durable composite material that is characteristic of bones and teeth (Boskey, 2007). Hydroxyapatite (HA) is the principal inorganic component of both dental and osseous tissues, although its precise structure differs between (or even within) tissue types. HA found in dentin and bone features a number of vacancies and substitutions in its matrix, leading to a Ca/P molar ratio that is distinct from the error-free crystal structure, while the mineral component of enamel is closer to stoichiometric HA and consequently displays a Ca/P ratio closer to the "ideal" value of 1.67 (Boskey, 2006, 2007).

Osseous tissues can recover from injury through the bone remodeling process, which involves the removal of damaged tissue and redeposition of a fresh protein matrix for subsequent mineralization (Sims and Gooi, 2008). While dentin can also undergo this process in response to pathogenic bacteria or the erosion of the enamel layer, its regenerative capacity is lower than that of bone. Enamel, in contrast to bone and dentin, is

entirely incapable of regeneration in the strict sense, although demineralized enamel can reaccumulate its inorganic matrix in a suitable environment (Featherstone et al., 1990). The defect-free composition and nonregenerative properties of enamel are a result of its unique and structurally demanding function: Enamel is the hardest structure in the human body (Beniash et al., 2009) and must continuously endure the stresses associated with the chewing of food, and its placement over the dentin layer precludes any location suitable for the maintenance of a living cell population (rodents regenerate enamel only in a specialized region at the base of the incisors (Ohshima et al., 2005)). The functional specialization of enamel is also evident in the structure of its constituent HA crystals: Bone and dentin typically feature rod-shaped crystals of approximately 20–50 nm in length and approximately 12–20 nm in width, while enamel crystals are 10-fold larger in all dimensions (Glimcher, 2006; Kirkham et al., 1998).

The embryonic formation of the tooth in general, and enamel in particular, is a well-regulated process. Tooth development begins with the formation of a dental lamina, which grows inward to the mesenchyme and eventually creates a tooth bud. Odontoblasts are produced from the mesenchymal cells that associate with the bud, while ameloblasts develop from the epithelial cells of the dental lamina (Peters and Balling, 1999). The secretion of enamel is closely linked to that of dentin: Although there is evidence that initial secretion of the two layers may be independent (Diekwisch et al., 1995), the coordinated activity of odontoblasts and ameloblasts is nonetheless necessary for tooth development and results in the formation of the dentinoenamel junction, where enamel extends within and fills the dentin structure. Odontoblast extensions that remain in this layer are embedded into the enamel, forming structures that are called enamel spindles and suspected to be sensory in nature (Simmer and Hu, 2001). The mature enamel contains very little organic material and is composed of well-ordered crystals.

7.3 DENTAL IMPLANTS

The natural process of tooth formation is complex, and the structure itself is capable of regenerating only to a limited degree. As such, defects of the tooth are typically treated by removing the damaged region in its entirety and replacing it with a variety of artificial materials. While simpler forms of damage, such as minor caries, are easily treated, more serious injuries warrant the removal of the tooth and its subsequent replacement with a

suitable *dental implant*. These implants are typically made of inert, alloplastic materials and may be embedded in the maxilla or the mandible; they are commonly used for the replacement of orofacial structures lost due to trauma, neoplasia, congenital defects, and other diseases (Pye et al., 2009). Dental implants can be classified based on their shape, material (metallic, ceramic, or polymeric), location (endosseous, transosseous, or subperiosseous), or the technique used for their placement (single-stage or two-stage).

A dental implant consists of a *crown*, which replicates the function of the tooth; an *abutment* region, which connects the crown to the implant proper; and the *implant* itself, which affixes the crown and abutment to the maxilla or mandible. The implant may be covered entirely by the jawbone or feature an additional length of material for the ease of attaching the abutment and the crown; this property determines the type of surgery used in implant placement. Implants that are positioned within the jawbone require two surgeries for tooth replacement, as they are typically left without crown or abutment to allow the jawbone to heal around the site of surgery. The overlaying gum tissue is stitched over the implant during this time period and necessitates a second, minor surgery for the subsequent attachment of the abutment and the crown. Single-stage implants, in contrast, use a longer, one-piece implant that protrudes through the gingiva, eliminating the need to stitch the gum tissue (although these implants are also left to heal prior to the attachment of the abutment and the crown).

Implants may be placed on, within, or through the jawbone; this also determines the suitable type of surgery for their attachment. *Subperiosteal implants* are typically positioned within the gum tissue and on the jawbone, and they are typically attached using single-stage procedures (Wingrove, 2013). They are advantageous in that they allow the secure attachment of dentures in individuals who do not have sufficient bone height. *Endosteal implants*, in contrast, are usually affixed in two-stage surgeries; they are shaped like a screw or cylinder and made out of metal, ceramic, or ceramic-covered metal. Designed to replace the roots of teeth, they are implanted into the jawbone, and they may be screw-shaped, threaded, cylindrical, smooth, or bladed depending on the tooth operated and the defect involved (Wingrove, 2013). Lastly, *transosteal* or *stable implants* are metallic and inserted through the jawbone, and they are useful when both teeth and the mandible are atrophied (Wingrove, 2013). Considerable variation exists in the exact sizes and morphologies of these implants, and some are custom-made depending on the injury in question.

Implant materials are likewise highly variable. Aluminum, silver, gold, porcelain, and platinum were among the first industrial materials used for

replacing teeth (Barfeie et al., 2015); however, most of these substrates raise substantial immune responses and trigger the formation of fibrous tissue. As such, they are rarely used in modern dentistry (Donath et al., 1992). Dental implant materials can be categorized according to their chemical properties or the biological responses they produce. From a purely chemical point of view, implants are typically either metallic, ceramic, or polymeric (Legeros and Craig, 1993). However, since implants remain embedded within live tissues for considerable periods of time, their biocompatibility is another important aspect for their practical use, and materials with similar chemical properties may trigger vastly different biological responses. As such, implants are also classified by their biocompatibility as *biotolerant* (the material is not necessarily rejected by host tissue, but is nonetheless surrounded by a fibrous capsule), *bioinert* (the material allows the close apposition of bone on its surface), and *bioactive* (the material triggers the formation of new bone on its surface and creates chemical bonds along its interface with the host tissue) (Pilliar, 1990).

Metallic, polymeric, and ceramic materials used in the construction of implants are listed as follows.

7.3.1 Metallic Implants

Titanium alloys, including Ti-6 aluminum-4 vanadium (Ti-6Al-4V), were among the first modern materials used for dental implants (Triplett et al., 2003). Upon contact with air, metallic titanium forms a surface oxide layer that can reach a thickness of 2–10 nm within a short period of time. This stable oxide layer renders titanium biocompatible (Ducheyne, 1987; Lautenschlager and Monaghan, 1993) and provides it with a high corrosion resistance (Donley and Gillette, 1991; Parr et al., 1985). The modulus of elasticity of titanium and its alloys are comparable to that of bone, which allows titanium implants to serve as implants in sites that regularly bear strong loads (Kasemo and Lausmaa, 1985; Meffert et al., 1992). Zirconium, gold, and Ti–aluminum–vanadium alloys are other metallic materials that have been used for osseointegration; however, some of these alloys are known to insufficiently support bone-to-implant connections (Triplett et al., 2003).

7.3.2 Ceramic Implants

Despite their low strength, ceramics are highly biocompatible and integrate well into host tissues, which make them popular candidates for the manufacture of dental implants (Triplett et al., 2003). In addition to their use in

stand-alone implants, ceramics can also be used as bioactive coatings to support the osseointegration of other materials. Plasma-sprayed HA is a common surface coating for bone implants, and it has been demonstrated that it and other bioactive ceramic coatings can enhance the chemical bonding of the implant with bone (Lacefield, 1998). Tricalcium phosphate and aluminum oxide ceramics are also currently utilized as plasma-sprayed coatings, usually over a metallic core (Triplett et al., 2003).

7.3.3 Polymeric Implants

Polymers are softer and more flexible than the other classes of biomaterials but can nonetheless be used in dental and bone implants (Triplett et al., 2003). A variety of polymers, including polyurethane, polymethyl methacrylate, polyamide fibers, and polytetrafluoroethylene, have been used to manufacture dental implants (Lemons, 1990). The low mechanical strength of polymers makes them prone to mechanical fractures under high loading forces. In addition, polymers have been reported to trigger adverse immunological reactions and display subpar adhesion capacities to living tissues (Chapman and Kirsch, 1989; Kawahara, 1983). As such, polymeric materials have very few applications in implant dentistry and are only used for the production of shock-absorbing components to be placed between the implant and its suprastructure (Triplett et al., 2003).

7.4 OSSEOINTEGRATION OF DENTAL IMPLANTS

An estimated one million endosseous dental implants are placed annually worldwide (Brunski, 1999; Jokstad et al., 2003). Not all implants are successful, however, as the implant must integrate into the jawbone in order to function. The Brånemark system was introduced for dental implants in 1971 and defines osseointegration as the ability of the bone-to-implant contact to function under load (Brånemark et al., 1983; Hobo et al., 1989). Osseointegration occurs in two steps: primary and secondary (Natali et al., 2009). Primary osseointegration is the mechanical attachment of an implant to the surrounding bone following its insertion, while secondary osseointegration (biological stability) involves bone regeneration and remodeling around the implant (Greenstein et al., 2008; Natali et al., 2009).

Primary stability is a critical determinant of the long-term success of dental implants (Rabel et al., 2007). The success of osseointegration is also affected by the material used in the implant, the machining conditions, the surface finish, the type of bone that receives the implant, the

surgical technique, the design of the prosthesis, and patient care (Elias, 2011). Surface properties of implants are extremely important for controlling the biological response that the implant will trigger and can be modified to improve the performance of the implant. These properties involve the attraction, repulsion, adsorption, and absorption capacity of the implant toward cells and proteins, as well as its roughness, wettability, electrical charge, chemical composition, surface energy, residual stresses, and morphology (Elias, 2011).

Various surface modifications have been used to enhance the osseointegration of implants. These modifications typically aim to provide metal implants with surface properties capable of facilitating the adsorption of proteins, adhesion and differentiation of cells, and integration into living tissues. The success of titanium implants, for example, has been shown to depend heavily on their surface topography (Le Guéhennec et al., 2007), including macroscopic, microscopic, and nanometric characteristics. These effects may be caused by the surface preferences and mechanosensory behavior of the cells responsible for facilitating osseointegration. Schwartz et al. have reported that osteoblast proliferation is increased on rough surfaces (Schwartz et al., 1996), while Albrektsson and Wennerberg likewise showed that the differentiation and adhesion of osteoblasts are enhanced on rough surfaces, although fibroblast adhesion was weaker (Schwartz et al., 1996).

7.5 USES OF NANOTECHNOLOGY IN THE DEVELOPMENT OF DENTAL IMPLANTS

Greater control over the topography and chemistry of implant surfaces would assist greatly in understanding the nature of biological interactions that occur on material surfaces and developing novel implants that display enhanced tissue-integrative properties. Such materials can be produced with the assistance of nanotechnology, since more textured surface topographies increase the surface energy at the nanoscale, which in turn enhances the wettability of the surface to blood and the adhesion of cells to the surface. Nanotopography can promote cell differentiation, migration, and proliferation and therefore enhance the wound healing and osseointegration process following implant placement (Dalby et al., 2008; Ehrenfest et al., 2010). Various methods exist for the fabrication of materials with nanometer-scale roughnesses; grit blasting, ionization, and acid etching are among the more common. Dental implants have also begun to use similar methods to increase surface roughness and promote protein adsorption

and cell adhesion. In addition, biomimetic calcium phosphate coatings and growth factor-releasing scaffolds are also under development for bone and tooth regeneration (Le Guéhennec et al., 2007).

7.5.1 Enhancement of the Osseointegration Process

The surface of Ti dental implants can be coated with bone-stimulating agents such as growth factors (transforming growth factor-β, bone morphogenetic proteins [BMPs], platelet-derived growth factors, and insulin-like growth factor [IGF]-1 and 2) and antiresorptive drugs (bisphosphonates) in order to locally enhance the bone healing process (Le Guéhennec et al., 2007; Tomsia et al., 2011). Schliephake et al., for example, have reported that a titanium implant coated with type I collagen and BMP-2 displayed greater peri-implant bone formation within the grooves of an endosseous screw, compared to an implant coated with collagen alone. Implant surfaces can also be loaded with molecules that modulate the bone remodeling process to further enhance their osseointegration.

The incorporation of bone antiresorptive drugs, such as bisphosphonates, into implants might be very relevant in clinical cases lacking bone support, for example, resorbed alveolar ridges. It has recently been shown that a bisphosphonate-containing titanium implant could locally increase bone density in the peri-implant region (Josse et al., 2004). The effect of antiresorptive drugs seems to be limited to the vicinity of the implant, and *in vivo* studies suggest that dental implants functionalized with bisphosphonates have little to no side effects despite only displaying a slight increase in osteointegrative capacity (Meraw and Reeve, 1999; Meraw et al., 1999). Plasma-sprayed HA-coated dental implants immersed in pamidronate or zoledronate, however, could trigger a significant increase in bone contact area (Kajiwara et al., 2005; Peter et al., 2005; Yoshinari et al., 2001). Bisphosphonates display a high chemical affinity for calcium phosphate surfaces, and their incorporation onto dental implants can be achieved easily by using a biomimetic coating method at room temperature. However, the dose of the drug will nonetheless have to be optimized on a case-by-case basis, as bisphosphonate-mediated increases in peri-implant bone densities are concentration dependent (Peter et al., 2005).

Growth factors and biomolecules can also be immobilized onto implants to enhance tissue growth and integration. TiO_2 nanotubes produced by anodization have been proposed as drug-eluting coatings for implantable devices (Popat et al., 2007). The surfaces of these tubes can be functionalized to attach biomolecules, such as bovine serum albumin. A Ti-based implant, for example, has been functionalized with BMP to enhance its

bioactivity and bone formation capacity (Puleo et al., 2002). The advantage of immobilizing BMP is that it allows the controlled administration of the hormone and avoids the problems associated with overdosing. Implants have been coated with nanocrystalline diamonds to increase the surface area and facilitate the immobilization of BMP (Kloss et al., 2008). The enhanced differentiation and proliferation of cells can be achieved without changing the overall texture of the implant using these diamonds (Specht et al., 2004).

Studies have also demonstrated that biphasic calcium phosphate grit-blasted surfaces can provide a more rapid osseointegration in comparison to smooth surfaces. Osseointegration can also be promoted by applying a calcium phosphate coat onto the implant through plasma spraying or biomimetic and electrophoretic deposition (Lavenus et al., 2010). Calcium phosphate residues on implant surface release calcium and phosphate ions to their immediate environment, potentially assisting in the precipitation of biological apatite nanocrystals and the adsorption of various proteins onto these structures. This protein matrix may in turn promote cell adhesion, osteoblast differentiation, and the synthesis of mineralized collagen. Osteoclast cells are also activated in response to calcium phosphate coatings, allowing the formation of bone tissue and the establishment of a direct bone-to-implant contact without an intervening layer of connective tissue (Lavenus et al., 2010). Another interesting approach involves the use of molecular self-assembled monolayers, which are formed by the spontaneous assembly of a single layer of molecules on a surface. These molecules expose only their end-chain groups to the environment, and these chains can be designed with osteoinductive or cell-adhesive properties, such as by the use of RGD peptides.

7.5.2 Pulp and Dentin Tissue Regeneration

Traumatic dental injuries are often irreversible and may require the excision of even the healthy portions of the tooth prior to their filling or replacement. The need for these surgeries would be reduced greatly if the natural regeneration of dental pulp cells can be enhanced. Regeneration of pulp tissue ordinarily proceeds at a slow pace, as the dental pulp has minimal collateral blood supply and the immune system cannot adequately defend against bacterial entry into the pulp (Huang, 2009). In addition, odontoblasts are postmitotic cells and exhibit only a limited ability to proliferate (Arana-Chavez and Massa, 2004). However, modern tissue engineering methods and especially the discovery of dental stem cells have allowed the development of techniques for the regeneration of pulp

and dentin (Huang, 2009). The interplay between nanotechnology and stem cell biology allows the selective differentiation of stem cells into specific lineages through artificial scaffolds that incorporate the active sequences of factors involved in the lineage commitment process. The administration of dynamic biological agents composed of stem cells, bioactive scaffolds, and/or nanoparticles to patients is an effective means of increasing the regenerative ability of damaged dental tissues, although many of these methods are still under development and do not currently see clinical use (Mitsiadis et al., 2012).

Scaffolds are three-dimensional structures that provide an initial framework for the growth and recruitment of cells (Muschler et al., 2004). They are commonly used in regenerative medicine and typically produced to mimic the gross morphology of the missing section of tissue. A number of factors should be taken into consideration for the design of tooth (and other tissue) scaffolds, including vascularization, cell–matrix interactions, growth factor incorporation, matrix degradation, mineralization capacity, and the risk of contamination or undesirable immune responses (Galler et al., 2011a). As with implants, a wide variety of scaffold materials can be used for the tissue engineering of teeth. These include long-lasting porous HA ceramics, inherently transpiring molecules (e.g., collagen and chitosan) and biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polyglycolic acid–poly-L-lactic acid (PGA–PLLA), and poly(lactic-*co*-glycolic acid) (PLGA) (Zhang et al., 2013). In addition to the effects of its raw material and implanted stem cells, a scaffold may also be functionalized through secondary chemical modifications and the slow release of regeneration-enhancing biological factors.

Certain types of isolated pulp cells have been shown to differentiate into odontoblast-like cells and generate a dentin-like mineral structure under *in vitro* conditions (About et al., 2000; Tsukamoto et al., 1992). These are collectively called dental stem cells and include dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), and stem cells from apical papilla (SCAP). These cells are derived either from mature pulp tissue or its embryonic precursors and may potentially serve as sources for the regeneration of pulp and dentin, especially when integrated into a scaffold suitable for their growth and differentiation (tissue banks have been founded to preserve the stem cells in deciduous teeth for that purpose) (Huang, 2009). DPSCs and SCAPs form a pulp–dentin complex when transplanted into immunocompromised mice (Gronthos et al., 2000; Sonoyama et al., 2006), while SHED produce a mineralized tissue matrix without a distinct pulp–dentin complex (Miura et al., 2003). The activity of these cells can also be enhanced through the

use of growth factors, which are integral to the formation and repair of dentin and pulp tissues. In addition to the factors involved in the embryonic development of teeth, the dentin matrix naturally contains odontoblast-secreted growth factors, primarily of the TGF-β superfamily (Smith et al., 2008), that play important roles in signaling events leading to the formation of tertiary dentin in mature teeth (Tziafas, 1995).

Although scaffolds used in regenerative medicine need not display nanoscale features, these features generally assist in the function of the scaffold by increasing its surface area and therefore enhancing its interaction with cells and the surrounding tissue. Wang et al., for example, tested the effects of a novel nanoscale bioactive glass (n-BG) on the proliferation, apoptosis, chemotaxis, mineralization, and differentiative capacity of human dental pulp cells (hDPCs) and found that a combination of these effects allows n-BG to induce dentin formation more effectively than a microscale equivalent (microscale bioactive glass (m-BG)), which was attributed to the greater number of binding sites, faster dissolution rate, and other biochemical properties associated with the larger surface area of n-BG (Wang et al., 2014). Nanostructured materials therefore allow more effective presentation of the active groups present on their surfaces.

Self-assembled peptide nanofiber gels are one of the more common forms of smart materials used in regenerative medicine. Rational design of the peptide sequence enables the precise control of material stiffness and allows the material itself to participate in biomineralization or induce the differentiation of surrounding cells. Cell adhesion motifs, enzyme-cleavable sites, and the incorporation of growth factors into the gel structure further enhance the ability of peptide amphiphile scaffolds to elicit specific cellular responses. Inductive scaffolds can also be seeded with stem cells to increase the rate at which the pulp–dentin complex is regenerated (Galler et al., 2011a). Peptide gels can also be designed to exhibit antimicrobial properties; for example, lysine-rich surfaces may facilitate the electrostatic interaction of the peptide with negatively charged bacterial surfaces, resulting in the disruption of the bacterial membrane (Salick et al., 2007). Cell-free approaches using peptide and other types of scaffolds have also been developed; these scaffolds recruit stem cells and growth and differentiation factors from the dentin, the pulp, or the periapical region (Galler et al., 2014).

A combination of the aforementioned approaches can also be utilized to increase the repair efficiency of a scaffold. Galler et al., for example, used a cell-adhesive, enzyme-cleavable hydrogel composed of self-assembling peptide nanofibers, DPSCs, and three growth factors (basic fibroblast growth factor, transforming growth factor β1, and vascular endothelial

growth) in tooth regeneration and further transplanted the gel within a dentin cylinder to enhance its integration into the native tissue. Their system was able to promote cell proliferation, differentiation, and angiogenesis and further supported the formation of a vascularized soft connective tissue with a structure similar to that found in the dental pulp. In addition to increasing the proliferation and differentiation of the seeded DPSCs, growth factors by themselves were also able to attract host cells into the peptide-based bioactive scaffolds (Galler et al., 2011b).

7.5.3 Whole Tooth Regeneration

Although implanted scaffolds are useful in cases where tooth structure stays partially intact, they cannot be used to replace teeth that have been lost in their entirety. The formation of complete replacement teeth would therefore be of great utility in regenerative dentistry. Such teeth could either be grown under *in vitro* conditions and subsequently implanted into empty sockets or produced *in vivo* directly on the maxilla or mandible, accomplishing in both cases the near-complete restoration of the original function of the tooth. As the development of teeth is a complex and well-regulated process, research in this area is still in its infancy and clinical applications are lacking, but the successful generation of tooth structures has been reported in both *in vitro* scaffolds and *in vivo* rodent and swine models. These efforts generally involve a combination of regenerative matrices, dental or mesenchymal stem cells, and growth factors that together mimic the environment in which the embryonic tooth is developed.

Tooth bud cells from rats have been used in the artificial generation of tooth crowns in a scaffold matrix implanted in rat omenta (Duailibi et al., 2004), while the fact that these stem cells could be cultured under *in vitro* conditions prior to implantation was highlighted as an indicator that the *in vitro* expansion of tooth bud cells is feasible (Duailibi et al., 2006). Likewise, SCAPs and periodontal ligament cells were able to produce the root structures necessary for anchoring an artificial crown (Sonoyama et al., 2006). Tooth bud implants in both pigs and rats appear to follow a developmental process similar to the embryonic teeth, as the times required for the production of functional teeth in implanted tooth buds are similar to the duration of tooth formation in the embryonic animals (Nakahara and Idei, 2007). Nondental stem cells were also shown to trigger the development of partial or complete tooth structures. Although embryonic and neural stem cells were unable to produce teeth, bone marrow cells could generate functional tooth crowns with layers of enamel, dentin, and pulp (Nakahara and Idei, 2007; Ohazama et al., 2004). Adipose-derived

stem cells have also been suggested as alternate cell sources for the regeneration of teeth (Jing et al., 2008).

7.6 CONCLUSIONS AND FUTURE PERSPECTIVES

The use of nanotechnology for the functionalization of dental implants has become widespread in the recent decade. Nevertheless, the development of safer and more effective coatings is still an active area of research, and advances in nanotechnology will no doubt uncover a greater diversity of material types and surface architectures for use in the modification of implants. In addition, biological signals are now being tested for their potential effect in modulating the osseointegration of implants; if successful, these materials may allow the implant surface to truly behave as native tissue for the attachment of cells. While these developments have greatly improved dental implants, efforts involving stem cells and regenerative scaffolds are tackling the problem from a different angle: By regrowing teeth from scratch, the entire rationale in using a foreign material as an implant would be eliminated, and although these methods are still in their infancy, their advancement may produce a new generation of tooth implants. Overall, nanotechnology has assisted in the production of more effective implants under more reliable methods, and while most of their applications are experimental, nanostructured materials are nonetheless promising candidates for use as implants in the following decades.

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