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Biomineralization is a natural process by which living organisms form minerals in association with organic biostructures to form hybrid biological materials such as bone, enamel, dentine, nacre, etc. Scientists have researched the fundamentals of these processes and the unique structures and properties of the resulting mineralized tissues. Inspired by them, new biomaterials for being used in tissue engineering and regenerative medicine have been developed in recent years.

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Dr Conrado Aparicio is Associate Professor at the University of Minnesota, USA with a research focus on the design, modification and characterization –physical-chemical, mechanical, and biological of biomaterial surfaces. His areas of research include biomineralization of advanced bio-inspired hydrogels and functionalized surfaces with biomolecules.

Dr Maria-Pau Ginebra is Professor at the Technological University of Catalonia, Spain with a research focus on designing calcium phosphates and developing biomaterials for bone regeneration and drug delivery. Her research contributions include characterisation of low-temperature calcium phosphates that mimic the mineral component of bone, including cements and foams, incorporating synthetic or natural biologically-active molecules.

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Aparicio and Ginebra

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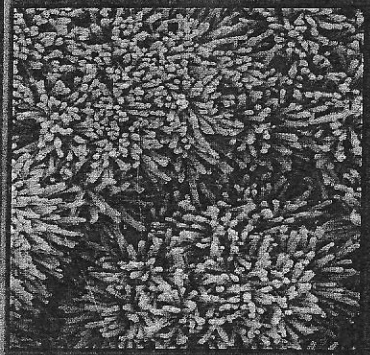

Biomineralization and Biomaterials

Fundamentals and Applications

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Biomaterials for catalysed mineralization of dental hard tissues

12

R. Osorio, M. Toledano
University of Granada, Granada, Spain

12.1 Introduction

12.1.1 *Necessity for enamel and dentine remineralization in restorative dentistry*

Dental caries is a multifactorial disease caused by the interaction of dietary factors, dental biofilm and the host's dental tissue within the oral environment. Enamel and dentine are not static tissues, and constantly undergo dynamic transformation. Enamel, in the oral environment, is covered by microbial deposits with metabolic activity resulting in periods of pH decrease and demineralization; reactive pH increases are produced in the course of remineralization processes. Under normal physiological conditions (pH 7), saliva is supersaturated with calcium and phosphate ions, making the progress of demineralization slow and facilitating remineralization. Saliva also incorporates buffering capacity and defensive elements into its composition. However, frequently the physiological remineralization fails, demineralization proceeds, enamel breaks and cavitation take place. Dentine is then exposed to demineralization (Fejerskov and Clarkson, 1996). The reaction of dentine to caries is further complicated mainly due to the high organic content of this tissue, the presence of multiple enzymes and by non-collagenous proteins playing a crucial role in this infectious disease (Chaussain-Miller et al., 2006). At this stage, restorative materials are needed.

Aesthetic and plastic restorative materials in dentistry are now in great demand, and are used in dental clinics. Most of these materials are resin based, and require demineralization procedures to be performed on dentine and enamel prior to resin application. Infiltration of hydrophilic monomers into demineralized dental tissue will create a hybrid layer that will couple resinous restorative materials to the demineralized dentine. Resin infiltration is never complete, and monomers will not be effectively polymerized in the presence of water. Therefore, a defective interface susceptible to collagen and resin degradation will be formed, jeopardizing the longevity of bonded restorations (Spencer et al., 2010). Dentine matrix metalloproteinases (MMPs) play a crucial role in collagen degradation at the hybrid layer, with the subsequent presentation of secondary caries under restorations. If effective inhibitors of MMPs are included in resin–dentine bonding interfaces, they may protect the seed crystallite-sparse collagen fibrils of the scaffold from degradation, and they could be

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remineralized. Reincorporation of mineral into the demineralized. dentine matrix is important since the mineral precipitated may become a constant site for further nucleation, and the remineralized subsurface of the tissue may be more resistant to subsequent acid attack (Liu et al., 2011b). Development of biomaterials able to catalyse remineralization of incompletely resin-infiltrated collagen matrices created by resin adhesives will represent a great advance in dental care.

12.1.2 Interaction of biomaterials with dental hard tissues for remineralization

Enamel remineralization is the process whereby calcium and phosphate ions are supplied to promote ion deposition into crystal voids in demineralized enamel, to produce net mineral gain. The ability of saliva to remineralize demineralized enamel crystals has been proved. It is made possible by its ability to supply calcium and phosphate ions. Ion stabilization will be produced by salivary phosphoproteins. Ion stabilization guarantees that precipitation will not occur and ions will remain available to diffuse into mineral, to allow for remineralization of demineralized enamel. However, the remineralization produced by saliva is small and is a slow process. An efficient remineralization system for enamel should supply stabilized bioavailable calcium, phosphate and fluoride ions that favour subsurface mineral gain rather than deposition only in the surface layer (Cochrane et al., 2010).

Demineralized dentine may be naturally remineralized. Two different models of *in vitro* mineralization can be found in the literature, classified as the top-down or classical and bottom-up or non-classical approaches. In the classical, dentine remineralization is obtained through the epitaxial growth of residual crystals, acting as nucleating sites. However, evidence exists indicating that it results in an extrafibrillar and non-functional remineralization. The bottom-up approach was suggested as alternative and does also require the use of synthetic substitutes for dentine matrix proteins. Two different analogues are needed: (1) a sequestering agent that facilitates amorphous calcium phosphate formation and (2) a dentine matrix phosphoprotein substitute that will facilitate positioning of hydroxyapatite at special sites of collagen, leading to intrafibrillar remineralization (Gu et al., 2011; Watson et al., 2014). Thus, a dual analogue biomimetic system functioning as sequestration analogue, and a phosphate-based templating analogue, may mimic the full role of non-collagenous proteins, resulting in a highly ordered intrafibrillar nanoapatite assembly (Li et al., 2013; Gu et al., 2011; Liu et al., 2011a).

Phosphoproteins are non-specific and may be substituted by other immobilized, naturally occurring phosphoproteins to induce apatite deposition within collagen (Gu et al., 2011). Many biominerals are formed by an amorphous precursor pathway mediated by non-collagenous proteins. The initial stage is the sequestration of the calcium and phosphate ions from solution into amorphous calcium phosphate nanoprecursors, the subsequent stage is templating the nucleation, and the growth of the nanoprecursors at the special sites of the collagen substrate by non-collagenous proteins (Li et al., 2013; Liu et al., 2011a).

12.2 Agents to facilitate remineralization through dental materials

Catalysed dentine bioremineralization will be attained through materials which ideally should be able to: (1) deliver mineral ions (calcium, phosphorus or silicon) in a sustained manner (bioavailability), (2) bind to collagen, acting both as sequestering/templating agent of calcium and phosphorus (bioactivity), and also perform as a nucleation promoter to aid in apatite crystallization, (3) protect collagen from degradation, and (4) provide an adequate pH to facilitate inhibition of collagenolityc enzymes and favour new mineral formation.

12.2.1 Ionic dissolution products from bioactive dental materials

Several inorganic materials have been shown to be bioactive, resorbable, and able to deliver remineralizing ions. However, the exact mechanisms of ion elution and interaction between the ionic dissolution products of such materials and dental tissues are not fully known. Due to the chemical similarity to the inorganic phase of dental tissue, different calcium phosphate-based compounds have been employed. The biodegradability of these materials depends on many parameters such as: crystallinity, porosity, chemical purity, surface roughness, pH in the media, other solubilized ions present in the biological fluid at the oral environment, etc. (Hoppe et al., 2011; Cochrane et al., 2010).

The main inorganic materials employed may be classified as: (1) Calcium phosphates or calcium sodium phosphosilicates, referred to as bioactive glasses – 45S5 Bioglass, Brushite, β-TCP or α-TCP. The main problem with applying these materials to the oral cavity to promote enamel or dentine remineralization is the poor solubility of the calcium phosphate phases. The dissolution of the calcium phosphate phase in saliva or at the dentine interface requires undersaturation of these ions with respect to that crystalline phase. In the normal pH range of biological oral fluid, these crystalline calcium phosphate phases would sparingly dissolve (Cochrane et al., 2010). (2) Unstabilized amorphous calcium phosphates and silicates where a calcium salt, phosphate salt, and silicates are delivered separately. Even when these amorphous calcium phosphates are unstable, they tend to be transformed into a stable crystalline phase. But, before phase transformation occurs, ions should be available to facilitate remineralization (Cochrane et al., 2010).

Ionic dissolution (mainly calcium, phosphorus and silicon) from these products is a key factor in understanding the behaviour of remineralizing materials. Since calcium and phosphorus are the main components of biological apatite, these ions play an essential role in remineralization. Other inorganic ions have been incorporated in new developed restorative materials due to their known effects in hard-tissue metabolism, as fluoride, zinc, strontium or magnesium (Sauro et al., 2013; Toledano et al., 2012).

The formula for stoichiometric hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. However, biological apatite is calcium deficient, and contains substantial amounts of carbonate. Carbonated apatite is a precursor of hydroxyapatite. In natural apatite, substitution by

foreign ions occurs frequently. Apatite may be substituted by many different trace elements in small concentrations. These ions include carbon, fluoride or zinc, and formed minerals are carbohydroxyapatite, fluorapatite or scholzite (Fejerskov and Clarkson, 1996; Osorio et al., 2014b). These new minerals usually have some different properties to those of hydroxyapatite, being very resistant to dissolution in acid environments or having antibacterial properties. An ideal caries preventive material should release these ions in the oral environment (Longbottom et al., 2009).

The above-mentioned ions have been shown to exert other beneficial effects on remineralization. Silicon is able to stimulate collagen formation and induce hydroxyapatite precipitation. The silanol groups (Si–OH) can interact with calcium ions and induce the formation of apatite crystals. Magnesium, strontium, and zinc may act as crystal growth inhibitors facilitating amorphous calcium phosphate stabilization and intrafibrillar remineralization of collagen. Zinc stimulates remineralization as a direct activator of the enzyme alkaline phosphatase. Magnesium, strontium and zinc have also been shown to inhibit osteoclast activity, and induce new bone formation through regulation of osteoblast differentiation (Hoppe et al., 2011; Ma et al., 2013). The effects of these ions on odontoblast activity remain to be ascertained.

12.2.2 Phosphopeptides and other templating, stabilizing and nucleating agents

Solubility of calcium and phosphate ions in biological systems is tightly regulated by proteins. Biological fluids containing high concentrations of calcium and phosphate ions also contain proteins (mainly phosphopeptides) to avoid ion precipitation. These proteins have a distinct similarity of structure, all containing negatively charged residues. These residues interact with calcium and phosphate ion clusters to stabilize them in aqueous environments. It results in biological fluids with remineralization potential (Cochrane et al., 2010).

These phosphopeptides are characterized by organic phosphate groups covalently bound to structural macromolecules, and are found in mineralized tissues. In dentine, many proteins and ions exist at the location of apatite formation, and the nucleation and crystal growth are regulated by these templates and ions at the same time. Promotion and inhibition of crystal formation both coexist. Some proteins have nucleation/promotion and inhibition sites (i.e. in collagen the positive net charge close to the C-terminal end of collagen promotes the infiltration of amorphous calcium phosphate, while the charged amino acids form nucleation sites that control the conversion from amorphous phase into orientated apatite crystals). The carboxyl groups on the template molecules are also an important nucleation site. These acidic groups have a strong interaction with calcium ions, and then phosphate will be attracted by the adsorbed calcium ions. Other nucleation sites are carbonyl, hydroxyl, and amino groups (Gu et al., 2011; Ma et al., 2013).

Non-collagenous acidic proteins inhibit apatite nucleation for amorphous phase stabilization. This occurs with some well-identified analogues of these proteins, such as polyacrylic acid and polyaspartic acid. These molecules are adsorbed on the collagen fibres and retard the mineralization process. The mineralization of collagen

is highly dependent upon the interaction of collagen with such acidic molecules. However, inorganic polyphosphates have to be used as a site-directing molecule in conjunction with a nucleation inhibitor for amorphous phase stabilization. The combination of polyphosphate salts and polyacrylic acid directs amorphous precursor phases into periodic apatite–collagen nanocomposites. The same mechanism of cooperative nucleation was proposed by studying the exposure procedure of collagen templates with polyaspartic acid, calcium, and phosphate ions (Zeiger et al., 2011). Polyaspartic acid is a biodegradable, water-soluble polyaminoacid with the potential to inhibit deposition of calcium phosphate salts and chelate metal ions. Polyaspartic acid and the polymer-induced liquid precursor (PILP) have been proposed as analogues for non-collagenous proteins and the biomimetic system for the natural process of mineralization of dentine and enamel (Burwell et al., 2012). The PILP process consists of adding liquid nanoprecursors encapsulated by polyanionic polymers. The anionic polymer sequesters calcium ions, which then build up a charge to sequester counter ions (phosphate or carbonate), which induces liquid–liquid phase separation in the crystallizing medium. The PILP process is also a pioneer work in bone mineralization (Olszta et al., 2003).

The presence of two phosphorylated non-collagenous proteins is important to attain dentine mineralization: (1) dentine phosphoprotein (DPP), in the presence of Ca^{2+}, tends to form a compact globular structure, with a specific affinity for collagen molecules. DPP localizes to the boundary between the gap, and overlap regions of collagen fibrils (acting as a crystal inhibitor); (2) dentine matrix protein 1 (DMP1) induces crystal nucleation, and regulates the phase transition from amorphous calcium phosphate to carbonated apatite. In conjunction, both lead to the formation of highly organized mineralized collagen fibrils. In contrast, in the presence of non-phosphorylated DPP and DMP1, no organized mineralization of collagen fibrils occurred. Phosphorylation is essential for proper collagen mineralization (Ma et al., 2013). Binding of biomimetic molecules containing multiple phosphate groups to collagen fibrils produces phosphorylated and negatively charged surfaces that attract calcium ions by electrostatic interaction. The phosphorylated collagen serves as a scaffold for homogeneous nucleation of apatite crystallites. Trimetaphosphate is one of the proposed analogues for phosphorylated non-collagenous proteins, as it may provide the interfacial linkage between mineral crystallites and collagen fibrils. Some of the phosphate groups immobilized on the collagen fibrils are also binding sites for calcium ions, and facilitate apatite nucleation onto collagen. Collagen and non-collagen proteins or their analogues, other ions and molecules have to work together in order to obtain formation of biological minerals (Gu et al., 2011).

Casein phosphopeptide (CPP), which is present in milk, is one of the most studied molecules. It has also been shown that its efficacy in remineralizing dental substrates is enhanced if combined with amorphous calcium phosphate and fluoride. CPP forms nanoclusters with amorphous calcium phosphate (CPP–ACP), thus providing a pool of calcium and phosphate which can maintain the supersaturation of saliva. The CPPs have a high binding affinity for apatite. The CPPs were shown to prefer binding to some faces of hydroxyapatite crystals, being able to change direction in crystal growth. CPP will release calcium, phosphate, and fluoride ions, and this process is promoted at low pH (Cochrane et al., 2010). Since CPP–ACP can stabilize calcium

and phosphate in the solution, it can also help in the buffering of plaque pH, and so calcium and phosphate levels in plaque are increased. Therefore calcium and phosphate concentrations within the subsurface lesions are kept high, which results in dentine and enamel remineralization (Wilson, 2007). There is now a large body of scientific evidence demonstrating that CPP–ACP and CPP–ACP combined with fluoride can promote the remineralization of enamel subsurface lesions.

The structural domains of proteins in human saliva, as statherin, are partly responsible for the protection and recalcification of tooth enamel, and analogues of these proteins have also been synthesized. The abilities of these fragments to adsorb at hydroxyapatite surfaces and to inhibit its mineralization in supersaturated solutions are determining factors in facilitating enamel remineralization, as it occurs with proteins involved in the dentine remineralization process (Raj et al., 1992).

12.2.3 Collagen protectors: Metalloproteinase inhibitors, crosslinkers and antibacterial agents

The organic phase in dentine is a templating scaffold for mineral crystals to grow, and dentine may be naturally remineralized as long as seed crystallites are present and non-collagenous proteins can act as nucleation sites. Therefore, collagen protection from enzymatic degradation is crucial to obtain remineralization.

MMPs have been suggested to play an important role in the destruction of dentine organic matrix following demineralization by bacterial acids, and in the control or progression of carious decay. During the caries process, the mineral part of dentine is dissolved, exposing the organic matrix to breakdown by bacterially derived enzymes, as well as by host-derived enzymes such as the MMPs present within the dentine (Chaussain-Miller et al., 2006). Demineralized exposed collagen, which is unprotected by intrafibrillar minerals, due to caries or to therapeutic acid treatment before resin bonding, can undergo degradation by endogenous MMPs. Effective inhibitors of MMPs included in resin–dentine bonding interfaces may protect the seed crystallite-sparse collagen fibrils of the scaffold from degradation, before they can be remineralized (Liu et al., 2011b).

Most first-generation MMP inhibitors are based on zinc- or calcium-chelating groups. Doxycycline and chlorhexidine digluconate have been shown to be efficient zinc chelators. These and other compounds may have potent effects but lack selectivity because of the strong homology between catalytic sites of MMPs. Some of these MMPs should be active in promoting further remineralization. Several studies have shown inhibition of MMPs by metals, metal salts, and zinc present in dental products. Zinc concentration strongly reduced MMP-mediated collagen degradation in etched dentine. A collagen protector effect, exerted through binding at the collagen-sensitive cleavage sites of metalloproteinases, has been advocated (Osorio et al., 2011b), so MMPs will be able to act in the subsequent remineralization process. The presence of hydrophilic monomers at the bonded hybrid layer may also inhibit MMP-mediated collagen degradation, through adsorption of MMPs (Osorio et al., 2011a). The coordination of the hydroxyl group of HEMA with zinc that is present in the catalytic domain of MMPs has been suggested as a reversible inhibitory mechanism.

Dentine collagen cleavage by cysteine proteases (also present in dentine) has been questioned, as only Cathepsin K may act extracellularly and, at the moment, it has not been isolated in the extracellular dentine matrix. Cathepsin-mediated collagen hydrolysis is restricted to an acidic pH environment, and thus confined to the sub-osteoclastic and lysosomal compartments. It is not possible to reach this pH, extracellularly, at the hybrid layer due to the buffer capacity of dentine (Kunawarote et al., 2010).

The mechanical performance and stability of collagen can be improved by crosslinking, thereby facilitating collagen remineralization (Gu et al., 2011). It seems that crosslinking opens intramolecular spaces and minimizes the excluded volume of collagen fibrils (Bleek and Taubert, 2013). Attempts to introduce primers with collagen crosslinkers have been researched. Proven dentine collagen crosslinkers include glutaraldehyde, riboflavin and carbodiimide (Ma et al., 2013).

The elimination of bacteria from the dental tissue interface, and providing a bacteria-tight seal between the dental tissue and oral cavity, are requisites for long-term success in dental restorative treatments. Antibacterial agents have been incorporated into dental restorative materials to achieve this goal. Antibacterials have been added to resin materials mainly by two strategies: antibacterial filler incorporation or attaching some antibacterial agent to resin monomers. Some of the researched antibacterial nanoparticles have been silver, zinc oxide, calcium and phosphate ion-releasing fillers (amorphous calcium phosphate or hydroxyapatite), alone or combined with barium-, boro-, or fluor-aluminosilicate glass particles. The main advantage of these composite resins containing calcium phosphate fillers is that they can release calcium and phosphate when the pH is reduced from neutral to cariogenic pH. Furthermore, these materials can rapidly neutralize the acidic medium, increasing the pH. The pH increase may also help the antibacterial activity. Of the antibacterial monomers, methacryloyloxydodecylpyridinium bromide has been incorporated into a commercially available adhesive system. It is a quaternary ammonium compound that may be attached to a methacrylate group of the resin monomer, forming a non-agent-releasing antibacterial dental resin (Imazato et al., 2014).

12.2.4 pH modulation

Remineralization is a dynamic process in which amorphous phase formation, phase stabilization, and transition of calcium phosphate continuously occur. pH value has a significant effect on these processes. At low pH the equilibrium concentrations of calcium and phosphate are greater than at high pH. In the pH range between 5 and 6, the equilibrium concentrations approximate the values for physiological fluids. Above that value, precipitation of mineral may occur from a supersaturated solution; below that value, dissolution of mineral occurs in an undersaturated solution. Calcium phosphate may precipitate in various crystalline forms differing in solubility (Ten Cate and Featherstone, 1996).

The alkalinity potential of the remineralizing materials should also be considered. Some calcium phosphate compounds in solution are able to induce an essential increase in pH (pH ranging from 9 to 10.5). This alkalinity is due to the progression of calcium hydroxide formation in calcium phosphate cements or to a rapid release of

Na^+ or K^+, and the incorporation of H^+ or H_3O^+ into glass particles (Sauro et al., 2013). This alkalinizing potential will facilitate an antibacterial effect, and will inhibit dentine MMPs which are activated under acidic conditions but act at neutral pH (higher than 6, optimum pH 7.5) (Fields, 2013). This pH increase may be beneficial for remineralization whenever it is maintained below 9, as 8–9 is the optimum pH range for alkaline phosphatase to act (Ross et al., 1951).

12.3 Applied dental restorative materials for catalysed bioremineralization

Dentine remineralization through materials is not easy to achieve. Using the previously described matrix protein analogues clinically is not realistic, and it is challenging to apply. Incorporation of amorphous precursors able to bind collagen is, at the moment, a more applicable approach to clinical dentistry (Watson et al., 2014).

12.3.1 Calcium hydroxide cement

Since its introduction to dentistry (1928), calcium hydroxide has been widely used as a mineralizing agent as well as an effective antimicrobial medication. Some relevant characteristics are found in this material, which may facilitate dentine remineralization. Calcium hydroxide is able to liberate calcium, and has been shown to behave as a bioactive surface able to precipitate calcium carbonate and calcium phosphate. As carbonated apatite represents the biological apatite phase seen in bone, cementum, and dentine, these crystals may aid in initiation of dentinogenesis.

Calcium hydroxide induces matrix formation and mineralization through the release of bone morphogenetic proteins, cytokines, and some specific tissue growth factors. Calcium hydroxide may also stimulate alkaline phosphatase activity.

The mineralizing action of calcium hydroxide has been attributed in part to release of hydroxyl ions and the resultant alkaline pH that may also neutralize acids, produce bactericidal effects and cause denaturation of bacteria-derived products (Sangwan et al., 2013).

12.3.2 Glass ionomer cements

Glass ionomers were first introduced to dentistry in 1975. They are composed of fluoro-aluminosilicate powder, a polyalkenoic acid (polyacrilyc acid) and water to facilitate the setting of the cement (acid–base reaction).

When this cement is placed on dentine or enamel an ion exchange process is produced. Aluminium, silicon and fluoride leach out of the cement. Phosphorus and calcium may move out of the underlying dental tissue due to the created acidic environment. This process seems to aid in dentine and enamel remineralization (Watson et al., 2014). A ligand exchange mechanism within the formed gel at the dental interface endows glass fillers not only with the ability to release but also to recharge fluoride ions. The formation of calcium polycarboxylate has also been demonstrated

in dentine and enamel, facilitating not only tissue remineralization but also chemical bonding, and it is a significant factor in the excellent long-term adhesion and mineralization ability of these materials (Falsafi et al., 2014).

The effect of polyacrylic acid as a stabilizing agent or mobilizing dentine biomolecule, facilitating remineralization, has not been fully explained. Some further glass ionomer modifications include incorporation of strontium, hydroxyapatite nanoparticles, liquid silica and other bioactive elements.

12.3.3 Calcium silicate cements

Portland cement was the first introduced calcium silicate cement. A similar material, mineral trioxide aggregate (MTA), was developed and recommended initially as a root-end filling material, and subsequently has been used for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apexes, repair of root perforations, and root canal-filling material. MTA powder contains fine hydrophilic particles that set in the presence of moisture.

This material is principally composed of di- and tricalcium silicate, tricalcium aluminate and tetracalcium aluminoferrite, and bismuth oxide. These cements set, after water mixing, through a hydration reaction. MTA is biocompatible, and a bioactive material with hard-tissue conductive and inductive properties. A high pH value (around 10) is obtained after mixing, and is maintained over time. The high pH value is due to the constant release of calcium forming calcium hydroxide. Both Portland cement and MTA contain tricalcium and dicalcium silicate, which on hydration produce calcium silicate hydrate gel, calcium hydroxide, and calcium carbonate, and thus are able to exert remineralizing effects (Parirokh and Torabinejad, 2010; Watson et al., 2014).

12.3.4 Advanced polymers for bioremineralization

New resin materials are being developed to facilitate dentin remineralization. Most of these incorporate inorganic fillers (bioglass, calcium phosphate and/or calcium silicate particles, hydroxyapatite and silicon nanoparticles) trying to produce calcium, silicon and phosphorus liberation in order to promote remineralization processes at the bonded interface (Sauro et al., 2013; Besinis et al., 2014).

Zinc (zinc oxide particles or zinc chloride) has also been successfully incorporated into dental resin adhesives, as an MMP inhibitor agent able to stabilize dentine collagen (Toledano et al., 2012; Osorio et al., 2014a) and promoting dentine remineralization at the adhesive interface (Toledano et al., 2013; Osorio et al., 2014b).

12.4 Future trends and research in bioactive dental restorative materials

An alternative strategy that is becoming the focus of research in the biomaterials field is the use of polymeric particles as calcium- and phosphate-sequestering materials (Osorio et al., 2014c). These polymers may also act as carriers of other biological

factors for the management of tissue mineralization, permitting a controlled ion release rate (Leonor et al., 2009; Wu et al., 2011; Musyanovych and Landfester, 2014). Surfaces of these polymers exhibit a high concentration of functional groups (i.e. $COOH^-$) able to form complexes with metal cations, and exert a potent quelant effect (Li et al., 2013; Osorio et al., 2014c). These functional groups may also be conjugated with various targeting molecules including proteins, growth factors or any other specific molecules. Studies have also shown that self-assembling polymers and biomimetic peptides based on dentine phosphophorin, which contain multiple phosphoseryl residues, will nucleate hydroxyapatite (Hartgerink et al., 2001).

A polymeric sphere system able to combine bioactivity with a capacity for controlled protein/drug delivery is ideal for teeth and also for bone regeneration (Wu et al., 2011). Filler nanoparticles will not only induce mineralization but also may be used for loading/delivering some antibiotic drugs, osteoinductive agents, or other proteins useful in the dental environment.

Biocatalysed mineralization is desirable within the dentine–resin hybrid layer, potentially reinforcing or stabilizing this zone. Biocatalysts can remain active within a polymer; a dental resin can potentially carry the catalyst for subsequent formation of particles in order to create a mineral gradient. The principle is that particles need to be transported, or could be formed *in situ*, as is done in nature (Goldberg et al., 2009).

There is a need to delve much deeper into dentine. Dentine is a biologically active tissue which contains trapped bioactive molecules which might be liberated if the dentine matrix is demineralized or solubilized by external agents. These molecules are released into the immediate vicinity and affect any reparative processes. Precise knowledge of these active molecules, their function and regulation mechanisms is necessary, if we really want to aid nature in dentine remineralization with newly developed materials.

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Designing biomaterials based on biomineralization for bone repair and regeneration

13

I.B. Leonor[1,2], A.I. Rodrigues[1,2], R.L. Reis[1,2]
[1]University of Minho, Guimarães, Portugal, [2]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

When Nature finishes to produce its own species, man begins using natural things in harmony with this very nature to create an infinity of species.

Leonardo da Vinci (1452–1519)

13.1 Introduction

Nature is full of biological structures, from nanoscale to macroscopic level, with a variety of shapes and forms. These structures are crystals, clays, inorganic/organic composites, seashells, pearls, bones and teeth, wood, silk, horn and collagen, among others (Boskey, 1999; Cuisinier, 1996; Dove et al., 2003; Green et al., 2002; Hou et al., 2004; Lowenstam and Weiner, 1989; Mann, 2001; Rubner, 2003; Sommerfeldt and Rubin, 2001; Zhang, 2003).

For centuries, these structures have inspired scientists to learn more about them and find ways to create them synthetically. Studying these natural structures has generated a growing interest in materials science and in the adaptation of biological processes that may lead to significant advances in designing complex structures and controlling sophisticated processing routes that lead to the final shape. To date, it has not yet been possible to fully replicate these structures using nonbiological processing methods. Learning from biological concepts (bioinspiration) and mimicking biological surfaces (biomimetic) has led to important advances in the manufacture of synthetic materials in recent years.

For instance, the abalone shell nacre, one of the most beautiful and delicate structures in the natural world, had inspired several research groups in the development of biomimetic-advanced materials (Ball, 2001). These shells serve as protective barriers for invertebrates, are impenetrable to the crushing action of predators, are resistant to the pressures in the deep ocean around hydrothermal vents where mollusks proliferate, and have toughened to avoid shattering in intertidal areas due to wave action (Kaplan, 1998).

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