Nanotechnology and Its Role in Caries Therapy

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ABSTRACT

The purpose of this review is to highlight recent nanotechnological developments for remineralization of incipient caries lesions as well as biomimetic strategies for enamel synthesis based on the application of nanotechnology. Analysis of *in vitro* data indicates that apatite nanoparticles might be effective in reversing lesion progression in the outer but not in the deeper part of early caries lesions. To control caries-induced demineralization, investigators have developed calcium and phosphate or fluoride ion-releasing nanofillers, enabling resin composites to release ions, if the pH decreases under *in vitro* conditions. Extensive *in vitro* investigations of apatite crystallization have been performed to mimic the hierarchical topology of natural enamel. Strategies for formation of highly organized biomineralized structures include oriented aggregation of nanocrystallites or the assembly of apatite nanoparticles mediated by organic scaffolds. Despite all these promising *in vitro* experiments, the effectiveness of such strategies for the control of demineralization processes as well as for caries therapy still needs validation by clinical studies.

Nanotechnology has been defined as the creation of functional materials, devices, or systems through control of matter on the nanometer scale (1-100 nm). Currently, nanotechnology is experiencing rapid growth, with many potential applications in dentistry. Nanotechnology has motivated mimicking of the nanostructural features of natural human enamel and development of bio-inspired strategies for remineralization and caries therapy, respectively.

The purpose of the review is to highlight recent trends and developments in the field of caries therapy based on the application of nanotechnology. Thereby, the focus will be on 3 topics: (1) remineralization of initial caries lesions, (2) caries-preventive nanofillers added to resin composites, as well as (3) strategies for biomimetic synthesis of enamel for repair of caries lesions with enamel-like nanomaterials.

REMINERALIZATION OF INITIAL CARIES LESIONS

Several nanotechnological approaches have been reported for remineralization of early caries lesions. Casein phosphopeptide-amorphous calcium phosphate nanocomplexes have been shown to promote enamel remineralization and provide anti-cariogenic activity in laboratory, animal, and human experiments. The casein phosphopeptides stabilize calcium and phosphate ions by formation of amorphous nanocomplexes. The calcium phosphate from these complexes is biologically available for remineralization of initial lesions. Details regarding the mode of action as well as clinical performance of these calcium-phosphate-based remineralization delivery systems have been described and discussed recently by Cochrane *et al.* (2010) and will also be covered by a (review) paper in this issue (see Cochrane and Reynolds, 2012).

In recent years, biomimetic treatment of early caries lesions by the application of various types of nano-sized hydroxyapatite or calcium carbonate has also received considerable attention (Huang S *et al.,* 2009, 2010, 2011; Nakashima *et al.,* 2009). An experimental dentifrice containing 1% nano-sized amorphous calcium carbonate particles (several tens to hundreds of nm), applied twice a day over 20 days, yielded statistically significant mineral gain and remineralization of artificial caries lesions in an *in vitro* system that used collagen-coated wells as a model for oral mucosal surfaces (Nakashima *et al.,* 2009). The authors conclude that the experimental dentifrice has the potential to remineralize incipient enamel lesions due to the unique properties of the nano-sized calcium carbonate, which had been retained on the collagen-coated surfaces in the *in vitro* model system and thus might also be retained on oral surfaces, thereafter releasing Ca ions into oral fluids for remineralization (Nakashima *et al.,* 2009).

Furthermore, several studies have indicated that nano-apatite, in principle, has the potential to remineralize, at least in part, initial enamel caries lesions under dynamic pH-cycling conditions *in vitro* (Huang S *et al.,* 2009, 2010, 2011). A 10% suspension of nano-hydroxyapatite particles (10-20 nm diameter, 60-80 nm length) promotes preferential remineralization of the superficial layer of artificial caries lesions, and thus might be effective in reversing lesion progression in the outer surface

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Key Words

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layer of initial caries lesions measuring 20 to 40 μm (Huang S *et al.,* 2010). However, little remineralization could be obtained by nano-hydroxyapatite in the body of the lesion (Huang S *et al.,* 2010, 2011). Interestingly, hydroxyapatite nanoparticles promote remineralizing effects under *in vitro* conditions, in contrast to a control solution containing an equivalent concentration of free ions as provided by the nano-HA solution at equilibrium (Huang *et al.,* 2011). These observations suggest that intrinsic characteristics of the nano-HA, such as size and structure or chemical composition, may be of considerable relevance for the remineralization process (Huang *et al.,* 2011). Apparently, not only the size of the apatite nanoparticles used for remineralization purpose but also the pH of the remineralizing agent will affect the process of mineralization (Huang *et al.,* 2011). More mineral was deposited in the body part of the lesion if the pH-value was reduced from 7.0 to 4.0 (Huang *et al.,* 2011). Nanoparticulate calcium carbonate or apatite might act as a calcium and phosphate reservoir, helping to maintain a topical state of supersaturation of these ions with respect to enamel minerals. In addition, deposition on the surface of the demineralized enamel might promote remineralization of the outer enamel caries lesion. However, diffusion of mineral ions into the body of the lesion will be strongly inhibited by a highly mineralized surface layer. The overall phenomenon of remineralization by application of nano-sized apatite or calcium carbonate particles has not yet been clarified, and, in particular, the mechanism of action on the atomic level is still open to debate. Further research is necessary to investigate in detail the size-dependent effects of the particles applied on the remineralization potential of nano-hydroxyapatite *in vivo*.

CARIES-PREVENTIVE NANOFILLERS IN RESTORATIVE MATERIALS

Secondary caries and restoration fracture are still the main reasons for dental restoration failure, thus limiting the longevity of (resin composite) restorations. To control caries-induced demineralization at the resin composite-tooth interface, calcium and phosphate ion-releasing nanofillers have been developed, such as nanoparticles of dicalcium phosphate anhydrous (112 nm in size) or of amorphous calcium phosphate (116 nm in size) (Xu *et al*., 2007a,b, 2010b, 2011; Moreau *et al.,* 2011). These additives enable the resin composite to release calcium and phosphate when the pH is dropped down under *in vitro* conditions, providing caries-inhibiting properties (Xu *et al.,* 2007a,b, 2010b, 2011; Chen, 2010). Nanocomposites containing 40% nanoparticles of amorphous calcium carbonate have been shown to rapidly neutralize a lactic acid solution of pH 4.0 by increasing the pH to 5.69 within 10 min (Moreau *et al.,* 2011). The mechanical properties of the calcium- and phosphate-releasing experimental composites match those of commercial hybrid composites (Chen, 2010; Moreau *et al.,* 2011; Xu *et al.,* 2011). In addition, fluoride release from restorative materials has been considered to inhibit tooth demineralization and caries development. The addition of CaF_2 nanoparticles (50-60 nm) to resin composites results in fluoride release similar to or even higher than that from commercial resin-modified glass-ionomer materials (Xu *et al.,* 2008, 2010a). Nano-CaF_2-containing composites with high flexural strength and sustained fluoride release may have the potential to reduce restoration fracture and secondary caries. Recently, nanocomposites containing CaF_2 and dicalcium phosphate anhydride, which can release F, Ca, and PO_4 ions for precipitation of fluoroapatite and potential caries-inhibiting capabilities, have been formulated with sufficient mechanical properties (Chen, 2010; Xu *et al.,* 2008, 2010a,b). Most recent developments are novel nanocomposites which contain antibacterial agents, such as chlorhexidine (10%) and quaternary ammonium dimethacrylate (7%) alone or in combination with silver nanoparticles (0.028%), in addition to calcium and phosphate ion-releasing nanofillers (Cheng *et al.,* 2012a,b,c). Incorporation of these antibacterial components into nanocomposites has been shown to yield antibacterial capabilities, thereby reducing the biofilm colony-forming unit counts, the metabolic activity, and lactic acid production of *Streptococcus mutans* biofilms under *in vitro* conditions (Cheng *et al.,* 2012a,b,c). However, the effectiveness of all these strategies for the control of demineralization processes still needs validation, on the one hand, by *in vitro* studies focusing on the caries-inhibiting potential of ion-releasing and antibacterial resin composites, as well as by subsequent clinical studies, on the other.

BIOMIMETIC SYNTHESIS OF ENAMEL – REPAIR OF CARIES LESIONS WITH ENAMEL-LIKE NANOMATERIALS

Due to its non-regenerative nature, enamel is unable to heal and repair itself after demineralization of the surface and subsequent cavitation. Biomimetic strategies for artificial enamel formation might have the potential to repair enamel surface damage and increase the longevity of teeth (Huang Z *et al.,* 2010). Extensive *in vitro* investigations of apatite crystallization have been performed to mimic the formation of hierarchically organized enamel-like nano- and microstructures using acellular nanotechnological approaches (Hannig and Hannig, 2010). Different kinds of synthesis methods have emerged for the preparation of amorphous or crystalline nanoparticulate hydroxyapatite (Hannig and Hannig, 2010). However, much more impressive than the synthesis of individual or agglomerated apatite particles on the nano-scale is the manipulation of nanoparticles to form highly organized structures resembling natural enamel. Potential mechanisms for formation of highly oriented biomineralized structures include guided crystal growth on templates, the aggregation of nanocrystals by organized attachment, or the assembly of inorganic nanoparticles mediated by organic scaffolds into aggregated structures (Table).

Combinations of nano-sized mineral particles, nano-crystal pastes, or calcium phosphate ion solutions with various biological additives or surfactants were adopted to form structures mimicking the hierarchical nanostructure of dental enamel. The application of surfactants as reverse micelles or micro-emulsions for the synthesis and self-assembly of nanoscale structures is one of the most widely used methods in nanotechnology (Chen *et al.,* 2005; Zhang *et al.,* 2010). This technology mimics the natural biomineralization process taking place during formation of enamel by modifying of hydroxyapatite nanorods with surfactants, allowing the nanorods to self-assemble into an

Table. Biomimetic Strategies for Enamel Synthesis Based on the Application of Nanotechnology under Near-to-Physiological Conditions

Author (yr)	Methodology	Key Findings
Fan *et al.* (2009)	application of amelogenin (recombinant rP172 at concentrations of 33 μg/mL or above) and fluoride in a calcium-phosphate solution using a slow and precisely controlled crystallization system	oriented bundle formation of fused needle-like fluoridated hydroxyapatite crystals
Onuma *et al.* (2005) Yamagishi *et al.* (2005)	paste of modified HA (acidic calcium phosphate solution containing hydrogen peroxide, fluoride-substituted hydroxyapatite)	layer of crystals (100-400 nm long, 20-80 nm wide) that have grown across the enamel-paste interface and are more or less regularly oriented to the enamel surface
Wang *et al.* (2009)	paste of fluorapatite nanorod powder and 17% phosphoric acid at ambient conditions	layer of self-assembled ordered bundle-like calcium phosphate crystals
Yin *et al.* (2009)	aqueous solution technique with a suspension of HEDTA-Ca, KH_2PO_4, and KF under near-to-physiological conditions (37°C, 1 atm, pH 6.0)	layer of fluoridated hydroxyapatite hexagonal nanorod-like crystallite structures (300-400 nm in diameter; 4-5 μm in length)

enamel prism-like structure (Chen *et al.,* 2005; Zhang *et al.,* 2010). However, the suspected biocompatibility of non-biological surfactants strongly limits their clinical application. In addition, attempts to mimic the nano- and micro-structure of tooth enamel in various hydrothermal conditions, including non-physiological temperature or pressure (Chen *et al.,* 2006; Zhang *et al.,* 2010), cannot be applied clinically.

A very promising route to achieve the arrangement of apatite nanoparticles in complex-oriented enamel-like materials is the process of self-organization induced by amelogenin. Amelogenin is the major extracellular matrix protein in the development of natural dental enamel and was adopted for the growth of biomimetic enamel-like apatite layers (Iijima and Moradian-Oldak, 2005; Fan *et al.,* 2009). Amelogenin promotes apatite crystallization and organization (Wang *et al.,* 2008 ; Yang *et al.,* 2010). Thus, the natural enamel protein amelogenin has been used *in vitro* to control calcium and phosphate crystallization, resulting in the growth of nano-sized rod-like apatite crystals (Iijima and Moradian-Oldak, 2005). With this method, remineralization of the etched enamel surface by formation of a mineral layer containing needle-like fluoridated hydroxyapatite crystals with dimensions of 35 nm has been demonstrated (Iijima and Moradian-Oldak, 2005). Follow-up studies revealed that self-organized microstructures, compositionally and morphologically similar to natural ones, are achieved in a slow and precisely controlled constant crystallization system, adopting amelogenin under ambient conditions (Wang *et al.,* 2008 ; Fan *et al.,* 2009). More recently, an oriented amelogenin fluoridated needle-like hydroxyapatite layer could be precipitated on etched enamel *in vitro* by the application of amelogenin and fluoride in a calcium-phosphate solution, indicating a synergistic interaction of fluoride and amelogenin (Fan *et al.,* 2009). However, synthesis of enamel-like structures adopting amelogenin-based approaches needs from several days to weeks and thus is not directly applicable in daily dental practice.

Currently, a biomimetic approach has been implemented in which single crystalline hydroxyapatite micro-ribbons were used as substitutes for amelogenin templates to control HAP crystallization at biophysical conditions at 37°C (Ma *et al.,* 2011). Hierarchical hydroxyapatite structures composed of nanocrystals on the micro-ribbons were synthesized. Thereby, the morphologies and orientations of the formed crystals were tunable by the fluoride concentration. The hydroxyapatite crystals change dramatically from disorderd aggregations of nano-flakes to bundles of nano-needles, which are almost perfectly aligned along the c-axis of the substrates, if the fluoride concentration is increased from 0-0.01 mM to 0.1-1.0 mM (Ma *et al.,* 2011).

Although there is clear evidence that organic templates or scaffolds would be a prerequisite for bioinspired formation of enamel-like structures, even approaches for apatite crystallization under ambient conditions have been proposed without organic additives (Onuma *et al.,* 2005; Yamagishi *et al.,* 2005; Wang *et al.,* 2009; Yin *et al.,* 2009). Yamagishi *et al.* (2005) described an acidic (pH < 2) paste consisting of fluoride-substituted hydroxyapatite, 35% H_2O_2, and concentrated phosphoric acid that could be used for the repair of small caries lesions. Within 15 min, an up to 20-μm-thick layer of F-HAP is formed seamlessly on the enamel surface (Onuma *et al.,* 2005; Yamagishi *et al.,* 2005). The regrown layer contains elongated crystals (100-400 nm long, 20-80 nm wide) that have grown across the enamel-paste interface and are more or less regularly oriented to the enamel surface (Yamagishi *et al.,* 2005). Unfortunately, up to now, no follow-up study has been published on the application of this paste regarding its *in vivo* longevity under clinical conditions. Interestingly, more recently, Wang *et al.* (2009) demonstrated direct growth of human enamel-like ordered micro-structures using a fluorapatite/phosphoric acid paste without the addition of H_2O_2. The newly formed crystals tend to be aligned in parallel to each other and self-assemble into ordered bundle-like structures. Yin *et al.* (2009) described a simple aqueous solution technique to form fluoridated hydroxyapatite hexagonal nanorod-like crystallite structures (300-400 nm in diameter; 4-5 μm in length) under near-to-physiological conditions (37°C, 1 atm, pH 6.0) using a suspension of HEDTA-Ca, KH_2PO_4, and KF. After formation times of several days, the reconstructed layer with a thickness of several micrometers was quite analogous to the natural enamel in chemical components, microarchitectural structure, and nanomechanical properties (Yin

et al., 2009). However, since HEDTA is not safe for consumption, this method cannot be applied directly under clinical conditions.

CONCLUDING REMARKS

Nanotechnology has promoted the development of bio-inspired routes for caries prevention and tooth-surface repair (Hannig and Hannig, 2010). At this point, to put the present knowledge and available data into a future perspective, one has to assess whether the biomimetic nanotechnological approaches for caries therapy will be suitable and applicable in daily dental practice. At the moment, there are many approaches tested *in vitro*; however, clinical validation is still lacking. Analysis of recent *in vitro* data indicates that apatite nanoparticles might be quite effective in reversing lesion progression in the outer part of artificial white-spot lesions, whereas few remineralizing effects will be achieved in the deeper areas of the caries lesions. Nanocomposites providing stress-bearing mechanical properties combined with caries-inhibiting capabilities due to the release of Ca, F, and phosphate ions reveal an interesting approach in modern restorative dentistry. Furthermore, biomimetic growth of organized, nano-apatite-based coatings that closely resemble the hierarchical architecture of human enamel has been demonstrated under close-to-physiological *in vitro* conditions.

Despite all these promising *in vitro* experiments, clinical application of the mentioned nanotechnological approaches for caries therapy is not yet conceivable. In particular, a facile and controllable strategy to develop apatite nanorods into large-size prism-like structures similar to that of human enamel still remains a major challenge for biomimetic synthesis of enamel. Unfortunately, up to now, the mechanical properties as well as the chemical stability of enamel-like restorations consisting of hierarchically organized apatite-nanoparticles under clinical conditions cannot be estimated, since valid data are lacking.

Furthermore, it should be kept in mind that formation of nano-scaled enamel-like structures by the currently available nanotechnological methods usually takes from hours to days to achieve surface coatings measuring only a few micrometers in thickness. Thus, concerning reconstruction of clinically visible enamel defects, further research is necessary to achieve an easy-to-apply, fast-growing enamel-like bioceramic for biomimetic tooth repair. One advantage of such restorative strategies might be the biocompatibility of the adopted nano-biomaterials. However, notwithstanding that nanotechnology-based biomimetic repair of enamel defects might provide fascinating novel strategies in restorative caries therapy, it should be kept in mind that tooth sites restored with nano-apatite and enamel-like materials might become damaged by caries attacks. The mode and progress of carious destruction in artificial enamel are unpredictable; data addressing this question are not yet available.

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