Nanomaterials in preventive dentistry

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The prevention of tooth decay and the treatment of lesions and cavities are ongoing challenges in dentistry. In recent years, biomimetic approaches have been used to develop nanomaterials for inclusion in a variety of oral health-care products. Examples include liquids and pastes that contain nano-apatites for biofilm management at the tooth surface, and products that contain nanomaterials for the remineralization of early submicrometre-sized enamel lesions. However, the treatment of larger visible cavities with nanomaterials is still at the research stage. Here, we review progress in the development of nanomaterials for different applications in preventive dentistry and research, including clinical trials.

The purpose of modern dentistry is the early prevention of tooth decay rather than invasive restorative therapy. However, despite tremendous efforts in promoting oral hygiene and fluoridation, the prevention and biomimetic treatment of early caries lesions are still challenges for dental research and public health, particularly for individuals with a high risk for developing caries, which is the most widespread oral disease. Recent studies indicate that nanotechnology might provide novel strategies in preventive dentistry, specifically in the control and management of bacterial biofilms or remineralization of submicrometre-sized tooth decay.

Nanomaterials for managing oral biofilms

Dental caries is caused by bacterial biofilms on the tooth surface, and the process of caries formation is modulated by complex interactions between acid-producing bacteria and host factors including teeth and saliva[1–3] (Figs 1a,b; 2). On exposure to oral fluids, a proteinaceous surface coating — termed pellicle — is formed immediately on all solid substrates[4]. This conditioning layer, which defines the surface charge and the nature of chemical groups exposed at the surface, changes the properties of the substrate[4]. Bacteria colonize the surface by adhering to the pellicle through adhesin–receptor interactions and form a biofilm, known as dental plaque. Maturation of the plaque is characterized by bacterial interactions (such as co-aggregation and quorum sensing), and increasingly diverse bacterial populations[5]. Each human host harbours different bacterial populations[5], and it is thought that the metabolic interactions between different bacterial species play a key role in the maturation process of the biofilm[5]. Therefore, the number of *streptococci* and *lactobacilli* bacteria that cause caries can increase, especially in the presence of dietary sugars[1–3]. These bacterial species produce acids as by-products from the metabolism of fermentable carbohydrates, and cause demineralization below the surface of the tooth[2,3] (Fig. 2).

Further to conventional oral hygiene, anti-adhesive surface coatings can be used to control the formation of dental biofilms because nanostructured surface topography and surface chemistry can both determine initial bioadhesion[6,7]. The classic lotus effect in ultrahydrophobic surfaces is an example of a self-cleaning surface[8,9]. However, such nanostructured surfaces are not suitable for application in the oral cavity because of surface wear and equilibration of the surface nanotopography by the ubiquitous pellicle layer[4].

To prevent the pathogenic consequences of tenacious intraoral biofilm formation over a longer interval, wear-resistant nanocomposite surface coatings have been developed for the modification of the tooth surface *in vivo*[10]. Easy-to-clean surface properties are achieved by integrating nanometre-sized inorganic particles into a fluoropolymer matrix[10]. These biocompatible surface coatings have a surface free-energy of 20–25 mJ m^{-2} — known as theta surfaces[11] — and therefore can facilitate the detachment of adsorbed salivary proteins and adherent bacteria under the influence of physiological shearing forces in the mouth (Fig. 1c)[10]. Easy-to-clean coatings are conceivable for patients with high caries risk, such as those suffering from mouth dryness owing to dysfunctional salivary glands — termed xerostomia — or for individuals who do not practise proper oral hygiene. Possible applications could be tooth sealants as well as coatings of restorations, dentures or transmucosal parts of implants. Even tooth fissures sealed with this material could be cleaned more easily by the shear forces from tooth brushing.

Other nano-enabled approaches for biofilm management are oral health-care products that contain bioinspired apatite nanoparticles, either alone or in combination with proteinaceous additives such as casein phosphopeptides[12,13]. Casein phosphopeptide (CPP)-stabilized amorphous calcium phosphate (ACP) nanocomplexes with a diameter of 2.12 nm (refs 14,15) seem to play a pronounced role in biomimetic strategies for biofilm management. There is *in vivo* evidence indicating that CPP–ACP complexes reduce bacterial adherence by binding to the surfaces of bacterial cells, the components of the intercellular plaque matrix and to adsorbed macromolecules on the tooth surface[16,17] (Fig. 1d). CPP–ACP-treated germanium surfaces that are applied in the oral cavity for up to one week have been shown to significantly delay the formation of biofilms[12]. However, it should be emphasized that because germanium is not a biomineral the clinical relevance of the study remains limited. Other *in vitro* experiments have shown that non-aggregated and clustered hydroxyl apatite nanocrystallite particles (average size 100 × 10 × 5 nm) can adsorb onto the bacterial surface[18], and interact with bacterial adhesins to interfere with the binding of microorganisms to the tooth surface.

These bioinspired strategies for biofilm management are based on size-specific effects of the apatite nanoparticles, and are thought to be more effective than traditional approaches that use micrometre-sized hydroxyl apatite in toothpastes. Hydroxyl apatite has been adopted for years in preventive dentistry; however, effective interaction of the biomineral with the bacteria is only possible if nano-sized particles that are smaller than the microorganisms are used (Fig. 1d).

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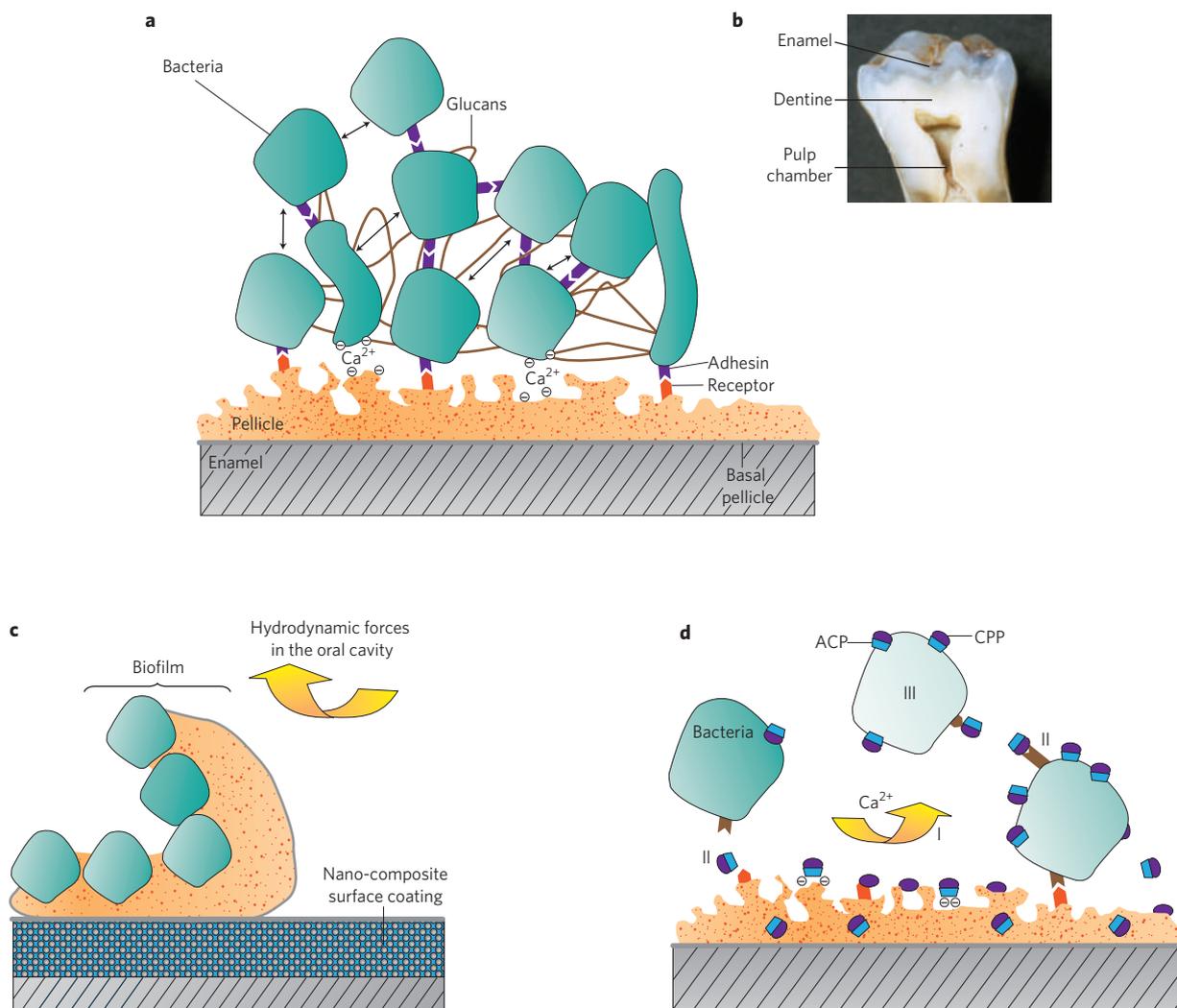
Figure 1 | Bioadhesion and biofilm management in the oral cavity. a, Bioadhesion in the oral cavity. Proteins interact with the enamel surface to form a proteinaceous pellicle layer. Bacteria adhere to this conditioning film through calcium bridges and specific adhesin–receptor interactions (purple and red). Bacteria are surrounded by an extracellular matrix of water-insoluble glucans, and they communicate through quorum sensing (arrows). **b**, Cross-section of a human molar tooth showing the enamel, dentine and pulp chamber. **c**, Easy-to-clean nanocomposite surface coating. The low-surface-free-energy coating (blue) causes poor protein–protein binding. Shear forces in the mouth (yellow arrow) can easily detach the outer layer of the pellicle and bacterial biofilm from the surface. **d**, CPP–ACP inhibits bacterial adhesion and oral biofilm formation. CPP attaches to the pellicle and limits bacterial adhesion. It competes with calcium for plaque–calcium binding sites (I), and decreases the amount of calcium bridging the pellicle and bacteria, and between the bacterial cells. Specific receptor molecules (red) in the pellicle layer and on the bacterial surfaces (brown) are blocked, further reducing adhesion and co-adhesion (II). This affects the viability of the bacteria (III).

Remineralization of submicrometre-sized tooth defects

Dental enamel is unique among the mammalian calcified tissues because it comprises 80–90% by volume of a calcium-deficient carbonate hydroxyl apatite. Other calcified tissues such as bone or dentine contain considerably lower amounts of inorganic minerals. However, like most other naturally mineralized tissues, dental enamel and dentine have hierarchical structures and surface features at the nanometre scale[6,7,19,20]. On the microscale, the enamel contains highly organized architectural units known as enamel prisms (Fig. 3c,e). On the nanoscale, the enamel consists of highly crystalline nanorod-like calcium hydroxyl apatite crystallites that are arranged roughly parallel to each other (Fig. 3a,b,d). Mature-human-enamel crystallites are 26.3 ± 2.2 nm thick, 68.3 ± 13.4 nm wide, and between 100 and 1,000 nm long (Fig. 3)[20,21]. In contrast, dentine is a hydrated tissue made up of approximately 50 vol.% mineral, 30 vol.% collagenous and non-collagenous proteins, and 20 vol.% fluids. The dentinal matrix is mainly composed of type I collagen fibrils forming a three-dimensional scaffold matrix, reinforced by hydroxyl apatite crystallites, measuring approximately 20 nm in size[19,22].

Initial carious lesions in the enamel caused by adherent biofilms yield a typical micromorphology with a pseudo-intact surface layer on top of the subsurface body of the lesion as a result of reprecipitating minerals (Fig. 2). In contrast to caries lesions caused by acids from bacterial metabolism, enamel erosions are induced by frequent consumption of acidic foods and beverages, or by gastric juice (Fig. 4). This direct and continuous surface demineralization is only slightly diminished by the pellicle present in eroded lacunae[23]. However, the approaches for remineralization are the same for submicrometre erosive and initial carious lesions.

Fluoride is an effective remineralizing agent, and has therefore been widely applied for the prevention of mineral loss[1]. CPP–ACP has also been shown to promote remineralization of initial enamel lesions and to prevent demineralization in laboratory, animal and human experiments[14,15,24–26]. In particular, CPP–ACP released by

chewing gum has been tested successfully *in situ* for remineralization of enamel subsurface lesions and for the prevention of demineralization[25,26]. Furthermore, CPP–ACP sugar-free gum significantly reduced progression and enhanced regression of proximal caries relative to a control sugar-free gum in a 24-month clinical trial with 2,720 children[24].

The casein phosphopeptides stabilize calcium and phosphate ions through the formation of amorphous nanocomplexes (diameter of 2.12 nm). Phosphorylated seryl-residues are regarded as responsible for the interactions between casein and the calcium and phosphate ions in the nanocomplexes, which ensure ions are available for biomineralization[16,27]. Oral health-care products containing CPP–ACP have been on the market for several years (for example, MI Paste, Recaldent and GC Tooth Mousse from GC company, Japan). It has been shown recently that a combination of CPP–ACP with fluoride will further enhance the remineralizing effects *in situ*[28].

Other biomimetic approaches for remineralization of initial submicrometre enamel erosions are based on nano-sized hydroxyl apatite particles[29,30]. *In vitro* data[30] indicate that repair at the enamel surface can be greatly improved if the dimensions of the apatite particles are adapted to the scale of the submicrometre- and nano-sized defects caused by erosive demineralization of the natural apatite crystallites (Fig. 4). Hydroxyl apatite with a size of 20 nm fits well with the dimensions of the nanodefects caused at the enamel surface during acidic erosion[30]. These particles adsorb strongly to the etched enamel surface under *in vitro* conditions[30] and, interestingly, retard further erosive demineralization[30]. Therefore, the use of well-sized nano-apatite particles could simultaneously repair and prevent initial enamel-erosive lesions. Nonetheless, these effects have not yet been confirmed in a clinical trial.

In another approach, biomimetic carbonate hydroxyl apatite nanoparticles that mimic the size of natural dentinal hydroxyl apatite (20 nm) or enamel apatite (100 nm) were used to repair micrometre-sized tooth-surface defects *in vitro*[29,31,32]. Clusters of these nanocrystals have been incorporated into toothpastes or mouth-rinsing solutions to promote the repair of demineralized enamel or dentine surfaces by depositing apatite nanoparticles in the defects. Commercially available dental prophylactic products that contain carbonate hydroxyl apatite nanoparticles to fill microdefects at the etched enamel surface (for example, BioRepair from Coswell Laboratories, Italy and from Dr. Wolff, Germany) have been proved to be effective *in vitro* after a ten-minute application onto enamel or dentine surfaces[31,32]. However, these promising effects have not yet been substantiated in a clinical study.

Nano hydroxyl apatite toothpaste with either spheroidal or needle-like particles as an active component was shown to enhance the remineralization of etched enamel better than sodium fluoride solutions[33]. However, the 5–10 day *in vitro* study had neglected the conditions of the oral cavity. In another *in vitro* study, nano-sized amorphous calcium carbonate particles that were applied twice a day for a period of 20 days promoted remineralization of artificial white-spot enamel lesions[34]. Reconstitution and remineralization of dentine using nano-sized bioactive glass particles and beta-tricalcium phosphate was also tested *in vitro*, however, the mechanical properties of original dentine could not be reproduced[35,36]. Owing to the complex organic and inorganic structure of the dentine, remineralizing dentine into a functional state remains one of the most difficult challenges in dental research[19].

Biomimetic synthesis of enamel and repair of microcavities

If carious or erosive enamel defects enlarge, they cannot be repaired by remineralization techniques. The established way to restore clinically visible loss of enamel is the application of resin composites in combination with dental adhesives. However, biomimetic synthesis of enamel could be an alternative reparative approach, and self-assembling hierarchically organized enamel-
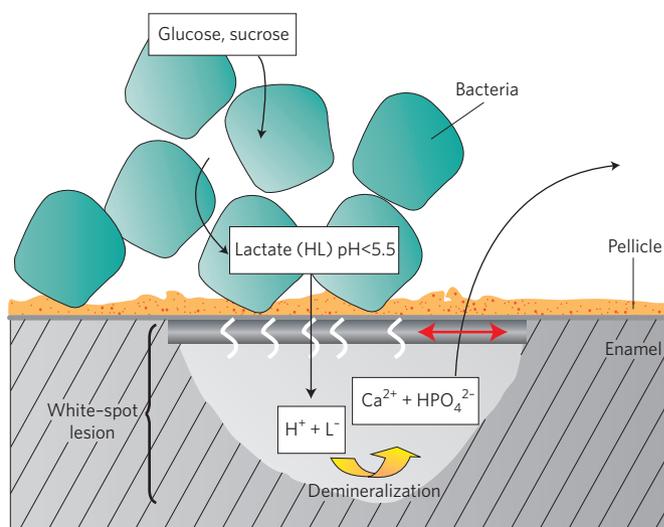

Figure 2 | Early stages of tooth decay caused by bacterial biofilm. Bacteria metabolize sugar and other carbohydrates to produce lactate (HL) and other acids that, in turn, dissociate to form H^+ ions that demineralize the enamel beneath the surface of the tooth; calcium and phosphate are dissolved in the process. This is known as a white-spot lesion. Owing to reprecipitation, a pseudo-intact surface layer (red arrow) is observed on top of the body of the carious lesion in this early stage of tooth decay. This pseudo-intact layer is permeable to ions (indicated by white chevrons).

like structures composed of hydroxyl apatite crystals would be ideal.

Various groundbreaking pilot investigations of apatite crystallization have been performed under purely *in vitro* conditions to mimic the formation of enamel-like microstructures in the presence of organic additives[21,31,37–44], or by using various hydrothermal conditions[45]. Growth of enamel-like nanocrystals in small cavities from a paste containing fluoride-substituted hydroxyl apatite was achieved *in vitro*. The artificially formed enamel-like layer was about 10 µm thick and was formed seamlessly on the enamel within 15 minutes. Unfortunately, the paste is highly acidic (pH 3.5) and contains high concentrations of hydrogen peroxide[46].

Formation of enamel-like structures at ambient conditions was also performed *in vitro* using different organic additives and scaffold-forming molecules, predominantly amelogenin, in slow and precisely controlled crystallization systems[21,37–40,42,43,47–49]. The amphiphilic amelogenin is a major extracellular matrix protein in physiological enamel development[38,39,42,47]. Amelogenin aggregates to supramolecular nanospheres and is required for the self-assembly of oriented parallel needle-like apatite bundles[38,50]. Accordingly, several *in vitro* attempts have been made to prepare enamel-like materials that contain nano- and microstructures using amelogenin to control the crystallization of biomimetic calcium and phosphate[21,37–40]. In particular, amelogenin oligomers mediate the controlled self-organized crystallization of a microstructured material that is compositionally and morphologically similar to natural enamel without using extreme hydrothermal conditions[21,37]. Furthermore, amelogenin promotes remineralization of etched enamel surfaces by forming a mineral layer containing needle-like fluoridated hydroxyl apatite crystals with dimensions of 35 nm (ref. 39).

Self-assembling anionic β-sheet peptides, based mainly on glutamic acid and glutamine from fibrillar networks, were able to increase remineralization and inhibit demineralization of the enamel[41]. Even amino acids, such as aspartic acid, alanine and arginine, could increase the bioactivity of synthetic hydroxyl
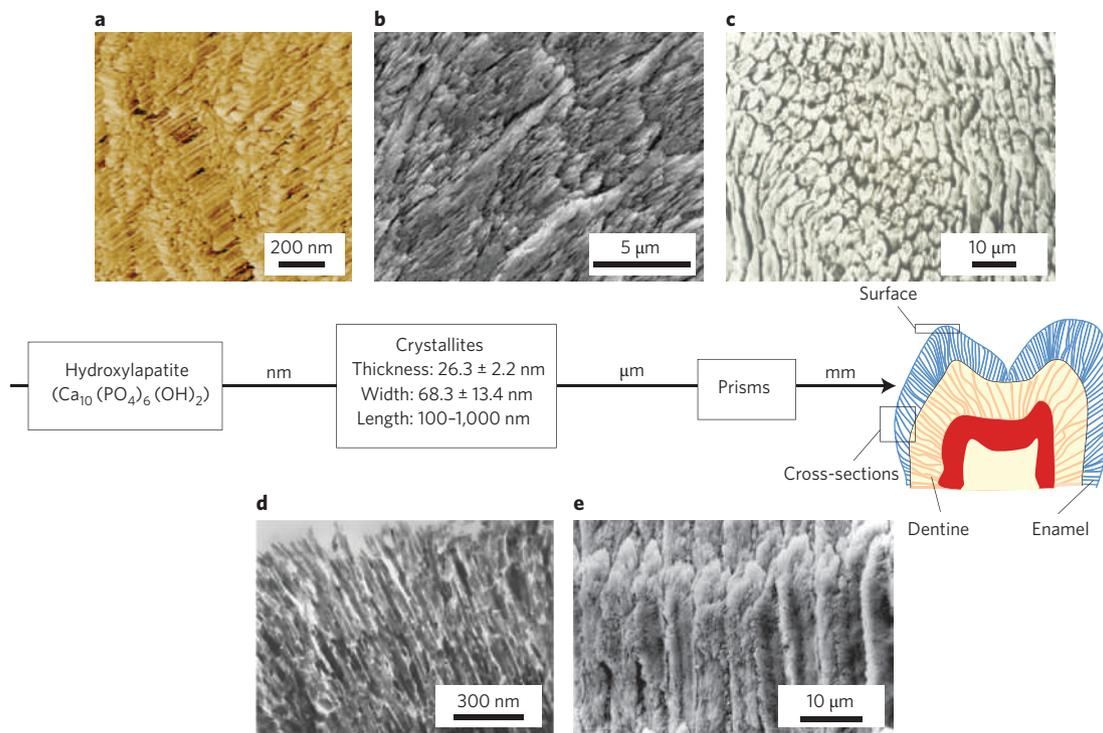
Figure 3 | Hierarchical structure of the dental enamel. Dental enamel is a masterpiece of bioceramics, containing structures at different hierarchical levels from the microscale down to the nanoscale. The enamel is composed of three-dimensionally organized nanosized hydroxyl apatite crystallites (**a,b,d**) that are arranged into micrometre-sized prisms (**c,e**). **a**, Atomic force microscope and **b,c**, scanning electron microscope images of the enamel surface. **d**, Transmission electron microscope and **e**, scanning electron microscope images of a cross-section of the enamel.
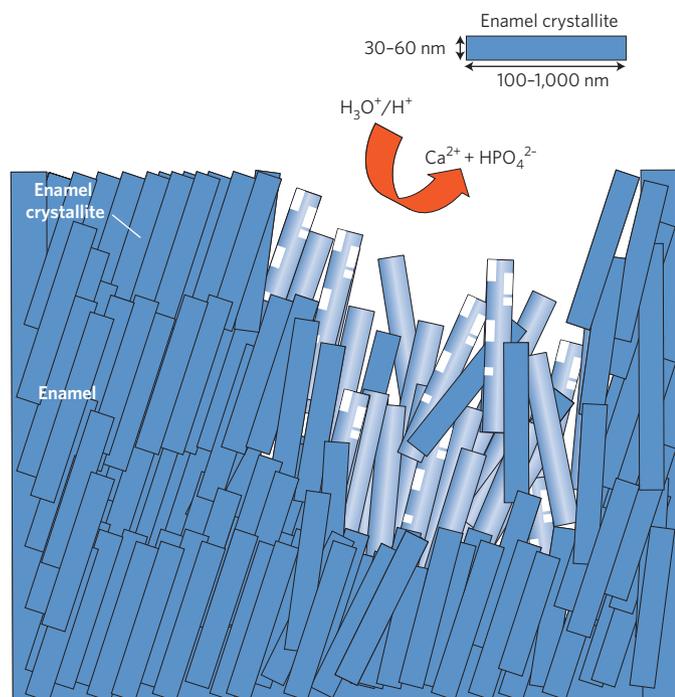

Figure 4 | Dental erosion caused by acidic beverages or food in the oral cavity. Low pH, caused by acidic beverages or gastric juices (pH 1–4), destroys the enamel surface by partial and complete dissolution of the enamel crystallites, resulting in the release of Ca^{2+} and HPO_4^{2-} ions. This loosens the microstructure of the enamel and hydroxyl apatite crystallites (pale blue) become demineralized, or are lost.

apatite and have been adopted as additives during the formation of biomimetic calcium-deficient hydroxyl apatite[31,44]; amelogenin-like effects were achieved in the presence of glycine[40].

Surfactants have also been used as reverse micelles or micro-emulsions to mimic the biomineralization process during the formation of enamel[43]. Hydroxyl apatite nanorods were modified with monolayers of surfactants to create specific surface characteristics that allow the nanorods to self-assemble into a prism-like enamel structure[43]. Furthermore, enamel-like microstructures were formed *in vitro* using sodium *bis*(2-ethylhexyl) sulphosuccinate as a structure-directing agent[40,42].

Despite all these promising *in vitro* experiments, the clinical application of these approaches for restoration of clinically visible cavities in the enamel is not yet conceivable. The stability and the mechanical properties of larger aggregates are not sufficient for tooth restorations, and the formation of the mineral structures often takes from several hours to days[21,40]. In all these studies, crystal formation was achieved under purely *in vitro* conditions that neglect the physiology of the mouth, where the saliva and the proteinaceous pellicle layer play an important role in biomineralization. Further research is necessary to achieve a real enamel-like bioceramic for clinically conceivable biomimetic tooth repair.

Conclusion

Oral health-care products based on bioinspired nanomaterials have moved from the laboratory to daily application — as a supplement to conventional approaches — for biofilm control and remineralization of submicrometre-sized enamel lesions. Easy-to-clean, wear-resistant and biocompatible nanocomposite surface coatings for biofilm management are close to being used in dental practice. However, biomimetic restoration and filling of small

clinically visible cavities with nanomaterials is not conceivable at the moment, and requires extensive further research with respect to clinical applicability. It should also be kept in mind that biomimetic enamel surfaces are still susceptible to caries if patients neglect conventional oral health-care such as tooth brushing or fluoride application.

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Additional information