Current trends in the use of nanotechnology in restorative dentistry

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Abstract: Nanotechnology is gaining tremendous impetus due to its capability of modulating matter at smaller and smaller level which drastically changes the chemical, physical and mechanical properties of materials. Nanoparticles have been introduced into oral health care products with good potential to be extensively used for various dental applications. The purpose of this review is to highlight recent nanotechnological developments for caries prevention and remineralization of incipient carious lesions. Possible uses of nanoparticles incorporated into various dental restorative materials, pit and fissure sealants and bonding adhesives is also reviewed. Furthermore, biomimetic growth of organized nanoapatite based coatings that closely resembles the hierarchical architecture of human enamel formed under close to physiological in vitro condition is discussed.

Keywords: nanotechnology, remineralization, restorative dentistry.

INTRODUCTION

Dental caries is a highly prevalent diet related disease and a major public health problem. Different from the traditional restorative approach the current opinion is that caries should be detected and monitored in its earliest stages when a nonsurgical reversal can still be achieved. The non-invasive management of early lesions involves remineralisation with bioavailable calcium, phosphate, and fluoride ions to restore the strength and esthetic appearance of the lesion and to increase resistance to future acid challenge. With the scientific ability to control physical processes at nanometer scale, we have entered the era of research and application of nanoscale phenomena. Recent years have witnessed an unprecedented growth in research in the area of nanoscience. There is increasing optimism that nanotechnology applied to dentistry will bring significant advances in the diagnosis, treatment, and prevention of disease. It has promoted the development of bio-inspired routes for caries prevention and tooth surface repair. Optimal delivery of molecules that facilitate tooth structure re-mineralization and forestall caries is an active area of nanostructure based research. Much of this work involves:

- Nanomaterials for caries prevention
- Biofilm management through eliminating its established colonies on tooth surface- Biocidal approach using nanotechnology
- Biofilm management by making the tooth surface more resistant to acid attacks and bacterial
colonization- Anti-adhesive approach using nanotechnology

- Nanomaterials for early caries remineralization
- Nanomaterials in restorative materials
  - Resin composites
  - Pit and Fissure Sealants
  - Bonding agents

- Approaches using nanotechnology for biomimetic synthesis of enamel or repair of caries lesion with enamel like nanomaterial.

The purpose of this review is to give an overview of these approaches and the current status of research in these areas.

I) Nanotechnology for caries prevention

Dental caries can be prevented by interfering with the transmission of Mutans Streptococci, and biofilm management through eliminating its established colonies on tooth surface and/or by making the tooth surface more resistant to acid attacks and bacterial colonization. Caries preventive approaches using nanotechnology have also worked on these aims.

Approaches:

IA. Prevention of caries by:

a. Biofilm management through eliminating its established colonies on tooth surface- Biocidal approach using nanotechnology

b. Biofilm management by making the tooth surface more resistant to acid attacks and bacterial colonization- Anti-adhesive approach using nanotechnology

IB. Remineralization of early carious lesions

The survival of micro-organisms within the oral cavity is dependent on their ability to adhere to surfaces and form a biofilm, which in turn is influenced by the physico-chemical properties of the underlying surface (1). Hence, mechanical plaque control aids need to be complemented with the use of antimicrobial agents. This is of particular importance within the oral cavity when these agents have to reach less accessible stagnation sites or through plaque to the enamel. In this regard, the use of nanoparticles as constituents of topical agents to control oral biofilms through either their biocidal or anti-adhesive capabilities is now emerging as an area worthy of serious consideration. These antimicrobial nanoparticles may be of particular value if retained at approximal tooth surfaces and below the gum margin.

The studies by Robinson and co-workers using the ‘Leeds in situ model’, demonstrated that plaque contains voids and channels, sometimes extending completely through the biomass to the underlying enamel (2). Such channels may have considerable influence on the transfer of nanoparticles through biofilms. Thus, the main considerations are the physico-chemical characteristics of the particular nanoparticles used, and the ability of the particles to adsorb to/be taken up at the biofilm surface. Within this context, nanoparticles are potentially useful because it is possible to alter their surface charge, hydrophobicity, and other physical and chemical characteristics (3Nel et al., 2009).

Eliminating its established colonies on tooth surface by biocidal approach:

Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium, and zinc have attracted particular attention, each having different properties and spectra of activity. Many oral products, including toothpastes, now incorporate powdered zinc citrate or acetate to control the formation of dental plaque (4). An inverse relationship between nanoparticle size and antimicrobial activity has been demonstrated, where nanoparticles in the size range of 1−10 nm has been shown to have the greatest biocidal activity against bacteria (5). Also, it appears that bacteria are far less likely to acquire resistance against metal nanoparticles than other conventional and narrow-target antibiotics (6).

The use of silver ions has been considered for a range of biomedical applications, including, within the dental field, as an antibacterial component in
dental resin composites (7). Silver is also known to exhibit a strong affinity for zeolite, a porous crystalline material of hydrated aluminosilicate which can bind up to 40% silver ions within its structure. Silver zeolite has been incorporated into tissue conditioners, acrylic resins, and mouthrinses. (8). Silver nanoparticles, either alone or as a composite with other agents, have shown particularly encouraging results as antimicrobials (8).

The use of nanoparticles for photodynamic therapy approach is also an area of exploration. Photodynamic therapy (PDT) is being actively utilized within the clinical setting in some countries for the control of bacteria in oral plaque biofilms where there is relatively easy access for the application of the photosensitizing agent and light sources to areas requiring treatment (9). The killing of micro-organisms with light depends upon cytotoxic singlet oxygen and free-radical generation, which are formed by the excitation of a photactivatable agent or sensitizer. Using nanotechnology, a complex of biodegradable and biocompatible poly(lactic-co-glycolic acid) (PLGA) and colloidal gold nanoparticles, loaded with methylene blue and exposed to red light at 665 nm, has been tested against planktonic E. faecalis and in experimentally infected root canals (10).

Biofilm management by making the tooth surface more resistant to acid attacks and bacterial colonization

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. Recently, a lot of attention has been targeted at the particles of nano- and micro-size based elements, for the rapid delivery of antimicrobial and anti-adhesive capabilities to the desired site within the oral cavity. The use of silica nanoparticles to polish the tooth surface may help protect against damage by cariogenic bacteria, presumably because the bacteria can be removed more easily. This has been investigated on human teeth ex vivo (11). Atomic force microscopy demonstrated lower nanometer-scale roughness obtained when silica nanoparticles were used to polish the surfaces of teeth as compared with conventional polishing pastes. It was also shown that adherent S. mutans could be removed more easily.

The surface of composite restorations have also been modified for attaining easy-to-clean surface properties by integrating nanometre-sized inorganic particles into a fluoropolymer matrix (12). These biocompatible surface coatings have a surface free-energy of 20–25 mJ m⁻² known as theta surfaces and therefore can facilitate the detachment of adsorbed salivary proteins and adherent bacteria under the influence of physiological shearing forces in the mouth. 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 practice 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.

This approach is also being investigated as a means of reducing bacterial and fungal adhesion to oral materials and devices, for example, incorporation of silver nanoparticles into denture materials (13) and orthodontic adhesives (14). Much of this current work is using in vitro methodologies, because concerns regarding nanoparticle biocompatibility have not been fully addressed as of yet. With regard to dental implants, numerous companies currently market novel synthetic hydroxyapatite (HA) materials as the optimal osteoconductive implant coating available, some of which have developed nanoscaled varieties. Some have used alternative coatings and application methods to the conventional coating techniques, including an HA material available in nanophase, and a nanocrystalline silver-based antimicrobial coating that, in theory, should reduce the potential for bacterial colonization. The antibacterial properties of an amorphous carbon film (15) incorporating silver nanoparticles in a 40- to 60-nm size range and deposited onto a standard titanium material have
been evaluated. A significant reduction in mixed biofilm counts was observed after 7 days using the coating with silver nanoparticles compared with the standard titanium material.

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(16,17). Casein phosphopeptide (CPP)-stabilized amorphous calcium phosphate (ACP) nanocomplexes with a diameter of 2.12 nm (18) 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. 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(16). 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, 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.

IB Nanotechnology and Remineralization of carious lesion:

Optimal delivery of molecules that facilitate tooth structure remineralization and forestall caries is an active area of nanostructure-based research. It is now recognised that demineralised but noncavitated enamel and dentine can be ‘healed’, and the conventional surgical approach is no longer tenable.

Recent studies indicate that nanotechnology can refine/motivate the current remineralization approach to make it more clinically acceptable and predictable. Nanotechnological principles applied on Casein phosphopeptide - amorphous calcium phosphate and hydroxyapatite or calcium carbonate have attained welcome in vitro success (20-22).

Casein phosphopeptide amorphous calcium phosphate nanocomplexes have been shown to promote enamel remineralization and provide anticariogenic activity in laboratory, animal, and human experiments. The casein phosphopeptides stabilize calcium and phosphate ions by formation of amorphous nanocomplexes having diameter of about 2.12 nanometers. Phosphorylated seryl-residues are regarded as responsible for the interactions between casein and the calcium and phosphate ions in the nanocomplexes, which ensure that ions are available for biominalization. CPP-ACP has been added to sugar free gum, dentifrices (alone and in combination with fluoride), lozenges, mouthwash and bovine milk and has been tested successfully in situ for remineralization of enamel subsurface lesions and reduced progression of proximal carious lesions (23).

Nano-sized hydroxyapatite or calcium carbonate has also received considerable success in biomimetic treatment of early carious lesion. Repair at the enamel surface can be significantly improved if the dimensions of the apatite particles are adapted to the scale of the submicrometre- and nanosized defects caused by erosive demineralization of the natural apatite crystallites. 20 nm hydroxyapatite crystals have been shown to fit well in these defects(24). These particles have high affinity to the etched enamel surface under in vitro conditions and help retard further erosive demineralization.8 These nano sized crystals have been formulated into toothpastes or mouth-rinsing solutions to promote the repair of demineralised enamel or dentine surfaces by depositing apatite nanoparticles in the defects(25).
Attempts to remineralize dentin are also on its way and nano-sized bioactive glass particles and bettricalcium phosphate were tested for the same (26).

II) Nanotechnology in restorative dentistry:

IIA. Resin Composites:

Resin composites are increasingly used for dental caries restorations because of their esthetics and direct-filling capability. Remarkable progress has led to esthetic composite restoratives with less removal of tooth structures, enhanced load-bearing properties, and improved clinical performance. However, secondary caries at the restoration margins is identified as a main limitation to the longevity of the restorations [27-28]. The replacement of existing restorations accounts for 50–70% of all restorations performed [29].

Delivery of re-mineralizing ions that can prevent caries-induced demineralization at the resin composite-tooth interface, and hence prevent caries is an active area of nanostructure-based research. Much of this work involves development of calcium and phosphate ion-releasing nanoparticles in conjunction with Resin Based Composite systems such as nanoparticles of dicalcium phosphate anhydrous (112 nm in size) or of amorphous calcium phosphate (116 nm in size) (30-34). 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 (35). 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 (34). The mechanical properties of the calcium- and phosphate-releasing experimental composites match those of commercial hybrid composites (33-35). In addition, fluoride release from restorative materials has been considered to inhibit tooth demineralization and caries development.

The addition of CaF2 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 (36). Nano-CaF2-containing composites with high flexural strength and sustained fluoride release may have the potential to reduce restoration fracture and secondary caries. Recently, nanocomposites containing CaF2 and dicalcium phosphate anhydride, which can release F, Ca, and PO4 ions for precipitation of fluoroapatite and potential caries inhibiting capabilities, have been formulated with sufficient mechanical properties (32,35,36). 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 (37-39). 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. 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.

II B. Pit and fissure sealants containing ion releasing nanofillers for caries inhibition

Pit and fissure sealing undoubtedly plays a fundamental role in preventing occlusal caries. Several types of resin, both filled and unfilled, have been employed as a pit and fissure sealants. Microleakage between the sealant and tooth surface causing bacterial invasion and secondary caries is one of the biggest deterrent to the success of the pit and fissure sealants. Over the years, research has been conducted on sealant materials and methods to improve their properties like retention, marginal integrity. Remineralization of carious lesions in the enamel can be achieved with use of pit-and-fissure sealants containing amorphous calcium phosphate. ACP is a non-crystalline form of calcium phosphate which when added as filler to sealants and
composites, may aid in the re-mineralization of enamel and dentin (40). During a carious attack, pH is lowered by bacteria, acid release, or food, and this drop results in ACP being converted to hydroxyapatite, which precipitates, thus replacing the hydroxyapatite lost to acid.

A study conducted by Skrtic and colleagues also concluded that sealants based on ACP-filled methacrylate composites have the potential to remineralize carious enamel lesions not only at the surface but also through the depth of the lesion (41). Meyer and Eanes showed that the solubility of ACP enables it to release supersaturating levels of calcium (Ca2+) and phosphate (P04) ions in proportions favorable for hydroxyapatite formation (42). Further, ACP hinders the colonization of dental surfaces by cariogenic bacteria (43). The extended time-release characteristics of ACP from sealants also may help to prevent demineralization along with remineralization of tooth structure (44). Aegis pit and fissure sealant is the first line of product that contains ACP filler in its formulation. It is an active "smart material" i.e., it releases Calcium and Phosphate ions in the surrounding only when the pH drops below 5.5, and ceases when the pH rises above 5.5. It neutralizes the acid and buffers the pH, has a long life, does not wash out and is non-reliant on patient compliance.

In recent years, biomimetic treatment of early caries lesions by the application of various types of nano-sized hydroxyapatite (n-HAP) has also received considerable attention. n-HAP has been used in toothpaste and pit and fissure sealants for remineralization of incipient caries lesions in-vitro(45,46). 

IIC. Bonding adhesives incorporating nanofillers:
Post operative sensitivity and marginal leakage constitute the most frequent causes of composite restoration failure. Recent reports have suggested that experimental composite adhesives (ECAs) containing silica nanofillers and silver nanoparticles can help prevent enamel demineralization around their surfaces without compromising physical properties(47).

III. Approaches using nanotechnology for biomimetic synthesis of enamel or repair of caries lesion with enamel like nanomaterial
Reconstructing enamel-like structures on teeth have been an important topic of study in the material sciences and dentistry. Mature enamel is acellular, has more than 95% mineral content and does not remodel. It consists of nanorod-like hydroxyapatite (HA) crystals arranged into a highly organized micro-architectural unit called an enamel prism. These special units play an important role in determining the unique physicochemical properties of dental enamel. However, unlike other mineralised structures like dentin and bone, there are no living cells in enamel and thus it has no ability to remodel and once lost, have to be replaced with restorative material. The investigation of in vitro biomimetic synthesis of enamel-like calcium phosphate structures under physiological conditions is therefore essential in dentistry as an alternative dental restorative material.

These approaches introduced for artificial enamel formation have shown the potential to repair enamel surface damage and increase the longevity of teeth (48). Many approaches for synthesis 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 (49-56), or by using various hydrothermal conditions. These approaches for in vitro synthesis of enamel-like hydroxyapatite (HAP) nanorods include: hydrothermal method with controlled release of calcium from Ca–EDTA (57), hydrothermal transformation of octacalcium phosphate (OCP) rod to HAP nanorods in the presence of gelatin (58), surfactant supported HAP self-assembly (54,55), hydrogen peroxide containing calcium phosphate paste (59) and electrolytical deposition taking place at 85 _C (60).

The majority of these synthesis methods were developed under the condition of high temperature, high pressure, and extreme acidic pH or in the presence of a concentrated solution of surfactants.
Potential mechanisms for formation of highly oriented biomineralized structures include
• guided crystal growth on templates,
• aggregation of nanocrystals by organized attachment,
• or the assembly of inorganic nanoparticles mediated by organic scaffolds into aggregated structures.

However, the synthesis of rod-like apatite crystals under physiological temperature is a challenging task. In vitro formation of enamel-like apatite crystals under relatively mild conditions was reported for the first time by Moriwaki et al. [61] in a mineralization device using a cation-selective membrane system. In such a device the direction of calcium ion transport was controlled and the crystals formed in the membrane isolated chamber contained bundles of needle-like OCP and HAP. Using a similar system, Iijima et al. applied a dual membrane device as a model of enamel formation to investigate the function of amelogenin proteins on calcium phosphate mineralization [62]. The co-existence of amelogenin and fluoride (F) was found to be crucial for the organized rod-like apatite crystal formation on the membrane [63].

Amelogenin is a major extracellular matrix protein in the development of natural dental enamel. It aggregates to supramolecular nanospheres and is required for the self-assembly of oriented parallel needle-like apatite bundles [51, 48]. 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. Accordingly, several in vitro attempts have been made using amelogenin to prepare enamel-like materials that contain nano- and microstructures by controlled crystallization of biomimetic calcium and phosphate [62, 65]. 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 (65). 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.

In another approach towards biomimetic synthesis, 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 [53]. Even amino acids, such as aspartic acid, alanine and arginine, could increase the bioactivity of synthetic hydroxyapatite and have been adopted as additives during the formation of biomimetic calcium-deficient hydroxyapatite [56]; amelogenin-like effects were achieved in the presence of glycine. Furthermore, enamel-like microstructures were formed in vitro using sodium bis(2-ethylhexyl) sulphon succinate as a structure-directing agent [52, 54].

Recently, a biomimetic approach has been tested in which single crystalline hydroxyapatite micro-ribbons were used as substitutes for amelogenin templates to control HAP crystallization at biophysical conditions at 37°C [65]. Hierarchical hydroxyapatite structures composed of nanocrystals on the micro-ribbons were synthesized. Thereby, the morphologies and orientations of the formed crystals could be modified by altering the fluoride concentration. The hydroxyapatite crystals change dramatically from disordered aggregations of nanoflakes 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 [65].

CONCLUDING REMARKS:

Nanotechnology has opened new spheres in the field of caries prevention and tooth-surface repair. At this point, to put the present knowledge and available data into a future perspective, one has to assess whether the bio-mimetic nanotechnological approaches for caries therapy will be applicable in daily dental practice. Oral health-care products based on bio-inspired 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. 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. In today’s date, despite promising results from the in vitro experiments, the clinical application of these approaches for restoration of clinically visible cavities in the enamel is not yet conceivable. The biomimetic aggregates lack sufficient stability and mechanical properties for being used as restorations and the formation of the mineral structure often takes from several hours to days. 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.

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