DOI:10.1002/cind.8210_6.x

Emerging technologies to enhance tooth remineralisation, and even to regenerate teeth, could make the visit to the dentist more bearable, Kathryn Roberts reports

In the UK, 2018 NHS data reveal that the single biggest reason for hospital admissions for children under six-years old is for tooth extraction. While the incidence of dental caries has declined in the past 40 years – mainly through widespread fluoridation of drinking water and use of fluoridated toothpastes – clearly there is still scope for improvement.

One of the first places to start is with toothpastes. Most fluoride-containing toothpastes contain sodium fluoride, which is soluble and soon washed away in the mouth. 'After you brush your teeth the fluoride concentration falls off exponentially with time,' says Robert Hill, professor of physical sciences and a specialist in dental materials at Queen Mary University of London. 'After around 90 minutes it will have dropped below the therapeutic level of 1ppm.' This is because saliva continually being produced and swallowed in the mouth quickly washes away the fluoride. Moreover, Hill says, an initial high concentration of fluoride, greater than 1000ppm,

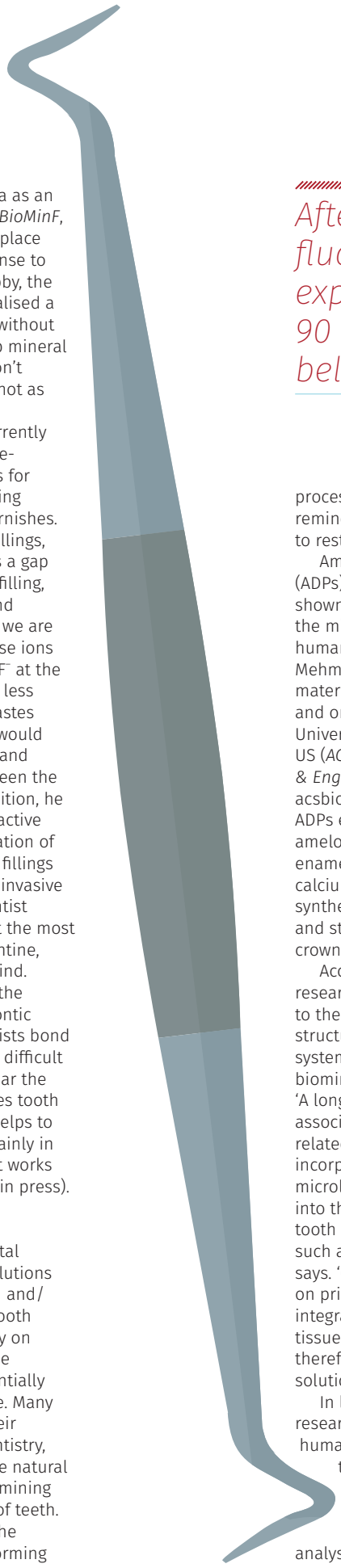
WIDE

results in the formation of CaF_2 rather than tooth remineralising fluorapatite.

Hill and his group have been investigating the use of 'bioactive glasses' in dental treatments. These man-made phosphor-silicate materials can bond to bone and connective tissue, making them suitable for clinical applications in the repair of bones, joints and teeth. Originally developed by Larry Hench, from the department of materials science at Imperial College London, in the late 1960s, as bone grafts and bone substitutes, the glasses degrade slowly upon contact with physiological fluid, making them useful for releasing therapeutic ions into the body.

Hill and his colleagues, and separately GSK, have developed bioactive glasses as additives for toothpaste and gels. Hill's calcium fluorosilicate glass releases fluoride slowly over 10–12 hours (*Dental Materials*, doi:#org/10.1016/j.dental.2017.08.185). 'With calcium and phosphate ions present in saliva, the chance of the stronger fluorapatite forming rather than the calcium fluoride increases significantly', explains Hill. The first step involves ion exchange of Ca^{2+} with H^+ ions in the mouth. As the exchange process proceeds, the pH in the mouth rises, which is advantageous because tooth decay occurs under more acidic conditions. The second stage involves the replacement of hydroxyl ions in the hydroxyapatite (HAp) enamel with F^- ions to form the more robust fluorapatite mineral.

Hill's bioglass was launched in 2016 and sold to companies in



India, the Far East and China as an additive for the toothpaste *BioMinF*, which is now on the marketplace in those countries. In response to the growing antifluoride lobby, the researchers also commercialised a bioglass additive, *BioMinC*, without fluoride to build up the HAp mineral in teeth for markets that won't accept F^-, but Hill says it is not as beneficial as *BioMinF*.

Hill and his team are currently developing different fluorine-containing bioactive glasses for use in dental adhesives, filling materials and protective varnishes. The problem with existing fillings, explains Hill, is that there is a gap between the tooth and the filling, where bacteria can enter and cause decay. 'The materials we are developing will slowly release ions such as Ca^{2+}, Sr^{2+}, PO_4^{3-} and F^- at the site of demand, rather than less specifically through toothpastes or remineralising gels; this would facilitate apatite formation and potentially fill the gap between the tooth and the filling.' In addition, he explains, the use of the bioactive glass, and thus remineralisation of the tooth, would mean that fillings would become a much less invasive procedure, because the dentist would need only to drill out the most decayed soft part of the dentine, leaving the harder part behind.

Hill's team has also put the bioactive glass into orthodontic adhesives. When orthodontists bond metal brackets to teeth it is difficult to remove all the plaque near the brackets and this encourages tooth decay. The bioactive glass helps to prevent this decay and certainly in lab experiments, says Hill, it works very well (*Dental Materials*, in press).

Biomimetic dentistry

In the past 10-20 years, dental research has focused on solutions to prevent demineralisation and/or remineralisation of the tooth structure, and more recently on treatments to regenerate the decaying tooth *in situ*, potentially the Holy Grail of dental care. Many researchers have turned their attention to biomimetic dentistry, looking at ways to mimic the natural processes involved in determining the structure and function of teeth. By taking inspiration from the body's own natural tooth-forming processes, they aim to encourage remineralisation of the dental tissues to restore damaged teeth.

After you brush your teeth the fluoride concentration falls off exponentially with time. After around 90 minutes it will have dropped below the therapeutic level of 1ppm.

Robert Hill *Queen Mary University of London*

Amelogenin-derived peptides (ADPs), for example, have been shown to facilitate the formation of the mineral layer on demineralised human root dentine, explains Mehmet Sarikaya, professor of materials science and engineering and oral health sciences, at the University of Washington in Seattle, US (*ACS Biomaterials Science & Engineering* (doi: 10.1021/acsbiomaterials.7b00959). The ADPs emulate the natural protein amelogenin, the key protein in enamel formation, which captures calcium and phosphate ions, and synthesises and controls the shape and structure of the mineral on the crown and the root of the tooth.

According to Sarikaya, while research to date has contributed to the general knowledge of tooth structure remineralisation, no system has emerged to promote biomimetic remineralisation *in vivo*. 'A longstanding practical challenge associated with demineralisation related to dental diseases is incorporating a functional mineral microlayer, which is fully integrated into the molecular structure of the tooth in repairing damaged tissue, such as enamel and dentine,' he says. 'Our approach is additive based on primary mineralisation which integrates with the existing sound tissue, *eg* enamel or dentine, and therefore facilitates a permanent solution.'

In lab experiments, the researchers demineralised human molars, creating lesions that mimicked white spot lesions. Scanning electron microscopy and energy dispersive X-ray spectroscopy analyses revealed that a 1100ppm F^- sample, found in many commercial toothpastes, did not deliver fluoride to the enamel. A higher F^- concentration of 2000ppm, as found in clinical varnish treatments, formed mainly CaF^2 on the surface. However, in the presence of ADPs, F^- deposited in both F^- – containing samples, forming a thin fluorapatite layer of the order of 1µm thickness. Interestingly, an ADP-only test sample resulted in remineralisation of a 10–50µm-thick layer containing HAp, which is similar to the structure of healthy enamel. In addition, the newly formed mineral layer was shown by SEM to be integrated into the underlying enamel.

The peptide-guided remineralisation approach, say the researchers, 'sets the foundation for future development of biomimetic products and treatments for dental health care'.

Other approaches by different research groups involve secondary mineralisation, Sarikaya says – *ie* deposition of mineral precipitates, or use synthetic materials – that don't produce integrated interfaces with the existing tissue and are therefore temporary.

More recently, Sarikaya's group reported an animal model for creating dental cavities in rats to evaluate their remineralisation strategies *in vivo* (*Journal of Dental Research*, doi: 10.1177/0022034518789898). They are currently testing the efficacy of their approach and developing a variety of formulations to target specific dental problems and move towards commercial products.

Meanwhile, Vivek Kumar and his team in the department of biomedical engineering at the New Jersey Institute of Technology, US, are working on an innovative technology based on self-assembling peptides

to keep a tooth alive after root canal treatment. The removal of soft living tissue, or dental pulp, which comprises nerves and blood vessels, is done to relieve the agony of an infection but results in a dead tooth. Despite the lengthy and invasive procedure, in which tiny rubber rods (gutta percha) replace the pulp, the remaining tooth is still susceptible to re-infection and, ultimately, could require extraction.

Kumar and his team are developing a hydrogel that could be injected into the pulp chamber in place of the gutta percha. This material would stimulate both angiogenesis, the growth of new blood vessels, and dentiogenesis, the growth of dental pulp stem cells within the tooth.

Kumar had previously made a hydrogel scaffold from self-assembling peptides that stimulated angiogenesis when placed under rodent skin, or in low blood flow areas, and which continued working for three months. The scaffold comprised a 31 amino acid polypeptide, similar to collagen, and incorporated a variety of biological moieties, including growth factors and cell-signalling proteins. 'This ability to incorporate such molecules into the scaffold, rather than adding them at a later stage, is unique to this system,' explained Kumar, speaking at the ACS meeting in Boston in August 2018. In addition, the peptides self-assemble through non-covalent bonds which break and form easily, allowing the researchers to inject or spray the peptides to the required location where they reform as the hydrogel.

Recently, by adding another peptide domain to the hydrogel scaffold – a protein that encourages dental pulp stem cells to proliferate – the researchers found, in lab experiments, that not only do the stem cells grow but they deposit calcium phosphate crystals. However, when they injected this gel under the skin of rodents, it degraded within one to three weeks, so they have redesigned the peptide backbone of the gel to make it more stable. They are now injecting this hydrogel into the teeth of dogs that have undergone root canal treatment to find out whether it will stimulate pulp regeneration in a living animal.

The team is also working on a version of the hydrogel that contains antimicrobial peptide domains. 'Instead of having to go in and rip out everything inside the tooth, the dentist could go in with a smaller drill bit, remove only a little bit of pulp and inject our hydrogel. The antimicrobial portion of the peptide would kill the infection, preserving more of the existing dental pulp while helping to grow new tissue.' Ultimately, says Kumar, they want to create a responsive tooth rather than one that decays and dies, and importantly a less invasive procedure at the dentist.

Decay and fluoridation

Dental decay is caused when acids dissolve tooth enamel – mainly hydroxyapapite (Hap, $Ca_{10}(PO_4)_6(OH)_2$) plus traces of protein and peptide – and the underlying dentine, comprising collagen and other insoluble proteins. The acid is produced by bacteria that live in the sticky deposit or plaque formed on teeth and gums after eating food. Early signs of caries are white spot lesions (WSL) and hypersensitivity. These WSL diffuse into the surface enamel and eventually bore through to the dentine layer, which is living tissue and therefore the target of bacteria. The decay 'balloons' when it reaches the dentine, which further undermines the surface layer of the enamel, and is most serious when it reaches the pulp containing the blood vessels and nerves.

Brushing teeth regularly removes most of the plaque. The rate at which the mineral phase – HAp – grows is affected by the presence of fluoride ions, increasing when fluoride ions are present. Fluoride ions can replace some of the hydroxyl ions in HAp, which strengthens the existing mineral or forms a new mineral phase, fluoroapatite, which is more resistant to plaque acids.

Fillings in the form of dental amalgam – a combination of silver, tin and mercury plus other metals – are used to prevent the spread of caries. White fillings, for the front teeth, are based on addition polymers, commonly bisGMA (bisphenol A-glycidyl methacrylate), filled with silica powders.

But fillings don't last forever; they too are prone to acid attack and eventually fall out. This is mainly because the amalgams and polymer composites do not integrate with the existing tooth structure. This results in small gaps where bacteria can get in and do their worst. The outcome for the patient is often more invasive treatment, either to repair broken teeth, root canal treatment to save a tooth, or extraction of the infected teeth. In addition, there is currently a big push from various bodies, including the World Health Organization and the EU, to replace amalgam fillings because of their Hg content. While the risk to patient and dentist is minimal, 80–90% of people in the UK are cremated on death, releasing toxic Hg vapours into the environment.