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Development of dental composites with reactive fillers that promote precipitation of antibacterial-hydroxyapatite layers

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ABSTRACT

The study aim was to develop light-curable, high strength dental composites that would release calcium phosphate and chlorhexidine (CHX) but additionally promote surface hydroxyapatite/CHX co-precipitation in simulated body fluid (SBF). 80 wt.% urethane dimethacrylate based liquid was mixed with glass fillers containing 10 wt.% CHX and 0, 10, 20 or 40 wt.% reactive mono- and tricalcium phosphate (CaP). Surface hydroxyapatite layer thickness/coverage from SEM images, Ca/Si ratio from EDX and hydroxyapatite Raman peak intensities were all proportional to both time in SBF and CaP wt.% in the filler. Hydroxyapatite was, however, difficult to detect by XRD until 4 weeks. XRD peak width and SEM images suggested this was due to the very small size (~10 nm) of the hydroxyapatite crystallites. Precipitate mass at 12 weeks was 22 wt.% of the sample CaP total mass irrespective of CaP wt.% and up to 7 wt.% of the specimen. Early diffusion controlled CHX release, assessed by UV spectrometry, was proportional to CaP and twice as fast in water compared with SBF. After 1 week, CHX continued to diffuse into water but in SBF, became entrapped within the precipitating hydroxyapatite layer. At 12 weeks CHX formed 5 to 15% of the HA layer with 10 to 40 wt.% CaP respectively. Despite linear decline of strength and modulus in 4 weeks from 160 to 101 MPa and 4 to 2.4 GPa, respectively, upon raising CaP content, all values were still within the range expected for commercial composites. The high strength, hydroxyapatite precipitation and surface antibacterial accumulation should reduce tooth restoration failure due to fracture, aid demineralised dentine repair and prevent subsurface carious disease respectively.

1. Introduction

Dental composites have been used for over 50 years as restorative materials [1]. Compared to dental amalgam, they trigger less safety concerns and provide improved aesthetics. Over the years there has been a significant increase in mechanical properties of commercial resin-based filling composites enabling a reduction in failure due to fracture and wear. Polymerisation shrinkage and lack of anti-bacterial activity, however, are continuing issues as they enable micro-gap formation between the tooth and restoration followed by bacterial microleakage. These bacteria can cause continuing disease and de-mineralisation of dentine underneath a restoration. Subsequent action by matrix metalloproteinases (MMPs) then degrades the demineralised dentinal collagen further widening the micro-gap. Recurrent caries is now the major reason for the shorter median survival lifespan (5–6 year) of composites in comparison with more antibacterial dental amalgam (13 years) [1–4]. Dental composites are typically composed of four major components: an organic polymer matrix (produced from dimethacrylate monomers such as UDMA, BisGMA, TEGDMA), inorganic fillers (e.g. glass, ceramic), coupling agents and the initiator–accelerator system. Much work has focussed upon varying these components to reduce shrinkage and improve mechanical properties [5–8]. To prevent bacterial microleakage, however, new components are additionally required to promote remineralisation (e.g. through calcium and phosphate release) and antibacterial action.

In the past 20 years a wide range of calcium phosphates (CaP) such as hydroxyapatite (HA) [9–11], amorphous calcium phosphates (ACP) [12–15], tetracalcium phosphate (TTCP) [16] and mono- and dicalcium phosphates (MCPM and DCPA) [17–19] have been studied as fillers in an attempt to produce calcium and phosphate — releasing dental composites. Both nano-sized and micro-sized HA particles have been investigated with the latter tending to give higher mechanical properties [9,17]. Acidic coupling agent optimisation could improve flexural strength but a maximum of only ~70 MPa was achieved [9,10]. ACP filled composites were shown to release calcium and phosphate at levels dependent upon the amount added to the formulations [20]. The biaxial flexural strength could be increased to ~75 MPa through hybridisation of the ACP with other elements (e.g. silicon and zirconium) but was still generally half that for base resin [13–15,21]. Initial low strengths

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could be attributed in part to poor dispersion and insufficient interaction between ACP and resin but might also be caused by the generation of pores upon component release and increased water sorption after water storage. The strength of TTCP filled composites was increased from ~50 to 100 MPa upon replacing 50% of the TTCP by silicon nitride whiskers. Calcium and phosphate release, however, was decreased by an order of magnitude [16]. Similar effects were observed with MCPM/whisker composites [17]. Replacing MCPM with less soluble DCPA increased strength but drastically reduced calcium phosphate release [17,20]. Furthermore, the addition of whiskers compromised optical properties preventing light cure feasibility.

More recently, reactive acidic and basic mono- and tricalcium phosphate fillers (MCPM/β-TCP) have been added together in dental composites [22]. The β-TCP enabled more control over the MCPM dissolution and composite water sorption. Highly soluble MCPM on the surface of the material dissolved but in the bulk it reacted with the β-TCP to form less soluble, water-binding brushite (dicalcium phosphate dihydrate) crystals. The strengths of these composites were subsequently improved through partial replacement of the reactive fillers with reinforcing fillers but these again compromised optical properties [23].

In the above studies, remineralisation potential was generally assessed through calcium and phosphate release determination [24]. Predicting the release levels required to promote remineralisation, however, is complex and dependent upon many other parameters. A dental restoration that promotes HA deposition could in addition to providing remineralisation of adjacent collagen, potentially also enable closure of gaps between the material and tooth and reduce bond deterioration over time. In this study, therefore, remineralisation potential was evaluated through calcium and phosphate release and their precipitation as HA layer on the surface. Some dental Portland cements, adhesives and ceramics have shown hydroxyapatite precipitates on their surfaces in simulated body fluids (SBF) [25–28]. In one study it was shown they could also re-mineralise adjacent human dentin [28].

Factors increasing rates of HA precipitation on the surface of a material include raised SBF supersaturation, pH and temperature. Material surface chemistry has also been shown to be important (e.g. by providing nucleation sites) [29–32]. In these studies, SEM, EDX, Raman, FTIR and XRD have all been employed to assess the hydroxyapatite precipitates. These studies, however, were largely only semi-quantitative. In addition material mass changes have been monitored to provide quantitative results. Such gravimetric methods are complicated in composites studies, however, because of large composite changes in mass upon water sorption and component release.

To provide anti-bacterial action, various agents including fluoride [20,33] and chlorhexidine (CHX) [15,34] have been added to composites. There is conflicting evidence over whether the addition of fluoride in commercial composites has any clinical benefit [24,35]. CHX was added into various experimental dental composites due its low minimum inhibitory concentrations against oral bacteria and ability to inhibit MMPs [34,36]. Composites with early release of chlorhexidine might reduce the need for extensive caries affected tissue removal as advocated in modern tooth restoration procedures [37]. The CHX, however, is not readily released from the bulk of conventional composites. This problem has been solved through combining CHX with reactive MCPM/β-TCP [23].

The aim of this study was therefore to develop methods that provide a quantitative assessment of any hydroxyapatite layer on the surfaces of systematically varying new MCPM, β-TCP and CHX-containing light curable composites. In addition, this study will assess if these new materials also have high CHX release and enhanced mechanical strengths. The precipitated HA-CHX layer was thoroughly investigated to provide a deep understanding to factors that determines precipitation kinetics. This study not only aims to form interactive composites that promote precipitation of HA-CHX layer, but also provides a detailed explanation to the nature of the precipitated layer and how it was formed. Furthermore, it is known that hydroxyapatite can promote the precipitation of chlorhexidine from solution [38]. This study will therefore address, how the formation of the HA layer affects the release of CHX and whether any of this antibacterial can be entrapped with the HA to potentially enable a long-term antibacterial restoration/dentine interface.

2. Materials and methods

2.1. Composite paste preparation

In this study, urethane dimethacrylate (UDMA, Esstech) was used as the base monomer. Triethylene glycol dimethacrylate (TEGDMA, Esstech) and hydroxyethyl methacrylate (HEMA, Esstech) were added as diluents and camphorquinone (CQ, Sigma-Aldrich)/dimethylparatoluidine (DMPT, Sigma-Aldrich) as initiator/activator respectively. UDMA:TEGDMA: HEMA:CQ: DMPT was 68:25:5:1:1 by weight.

The composite filler consisted of radiopaque barium–alumino–silicate glass with an average particle diameter of 7 μm (1 to 20 μm diameter range by SEM) (DMG, Hamburg, Germany). Its refractive index (0.52) matched well that of the monomer phase (0.48) to enable good depth of cure. Chlorhexidine diacetate salt hydrate (CHX, Sigma-Aldrich) and borosilicate glass fibres (15 μm diameter × 300 μm length) (MO-SCI Healthcare L.L.C. Rolla, USA) levels were fixed at 20 and 10 wt.% of the total filler respectively. Reactive calcium phosphate (CaP, equal masses of β-tricalcium phosphate (β-TCP, Plasma Biotal) and monocalcium phosphate monohydrate (MCPM, Himed)) levels were 0, 10, 20 or 40 weight % (wt.%) of the filler. The base powder phase therefore contributed 30, 50, 60 or 70 wt.% of the filler. Powder and liquid phases were combined at a ratio of 4:1.

2.2. Composite disc preparation

To prepare disc-shaped specimens, pastes were placed in metal rings (1 mm deep and 10 mm internal diameter), covered top and bottom with acetate sheet and light cured with blue light (Demi Plus, Kerr) with 1100 mW/cm^2 output for 40 s top and bottom. This long cure time ensures maximum polymerisation of the whole disc and greater than 70% conversion irrespective of formulation as assessed by FTIR. The resultant composite discs were removed from the moulds and their edges polished with 1000 grit paper to remove any loose chips. They were subsequently stored dry in sterilin tubes overnight before testing or immersion either in water or a simulated body fluid (SBF) (as in ISO 23317:2007).

2.3. Characterisation of hydroxyapatite deposition

The morphology, elemental composition, chemical changes, crystallinity and mass of any deposited layer on the surface of composites were assessed after immersion in 10 ml of water or SBF for periods ranging from 1 day up to 12 weeks using the techniques below. During the storage periods the solutions were left unchanged to mimic accumulation of components as might occur underneath a sealed tooth restoration.

2.3.1. Scanning electron microscopy and energy dispersive X-ray analysis

To assess the morphology and elemental composition of the precipitated layer, scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis was employed. Specimens stored for 1 day or 1, 2, 3, 4 and 8 weeks were mounted onto stubs with fast setting epoxy adhesive. The mounted specimens were then sputter coated using gold and palladium alloy. All SEM images were captured at 5 kV accelerating voltage using a Scanning electron microscope (Phillip XL-30, Eindhoven, The Netherlands) and INCA software.

EDX analysis was performed using an Inca X-sight 6650 detector (Oxford Instrument, UK) at 20 kV accelerating voltage to quantify the average and homogeneity of calcium versus silicon content of the