The effect of surface treatment of hydroxyapatite on the properties of a bioactive bone cement

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Bioactive bone cements based on a paste–paste system for orthopaedic applications have been developed. They consist of hydroxyapatite (HA) filler particles in a methacrylate matrix comprising urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA). To improve the interface between inorganic filler and organic matrix the HA particles were subjected to two different surface treatment methods, using polyacrylic acid (PAA) and γ–methacryloxypropyltrimethoxy silane (γMPS). The aim of the present study was to determine the influence of surface treatment on the mechanical properties, namely compressive strength (CS), diametral tensile strength (DTS) and three-point flexural strength (FS) of the cements and the effect of ageing in simulated body fluid (SBF). Comparing the mechanical properties of the two cements after fabrication, the γMPS–HA cement showed higher strength values for all tests conducted (CS = 185 ± 19.6 MPa, DTS = 27 ± 2.5 MPa, FS = 50.2 ± 4.9 MPa), whereas PAA–HA containing cement had strength values around 20% lower. However, poly(acrylic acid) surface treatment was found to be more effective in improving the interface, and PAA–HA cements maintained their mechanical properties after immersion in SBF whereas γMPS–HA cement showed a reduction in strength values post ageing. From the results of this study, it is concluded that PAA treatment of the HA filler is a viable alternative to silanation with γMPS which may provide increased durability in aqueous environments.

1. Introduction

The incorporation of bioactive fillers, such as hydroxyapatite (HA), bioactive glass or glass–ceramic particles into a methacrylate matrix as an alternative to poly(methylmethacrylate) (PMMA) bone cement was first documented by Hennig *et al*. in 1979 [1]. Since then many research groups have looked at developing bone cements containing HA or bioactive glass as inclusions [2, 3]. The rationale of incorporating a bioactive filler into PMMA cement is two-fold: on the one hand biocompatibility as well as osteoconductivity and interlocking with bone are enhanced, on the other hand mechanical properties, such as wear resistance, strength and hardness can be improved. Other advantages include the decrease in polymerisation exotherm and shrinkage. The ability of osteoblast attachment and proliferation on a PMMA/HA composite with preferential attachment to HA rather than the PMMA matrix has also been demonstrated [4–6]. Nevertheless, small additions of HA have resulted in a lack of bioactivity whereas large additions (60% by weight) had an adverse effect on the handling properties of the material [2]. The PMMA is itself a brittle material, hence further reinforcement with ceramics may embrittle it further [7], especially if the microstructure of the material and the interface between reinforcement and matrix are not optimised.

An orthopaedic cement with improved mechanical and biological properties by combining ceramic particles and high molecular weight monomers such as Bisphenol-A-glycidylmethacrylate (BisGMA) has also been reported in literature [8, 9]. A clinical study by Raveh *et al*. [8] in 1982 found that a titanium made alloimplant containing BisGMA based bioactive cement placed in the mandible of rhesus monkey was completely covered with new bone in a continuous layer after 12 months. Yamamuro *et al*. [9] reported a successful clinical trial with a BisGMA-based bioactive bone cement containing HA particles, however, poor ductility and rigidity were reported in some cases [9].

The interface between the inorganic filler and organic matrix in a particle-filled composite plays an important role as it influences the mechanical properties of composites [10]. Methacrylate-functionalised silanes, for example, γ–methacryloxypropyltrimethoxysilane (γMPS), are commonly used to create covalent bonds between filler and matrix. Various studies have demonstrated that the improved adhesion between filler and matrix resulted in enhanced mechanical properties of the

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cement [11–14]. The silanation of HA particles also reduces the amount of monomer required to sufficiently wet the filler, hence larger amounts of filler can be incorporated without compromising the handling properties. The hydrophobic nature of the silane improves the water resistance of the composites [11, 15] however, there is also some concern regarding the durability of the coupling agent in aqueous environments [16]. Poly(acrylic acid) (PAA), a water soluble ionic polymer, has also been shown to function as an effective coupling agent and to improve the properties of nano-sized HA–PEG/PBT composites [17].

In the present study, PAA and γMPS have been used to surface treat hydroxyapatite and as a filler for the bone cements based on methacrylate monomers. As a novelty, a urethane dimethacrylate/triethylene glycol dimethacrylate (UDMA/TEGDMA) matrix was used which has a lower viscosity than BisGMA thus facilitating incorporation of HA. The aim of the present study was to determine the influence of change of one compositional variable, namely the surface treatment of the filler, on the mechanical properties of the composite cements. The HA surface treatment methods were optimised and a direct comparison between the two methods was carried out in terms of mechanical properties of the bioactive bone cements achieved. The mechanical properties were also determined post ageing in an aqueous environment to assess the effect of the two different surface treatments of HA.

2. Materials and methods

2.1. Materials

The sintered HA powder with a mean particle size of 7 μm was used as received from Plasma Biotal UK. The monomers urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) were supplied by ESSTECH Corp. USA. The initiator benzoyl peroxide (BPO), the activator *N*,*N* dimethyl-*p*-toluidine (DMPT) and the PAA (with an average MW of 5100, 50% water solution) were procured from Sigma-Aldrich and used without further purification. The γMPS was supplied by Witco Europe.

2.2. Surface treatment of HA filler particles with γMPS

The silanation process using γMPS was conducted as described earlier [11]. For the optimised silane treatment, 12.5 wt % γMPS was dissolved in a 70 : 30 mix of acetone and water and the HA particles were added. The mixture was heated to 40 °C for 3 h under constant magnetic stirring, followed by further treatment at 60 °C for 5 h. The mixture was then transferred to an oven at 120 °C for 2 h to evaporate excess water and solvent. The powder was left in an oven at 40 °C for 48 h and stored in a desiccator prior to use.

2.3. Surface treatment of HA particles with PAA

For the optimised PAA surface treatment, 40 g of HA powder were transferred into 900 ml of 2 mM PAA sodium salt (MW = 5100) solution with the pH adjusted to six using 1 M HCl and stirred for 24 h. The pH of the suspension was then reduced to five and washed with ethanol to remove unabsorbed PAA. The HA powder was then washed with acetone and dried at 40 °C for 24 h.

2.4. Preparation of the composites

A paste–paste system previously developed was used, one paste containing the initiator benzoylperoxide (BPO) and the other the activator DMPT. Each paste consisted of equal quantities of UDMA (25 wt %) and TEGDMA (15 wt %) and HA treated with PAA or γMPS (60 wt %). After the monomer constituents had been mixed, the treated HA powders were added in small portions. The cement samples were fabricated by mixing equal amounts of each paste.

2.5. Mechanical testing

Mechanical testing of the fabricated specimens, including compressive strength (CS), diametral tensile strength (DTS) and three-point flexural strength (FS) were performed using an Instron Universal Testing Machine (1193) with a crosshead speed of 5 mm min^{-1}. CS/DTS specimens were prepared in stainless steel cylindrical moulds of 6 mm height and 4 mm diameter. Rectangular bar specimens of dimension (length = 25 mm, width = 2 m and thickness = 1 mm) were prepared for flexural strength determination. A three-point bend test was carried out for both as-fabricated and aged samples. The two types of cements were tested in dry conditions (24 h after preparation) and after immersion in simulated body fluid (SBF) for six weeks. The specimens were immersed in SBF at 37 °C for up to six weeks in static conditions. Six specimens were tested per each condition and a one-way analysis of variance was performed for the analysis of results.

2.6. Investigation of fractured surfaces before and after immersion in SBF

The scanning electron microscope (SEM) (Oxford Instruments) was used to study the fractured surfaces of samples after mechanical testing before and after immersion in SBF. The samples were gold coated for scanning electron microscopy.

3. Results

3.1. Characteristics of HA particles

The SEM micrographs (Fig. 1(a)–(c)) show the as-received, silane treated and PAA treated HA powders. There was a negligible change in particle size after surface treatment of the HA particles. While silanation of the powder resulted in rounder and smoother particles (Fig. 1(b)), the PAA surface treatment resulted in a more porous structure especially for the larger HA particles as shown in Fig. 1(c).

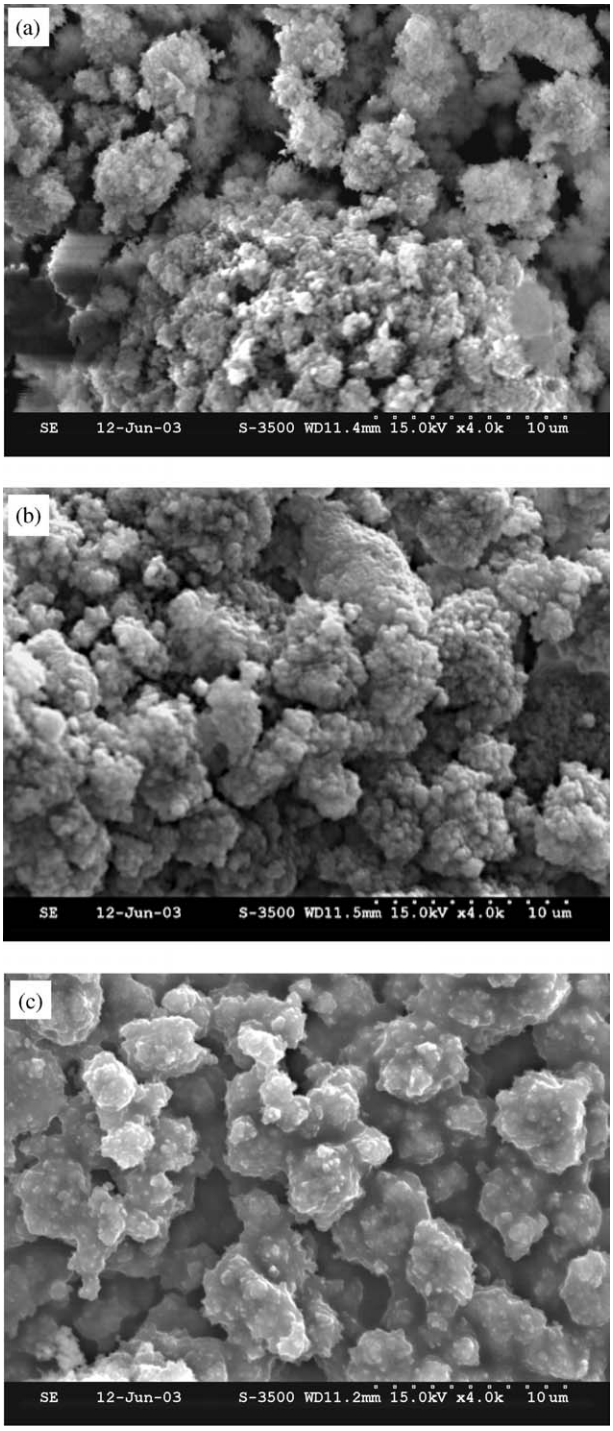

Figure 1 (a) HA particles; (b) HA particles treated with γMPS; (c) HA particles treated with PAA.

3.2. Mechanical testing

3.2.1. Compressive strength, diametral tensile strength and flexural strength

The average CS of the two cements are shown in Fig. 2. A one-way ANOVA analysis was conducted which showed that there was a statistically significant difference ($p < 0.001$) between γMPS–HA and PAA–HA cements when tested in dry conditions, whereas no statistically significant difference was found post incubation in SBF for six weeks for the two cement groups. A statistically significant lowering ($p < 0.050$, Tukey test) in the CS was observed between as-fabricated and wet samples for the γMPS–HA cements, however, no significant differences were observed for the PAA–HAA cements. The PAA–HA cements exhibited lower DTS values in comparison to the γMPS–HA ($p < 0.050$, Tukey test) cements, however, no statistically significant difference were observed post immersion in the two groups as shown in Fig. 3. Fig. 4 shows the flexural strength of cements with γMPS–HA and PAA–HA cements. In dry conditions, PAA–HA cements had statistically significant lower strength ($p < 0.05$, Tukey test) values in comparison to the γMPS–HA cements, however after storage in SBF the PAA–HA cements were able to maintain their strength.

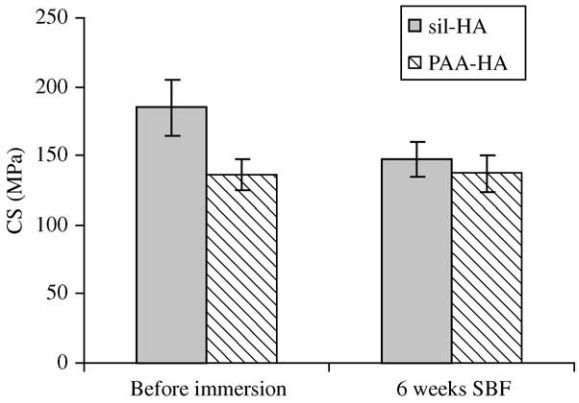

Figure 2 Compressive strength of bioactive cements containing HA particles pretreated with A174 and PAA before immersion and after immersion in SBF for six weeks.

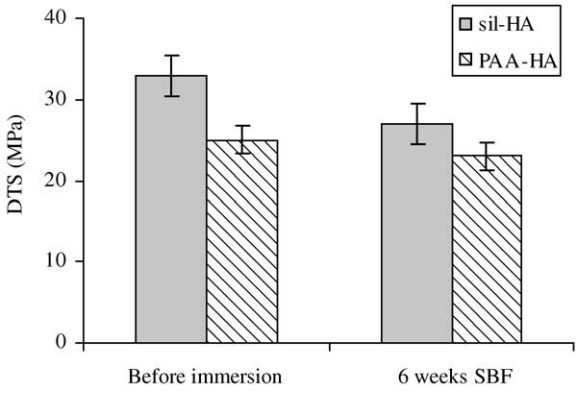

Figure 3 Diametral tensile strength of bioactive cements containing HA particles pretreated with A174 and PAA before immersion and after immersion in SBF for six weeks.

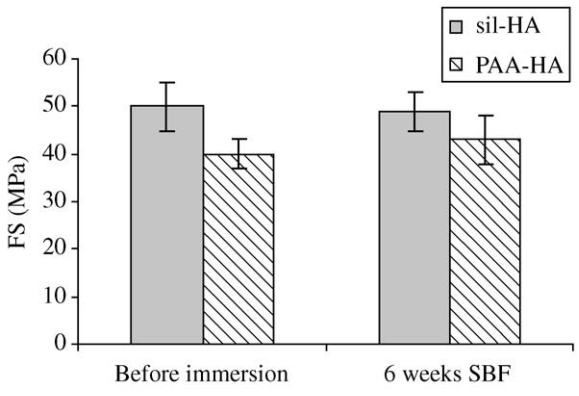

Figure 4 Three-point bend strength of bioactive cements containing HA particles pretreated with A174 and PAA before immersion and after immersion in SBF for six weeks.

3.3. Fractography study by SEM

Figs. 5 and 6 show the fracture surfaces of the dry PAA–HA and γMPS–HA, respectively. The as-fabricated PAA–HA composites had a very different appearance from the γMPS–HA composite. The PAA–HA particles appear to be uniformly embedded within the polymeric matrix which is clearer at a higher magnification (Fig. 5(b)). The fractured surfaces of the PAA–HA cement were relatively flat compared to the γMPS–HA cement surfaces which were noticeably rougher. The analysis of the fracture surface of the γMPS–HA cements showed that the particles were evenly distributed in the matrix and pull-out of HA particles was apparent (Fig. 6(a)). The HA–PAA particles appeared well bonded (Fig. 7(a), (b)) to the matrix in the cement aged in SBF in comparison to the γMPS–HA cements (Fig. 8(a), (b)).

4. Discussion

The surface treatment of HA was carried out using a dilute solution of PAA at a pH of six and excess acid was removed. Subsequent analysis with SEM showed that an interaction had occurred between PAA and HA. The interaction of polyalkenoic acids such as PAA with HA can be explained on the basis of either diffusion of the poly acid into the surface of HA particles or via the formation of ionic bonds with calcium present within the HA. Recently, Fukada *et al*. [18] showed that copolymers of acrylic–maleic acid were self adhesive to hydroxyapatite through the formation of ionic bonds between the carboxyl group and calcium present in HA. The extent of the interaction of the PAA depends on the concentration of the polyacid and pH of the reacting medium. However, in the present study, the effect of PAA treatment caused a minimal change in the particle size of the HA powder. The treatment of HA powder with γMPS resulted in rounder and smoother filler particles that could be more easily incorporated into the matrix compared to untreated HA filler. The better wetting of the filler particles by the organic phase is typically due to the silanation of the HA surface.

The use of PAA as a coating is interesting due to the

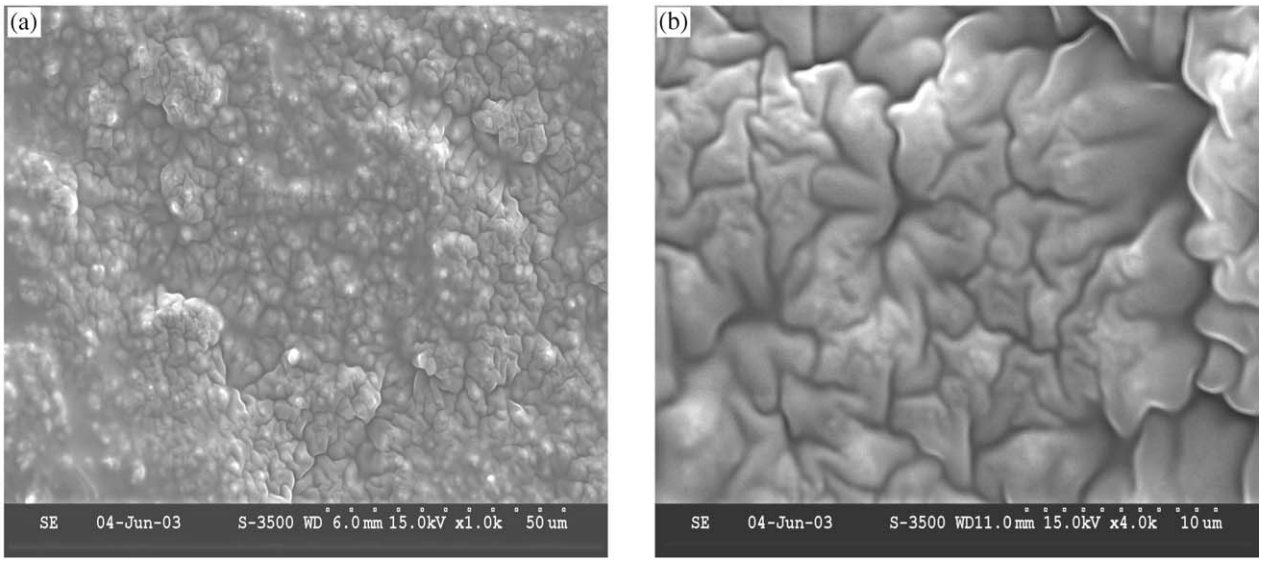

Figure 5 (a) Fractured surface of a dry sample of PAA–HA × 1 K; (b) same image at × 4 K.

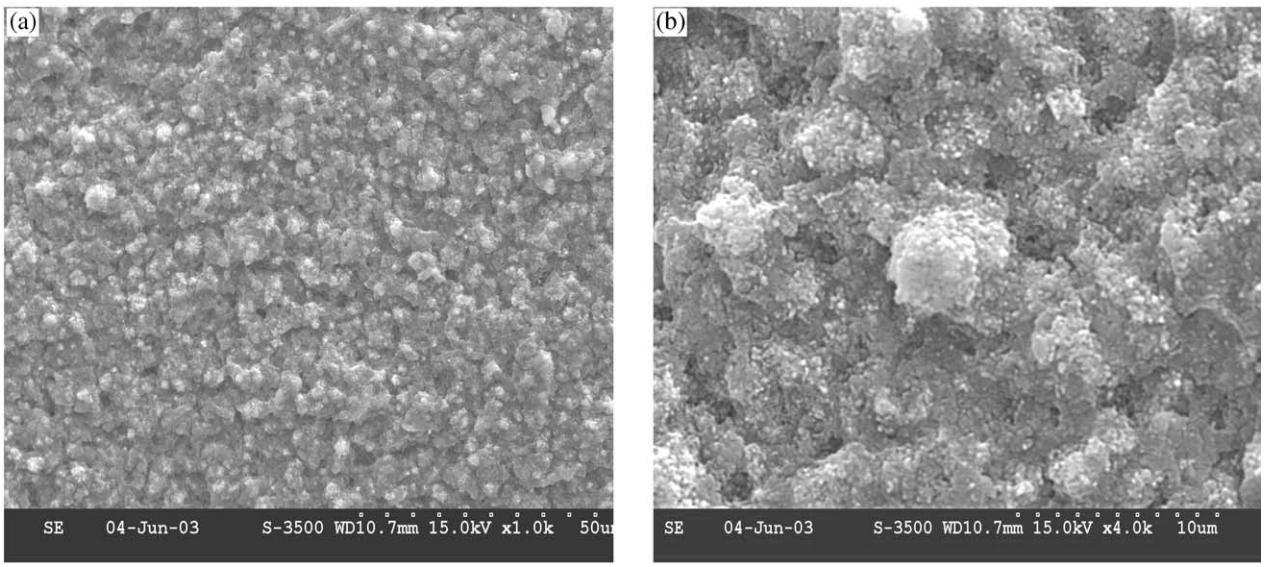

Figure 6 (a) Fractured surface of a dry sample of γMPS–HA × 1 K; (b) same image at × 4 K.

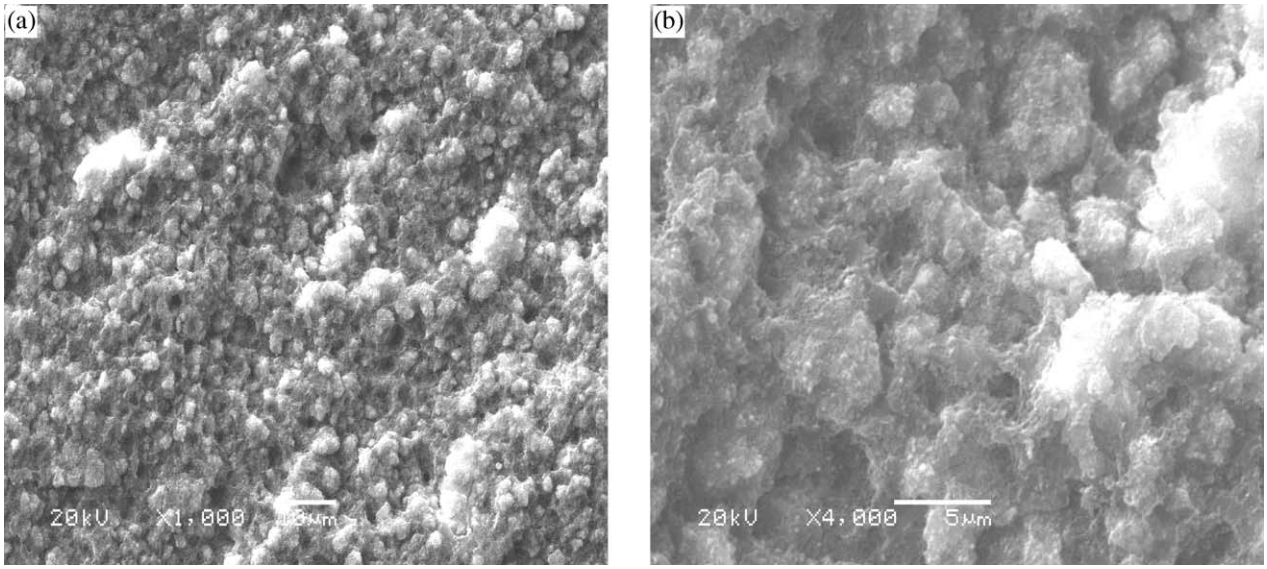

Figure 7 (a) Fractured surface of PAA–HA cement after immersion in SBF for six weeks (× 1 K); (b) same image at 4 K.

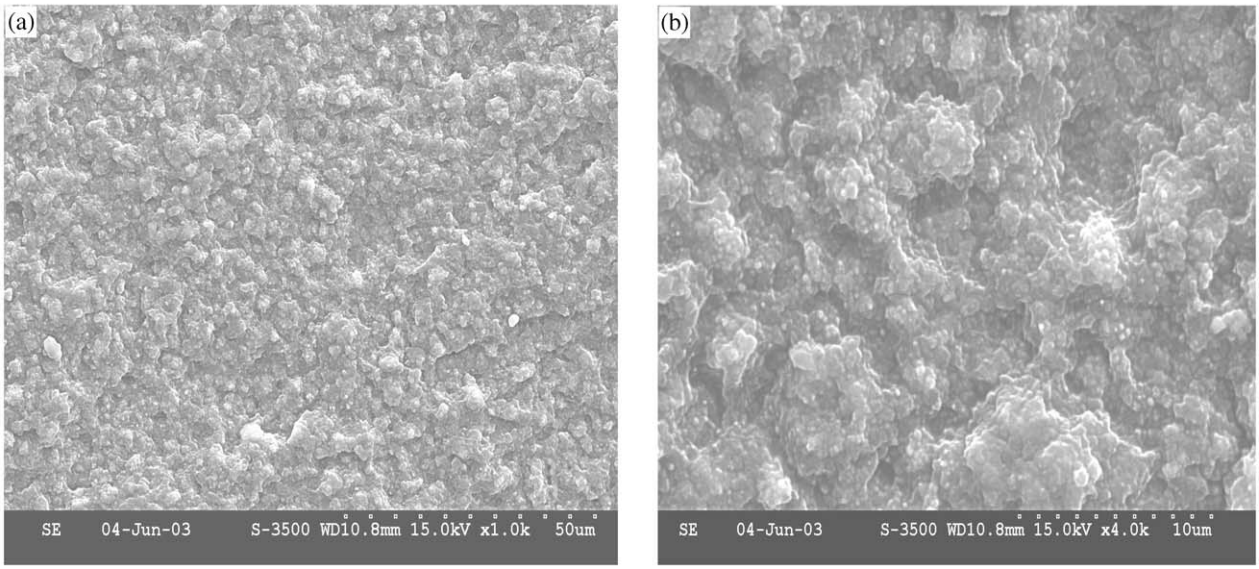

Figure 8 (a) Fractured surface of γMPS–HA cement after immersion in SBF for six weeks (× 1 K); (b) same image at 4 K.

fact that PAA can be easily adsorbed onto the surface of HA via ionic interactions between the calcium ions in the HA and the carboxylic group present in PAA. The ionic interaction between the HA and PAA causes the polyacrylic acid to uniformly coat the surface of the particles such that the acrylate units are oriented towards the surface. The addition of the PAA–HA particles to the UDMA/TEGDMA monomer then results in effective wetting of the surface by the monomers. Subsequent polymerisation leads to the formation of the polymeric chains with the particles embedded within the matrix. The stiffening effect thus is not as pronounced as in the case of γMPS–HA where the HA particles are not embedded within the matrix. This can also be observed from the SEM micrographs as shown in Figs. 5 and 6.

The interface between the filler particles and matrix plays an important role in determining the ultimate mechanical properties of particle-reinforced polymer composites. For all mechanical properties investigated, that is, CS, DTS and FS strength, the as-fabricated specimens exhibited higher values for γMPS–HA cements than for PAA–HA cements. The improved properties of the dry cements containing the silanated HA can be explained on the basis of the presence of the sileceous coating on the filler particles which allow the hydrophobic methacrylate matrix to wet the surface effectively.

However, when tested in an aqueous environment the PAA coupled HA composites were better able to maintain their mechanical properties in comparison to the γMPS–HA composites. It has been proposed that silane bonds are known to degrade with time; according to Soderholm's hydrolysis theory silane coupling agent may degrade due to water dissolution, leading to loss of filler and subsequent acceleration of wear. Lin *et al*. [16] showed that in a BisGMA/TEGDMA matrix reinforced with silanated fillers the degradation in DTS values increased with increasing amount of silanated filler in a 75% ethanol solution, whereas for unsilanated fillers the degradation in DTS values was less pronounced. In our investigation, although for as-fabricated specimens the treatment with silane coupling agent enhanced the

mechanical properties in comparison to treatment with PAA, the results for the mechanical properties post ageing in SBF indicated that the interface of PAA–HA cements was less sensitive to degradation by moisture.

The analysis of the fracture surfaces of the present composites showed significant differences for the PAA–HA and γMPS–HA cements. The PAA–HA cements exhibited a more intimate contact between particles and matrix and the absence of discreet HA particles in the as-fabricated specimens can probably be explained on the basis of the ionic interaction of PAA and HA, allowing the monomer to provide a coverage on the particulate surface that on polymerisation forms a network with the particles embedded within the matrix. The appearance of HA-like particles on the fractured surfaces of the specimens aged in SBF could arise from the formation of hydroxyl carbonate apatite due to the interaction with SBF, however, further investigation is required to confirm these findings.

5. Conclusions

Composites based on urethane dimethacrylate containing hydroxyapatite were prepared for application as orthopaedic bone cements. The surface modification of hydroxyapatite particles using PAA or γMPS was demonstrated to be an effective way to improve the interface between the polymer and HA. The present findings demonstrate that when HA was surface treated with PAA, the cured cement was more suitable to maintain the mechanical properties after storage in an aqueous environment.

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