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Hydroxyapatite as a filler for dental composite materials: mechanical properties and *in vitro* bioactivity of composites

C. SANTOS[1], Z. B. LUKLINSKA[2], R. L. CLARKE[3] K. W. M. DAVY[3]*
[1]St. Bartholomew's and the Royal London School of Medicine and Dentistry, Department of Biomaterials in Relation to Dentistry, Queen Mary and Westfield College, University of London, Mile End Road, London E1 4NS, UK
[2]Materials Department/IRC in Biomedical Materials, Queen Mary and Westfield College. University of London, Mile End Road, London E1 4NS, UK
[3]IRC in Biomedical Materials, St. Bartholomew's and the Royal London School of Medicine and Dentistry. Department of Biomaterials in Relation to Dentistry, Queen Mary and Westfield College, University of London, Mile End Road, London E1 4NS, UK

Hydroxyapatite (HAp) powder was treated with γ-methacryloxypropyltrimethoxy-silane (γ-MPS) using standard techniques in both non-polar and polar systems. Infrared spectra (DRIFT) and thermogravimetric analysis (TGA) confirmed the presence of γ-MPS on the surface of the HAp filler particles. Series of experimental composites consisting of bisphenol-a-glycidyl methacrylate (BisGMA) based resin and untreated or treated HAp filler were produced to determine the mechanical properties and *in vitro* bioactivity. The incorporation of HAp filler into the BisGMA base resin had an enhancing effect on the flexural strength and Young's modulus of the base resin, the latter being increased by a factor of three. The mechanical properties of the filled resin were not affected by the surface treatment of the HAp, but filler loading was found to have a significant effect on Young's modulus. Higher proportions of silane-treated HAp of smaller particle size could be incorporated in the monomer phase giving rise to composites of higher stiffness. Examination of the fracture surfaces showed that the silanized HAp particles maintained better contact with the polymer matrix. *In vitro* study revealed that the composites incorporating silanized HAp formed a compact and continuous calcium phosphate layer on their surface after 4 weeks immersion in a simulated body fluid (SBF).

1. Introduction

Hydroxyapatite (HAp) powder is an appropriate reinforcement for organic polymers in terms of mechanical and biological properties. Several researchers have developed HAp-filled composites using organic polymers for use as bone cements [1–3], dental implants [4] or bone replacement material [5, 6].

There are several reasons for which the use of HAp as a filler may be suitable for dental composites. HAp is the structural prototype for the principal inorganic crystalline constituent of tooth. It is also radio-opaque, highly resistant to moisture and possesses the ideal hardness, which is used as a standard for all composite resin filler particles [7].

The service life of dental composites is assumed to be strongly dependent on the bonding between the resin matrix and the reinforcing filler. Bonding is achieved by the surface treatment of the filler with a coupling agent such as organofunctional silanes. Therefore, both the silane coupling agent and the silanization procedure are important factors that determine the quality and durability of the resin/filler interface and the resultant physical and mechanical properties of the composite. The interfacial bonding between HAp and the polymer matrix still remains an issue of concern. There is conflicting evidence regarding the efficacy of the surface treatment of the HAp with a silane coupling agent [4, 8, 9].

On the other hand, it is well known that variables such as volume fraction of the inorganic filler or the particle size distribution also have a significant effect on the properties of dental composites.

In this study, surface treatment of HAp powders was carried out with a silane coupling agent using both a polar and a non-polar method of silanization. The mechanical properties of BisGMA based composites using HAp as a filler were investigated to determine the effectiveness of the process of silanization. The effect of the particle size and increasing volume fraction of the treated filler was also investigated.

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*Now deceased, his contribution is sadly missed.

In vitro bioactivity of the silane treated HAp was assessed in simulated body fluid (SBF) experiments.

2. Materials and methods

2.1. Materials

Synthetic HAp powders were incorporated into a 2,2-Bis(4-(2 hydroxy-3-methacryloxy propoxy) phenyl) propane (BisGMA) (Röhm Gmbh, Chemische Fabrik, Germany) based resin to produce composites for dental applications.

Polycrystalline HAp was obtained by a precipitation route [10] from reagent grade H_3PO_4 and $Ca(NO_3)_2 \cdot 4H_2O$ in a basic medium. After drying at 110 °C, the resultant compact material was thermally treated in an electric furnace at a rate of $10° \, min^{-1}$ to 900 °C and held at this temperature for 1 h. It was then ball-milled and sieved through a 38 mesh screen to provide HAp powder with two different particle sizes. The particle size distribution was measured using a X-ray grain-size analyzer (Malvern MasterSizer, UK). X-ray diffraction (XRD) was performed in a Siemens D-5000 diffractometer to determine the phase composition of the synthesized powder.

2.2. Surface treatment of HAp powder

HAp powders were treated with γ-methacryloxypropyl-trimethoxy-silane (γ-MPS) known as A-174 as supplied by Union Carbide, Harefield, UK. Two methods of silanization were used namely, non-polar (nP) and polar (P). The silanization solution for method nP was prepared by dissolving 10 wt % of A-174 in cyclohexane with 2 wt % of n-propylamine to increase the coupling effectiveness of the silane [11]. For method P, 10 wt % of A-174 was dissolved in a 95/5 by volume methanol/water mixture. HAp powder was immersed in each of these solutions and, after the solvents were evaporated off, the treated HAp was heated under vacuum at 120 °C for 24 h to obtain the resultant condensed silane on the surface of the HAp particles.

One half of the silanized HAp powder produced by methods nP and P was washed with dichloromethane (DCM) to remove weakly attached products derived from the reaction of γ-MPS. The DCM washed HAp powders were labeled method nP1 and P1.

2.3. Preparation of composites

The monomer phase was prepared from BisGMA and TEGDMA (triethylene glycol dimethacrylate) manufactured by Aldrich Chemical Company Inc., USA, in a w/w ratio of 66.5/33.5. Benzoyl peroxide was used as an initiator in the proportion of 1 wt %.

Untreated and surface treated HAp powders were blended with the monomer preparations in different proportions resulting in pastes with adequate handling characteristics. Higher proportions of filler could be incorporated in the monomer with silane treated HAp. Rectangular specimens (2 × 10 × 50 mm) of each paste were heat cured at 80 °C overnight under a pressure of 2.5 bar. Three series of experimental composites as listed in Table I were obtained and used in mechanical testing and *in vitro* experiments.

2.4. Spectroscopy analysis

DRIFT (diffuse reflectance infrared Fourier transform) spectra of the untreated and treated HAp powders were obtained using a Nicolet 730 spectrometer. The absorption bands in DRIFT spectra were identified to confirm the presence of the silane coupling agent on the surface of HAp particles.

2.5. Thermogravimetric analysis

Thermogravimetric analysis was performed using a Shimadzu TGA-50 thermogravimetric analyzer on untreated and treated HAp powders under oxygen at a heating rate of $10 \, °C \, min^{-1}$ (ULIRS Thermogravimetric Service, Department of Chemistry, Birbeck College, University of London).

2.6. Mechanical testing

Flat specimens for Young's modulus and flexural strength measurements (50 × 10 × 2 mm) were polished with silicon carbide paper (320, 400, 600) and the dimensions measured accurately to ± 0.01 mm using a

TABLE I Formulations of experimental Bis-GMA based composites using hydroxyapatite powder as a filler

Series	Material	Hydroxyapatite			
		Silane treatment	% w/w	% v/v	$D_{0.5}$ (μm)
Silane treatment	a	None	62.5	37.4	8.45
	b	non-Polar (nP)	62.5	37.4	8.45
	c	non-Polar 1 (nP1)	62.5	37.4	8.45
	d	Polar (P)	62.5	37.4	8.45
	e	Polar 1 (P1)	62.5	37.4	8.45
% Filter	f	P	53.0	28.9	8.45
	d	P	62.5	37.4	8.45
	g	P	62.0	40.0	8.45
	h	P	62.5	37.4	3.15
	i	P	68.0	43.3	3.15
Particle size	d	P	62.5	37.4	8.45
	h	P	62.5	37.4	3.15

$D_{0.5}$: Median particle size.

micrometer (Moore and Wright, UK). Testing was carried out using a three-point bending jig in a Lloyd's M30K mechanical testing machine (J.J. Lloyds Instruments Ltd, Southampton, UK) with an effective length between lower supports of 30 mm at a cross-head speed of 5.0 mm min^{-1}. The modulus (E) was calculated from the linear portion of the load/deflection curve and the flexural strength (σ_F) from the load at break using the respective standard formulae:

$$E = l^3F/4bd^3y \quad \sigma_F = 3l\mathrm{F}/2bd^2$$

where l is the length between the supports, F is the applied load, b is the width of the specimen, d is the thickness of the specimen and y is the deflection.

In all mechanical tests a minimum of 6 specimens were used for each composition, values being expressed as the mean ± standard deviation. Distribution of data was found to be normal by Kolmogorov–Smirnov test for both Young's modulus and flexural strength results. Comparisons were made among the composites within each experimental series for each mechanical test using one-way ANOVA analyses. Subsequently, a one-way ANOVA was performed to compare the base resin with the composites within the silane treatment series. Probability values of less than 0.05 were considered to be statistically significant.

2.7. SEM analysis

Fracture surfaces of the composites were examined by scanning electron microscopy (SEM) using a JEOL, JSM 6300, fitted with energy dispersive X-ray spectrometer (EDS), at 10 kV. Samples were sputter coated with gold and examined by secondary electron imaging.

2.8. *In vitro* bioactivity study

This study was conducted to evaluate the apatite layer forming ability of the composites formulated with surface treated HAp powder. The specimens were soaked for a period of 4 weeks in a simulated body fluid (SBF) of the following composition (in mM): Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, CT 148.8, HPO_3^- 4.2, HPO_4^{2-} 1.0 [12]. The temperature was kept constant at 37 °C. After soaking, the surfaces and polished cross sections of the specimens were examined by SEM. The chemistry of the new formed layer was analyzed using energy dispersive X-ray detector in SEM and elemental maps for Ca and P as well as line scans of the cross sections were obtained.

3. Results

3.1. Characterization of HAp powder

Fig. 1 shows the X-ray diffraction pattern of the synthesized powder, which was composed of hydroxyapatite as the only crystalline phase.

Two HAp powders of different particle size were used in this study (Table II). The median particle sizes were 8.45 and 3.15 μm.

TABLE II Particle size (μm) of the synthetic hydroxyapatite powder

$D_{0.5}$	$D_{0.1}$	$D_{0.9}$
8.45	0.98	36.78
3.15	0.69	12.36

$D_{0.5}$: Median particle size.
$D_{0.1}$ and $D_{0.9}$: Sizes below which 10 and 90% of the particle diameters lie, respectively.

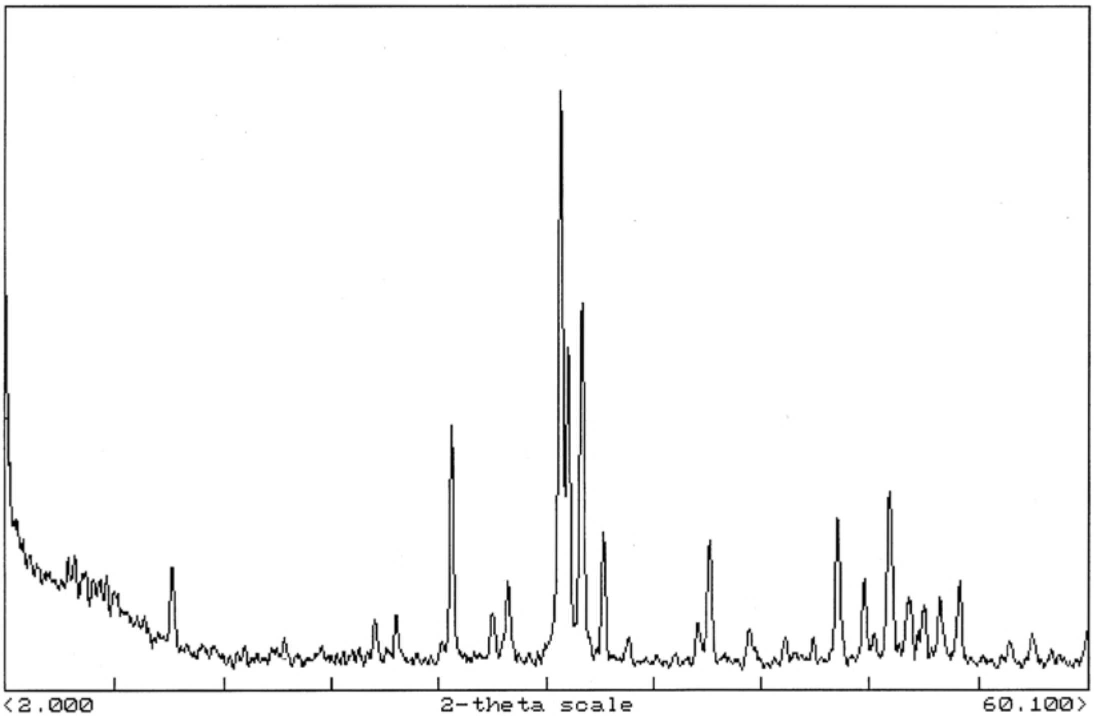


Figure 1 XRD patterns of HAp powder after firing at 900 °C for 1 h.

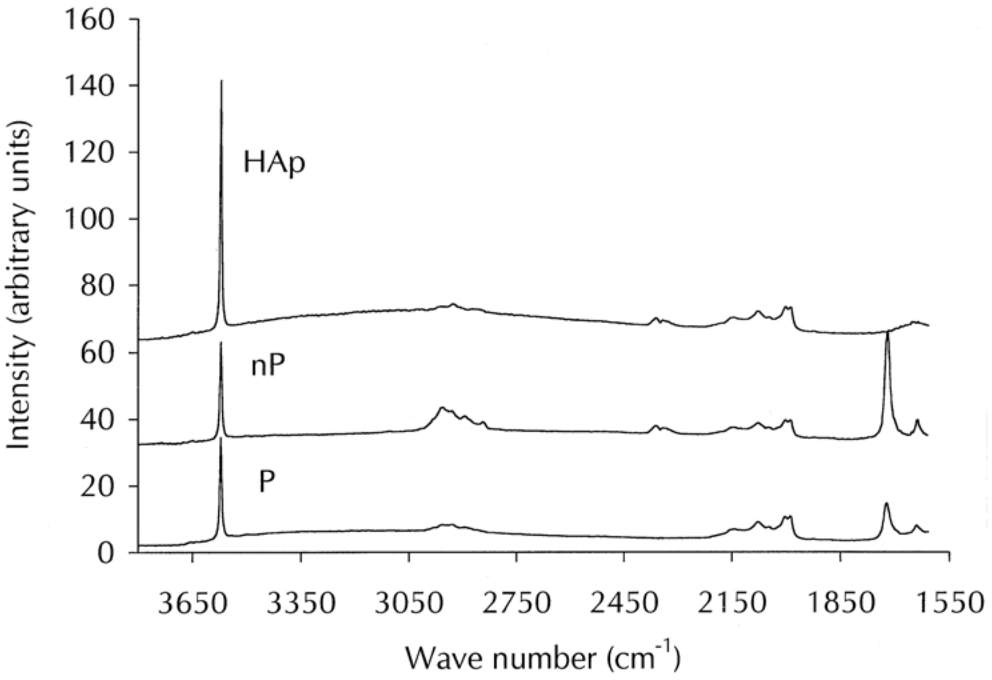


Figure 2 DRIFT spectra in the range 3800 to 1550 cm^{-1} of the unsilanized HAp, silanized HAp under non-polar conditions (nP) and silanized HAp under polar conditions (P).

3.2. Spectroscopy analysis

Fig. 2 shows the DRIFT spectra in the range 3800 to 1550 cm^{-1} of unsilanized and silanized HAp in both non-polar and polar systems. Hydroxyl stretch, belonging to HAp, was observed at 3570 cm^{-1}. An intensity reduction of the O-H stretching peak was observed in both silanized HAp samples as compared to untreated HAp.

Both treated HAp spectra showed the characteristic absorption bands of the adsorbate silane species. The presence of a band at 1720 cm^{-1} was attributed to the carbonyl group and the band at 1635 cm^{-1} to the stretching vibration of the C=C bond in the coupling agent γ-MPS.

3.3. Thermogravimetric analysis

TGA was applied to investigate the amount of coupling agent adsorbed on the HAp powder. Fig. 3 shows the

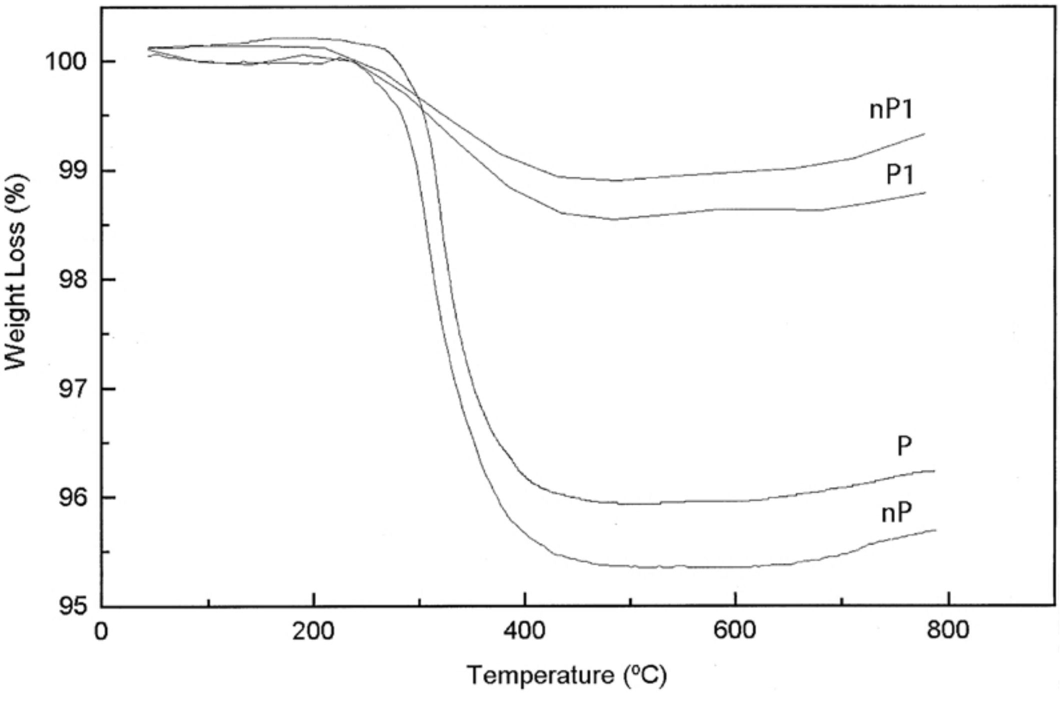


Figure 3 TGA curves for HAp powders treated with coupling agent using different methods. nP = non-polar, P = polar, P1 = polar + DCM and nP1 = non-polar + DCM.

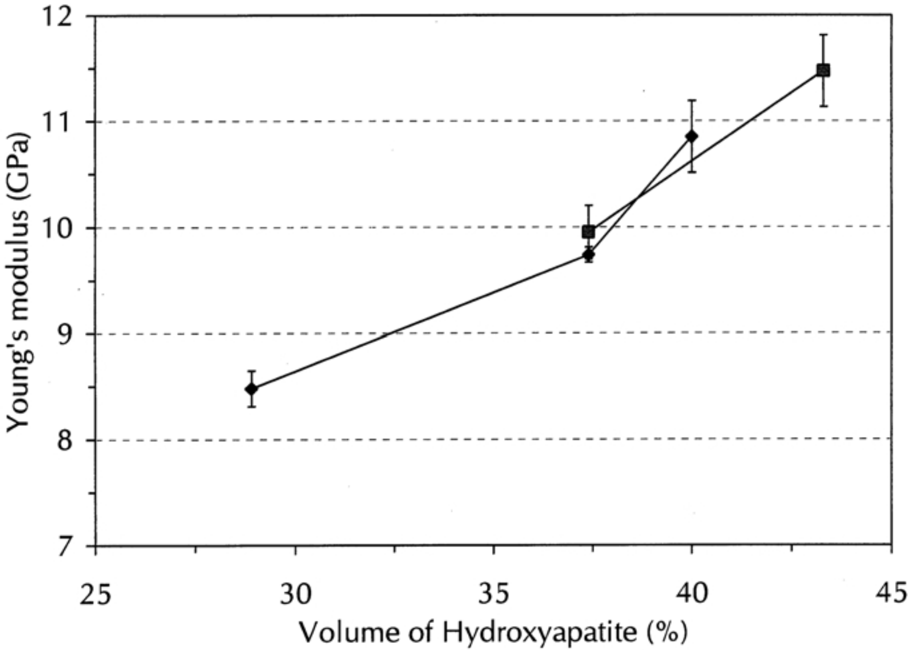
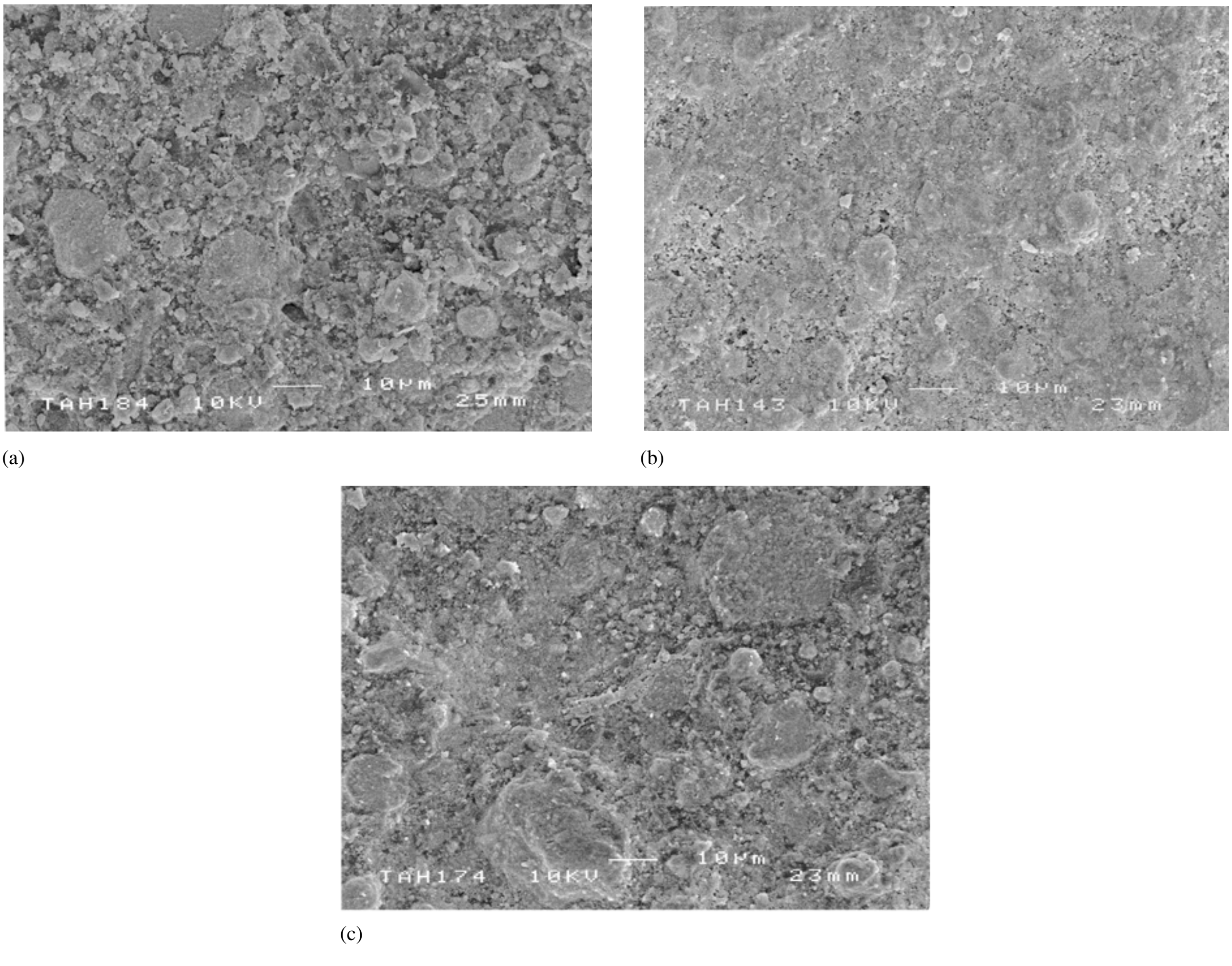


Figure 4 Young's modulus as a function of HAp volume for composites formulated with HAp silanized under polar conditions (method P) and different particle size. (◆) $D_{0.5}$: 8.45 μm; (■) $D_{0.5}$: 3.15 μm.

TGA curves of treated HAp powders by both methods (nP and P) and after washing these silanized fillers with dichloromethane (nP1 and P1 methods).

TGA curves revealed a weight loss in the temperature range 200–520 °C owing to the elimination of the coupling agent. A weight loss of 4.62% and 4.54% was detected for silanized HAp under non-polar and polar conditions, respectively. The weight loss was smaller for the silanized fillers washed with dichloromethane, this being about 1.5%.



(a)

(b)

(c)

Figure 5 SEM images of the fracture surface of the composites, (a) unsilanized HAp filled resin, (b) filled resin with HAp silanized under non-polar conditions, (c) filled resin with HAp silanized under polar conditions.

TABLE III Mechanical properties of Bis-GMA base resin and hydroxyapatite filled composites

Series	Material	Flexural strength, σ_F (MPa)	Young' modulus, E (GPa)
Base Resin	BisGMA/TEGDMA	72.43 ± 2.27	3.46 ± 0.18
Silane treatment	a	82.87 ± 6.14*	10.25 ± 0.61*
	b	84.83 ± 8.44*	9.42 ± 0.41†
	c	93.56 ± 2.44*	10.15 ± 0.33*
	d	88.47 ± 4.67*	9.74 ± 0.07†
	e	92.65 ± 3.77*	10.79 ± 0.16*
% filler	f	90.71 ± 4.26*	8.48 ± 0.17*
	d	88.47 ± 4.67*	9.74 ± 0.07†
	g	79.21 ± 10.15†	10.85 ± 0.34‡
	h	95.09 ± 3.04*	9.95 ± 0.25*
	i	94.64 ± 5.34*	11.47 ± 0.34‡
Particle size	d	88.47 ± 4.67*	9.74 ± 0.07*
	h	95.09 ± 3.04†	9.95 ± 0.25*

No statistically significant difference was found between groups within a series for each mechanical property indicated with the same superscript.

3.4. Mechanical properties

The results for mechanical testing of the three series of experimental composites and the base resin BisGMA/TEGDMA are shown in Table III.

Both the flexural strength (σ_f) and Young's modulus (E) of the materials containing HAp filler were significantly higher $(p < 0.05)$ than those of the base resin. The E values of the composites were in the range of 8.5–11.5 GPa, which were approximately three times greater than that of the base resin.

No significant difference was noted between the mechanical properties of the composites formulated with untreated and treated HAp under both non-polar and polar conditions. It could be seen that there was a very slight enhancement of σ_f and E when the treated filler was washed with dichloromethane (Table III, c, e).

The influence of the filler volume fraction and particle size of HAp was studied in composites incorporating silanized HAp by method P. The percentage of silane treated filler had a significant effect on modulus, which increased as the proportion of HAp added to the base resin increased for both 8.45 and 3.15 μm size particles (Fig. 4). However, the percentage of HAp in the composites did not significantly affect the value of σ_f though a decrease in the flexural strength was observed for HAp with larger particle size when the filler volume fraction was increased (Table III, g).

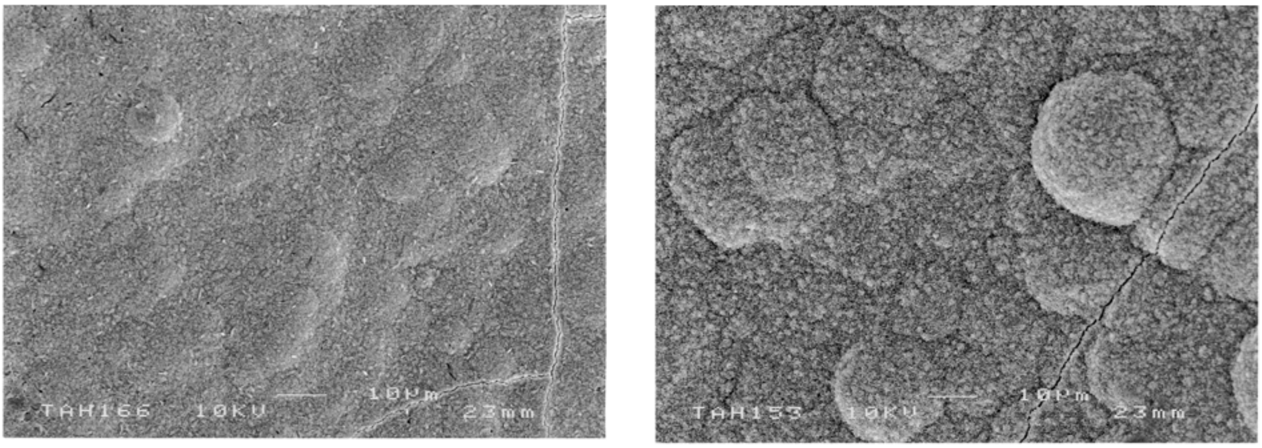
The flexural strength of composites with the same filler volume fraction (Table III, d, h) was significantly increased by a reduction in the particle size of the filler.

3.5. SEM analysis

3.5.1. Fracture surface of composites

The study of the fracture surface of the materials showed small morphological differences between the composites formulated with untreated HAp particles compared to the surface treated HAp particles.

The composites containing untreated filler showed more irregular granular and porous surface than the HAp treated composites (Fig. 5(a)). Voids where loosely embedded HAp particles have been plucked out during fracture could be observed indicating poor bonding between the filler and the matrix polymer phase. The composites prepared with surface treated HAp particles showed more regular surface features (Fig. 5(b, c)). The particles also appeared to exhibit a more intimate contact with the polymer matrix.



(a) (b)

Figure 6 SEM images of the surface of the composites after soaking in SBF for 4 weeks, (a) filled resin with silanized HAp under non-polar conditions, (b) filled resin with silanized HAp under polar conditions + DCM.

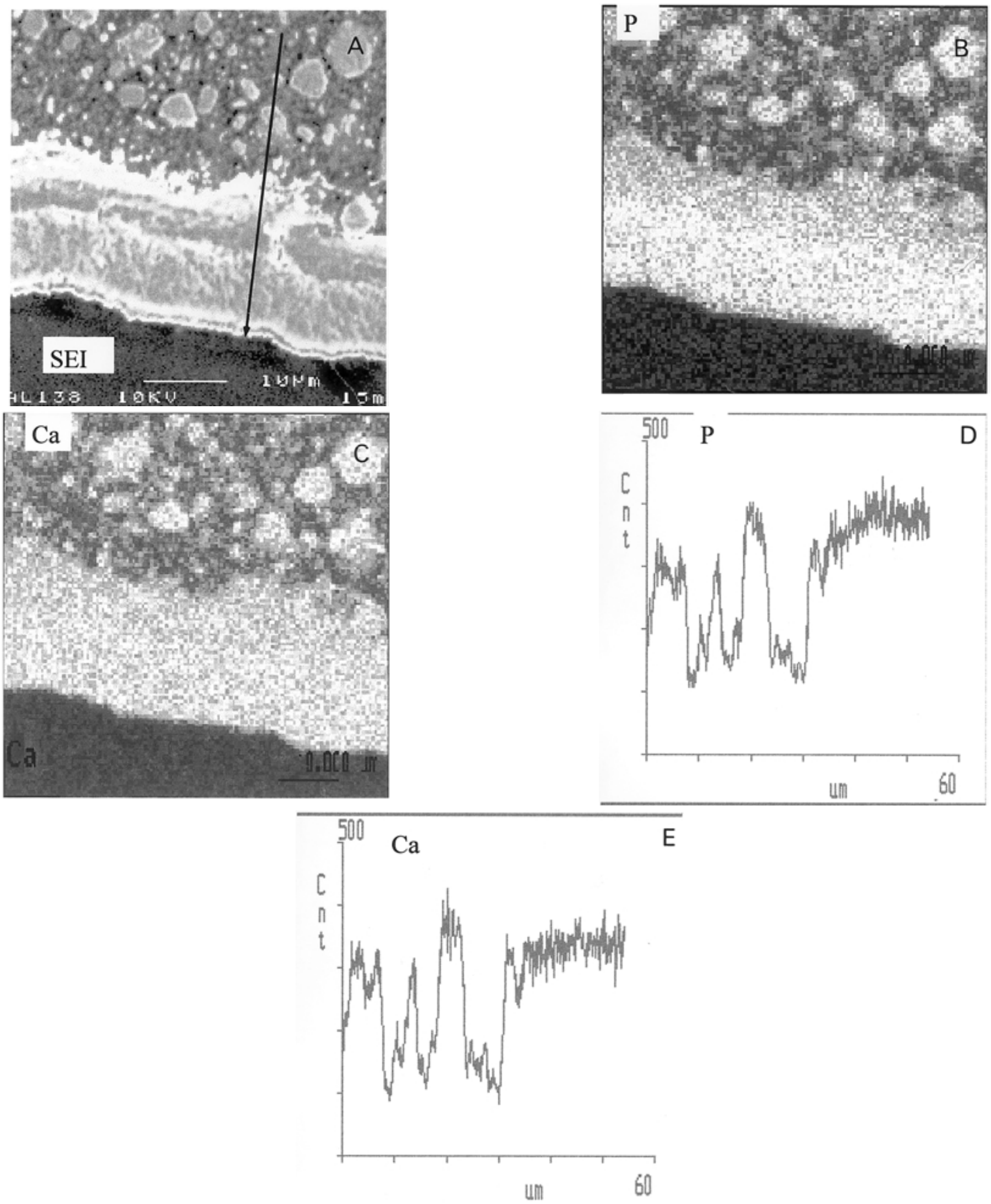


Figure 7 (A) SEM secondary electron image of the cross section of the composite incorporating silanized HAp under polar conditions + DCM after soaking in SBF for 4 weeks (→) scan line, (B) P X-ray map, (C) Ca X-ray map, (D) line scans for P, (E) line scans for Ca.

3.5.2. In vitro bioactivity

Morphological study of the treated HAp-based composite surfaces soaked for 4 weeks in SBF showed a calcium phosphate layer formed on the whole surface of the composites, as shown in Fig. 6(a) and 6(b). Fig. 7(A) shows the cross section of a composite containing treated HAp, using method P1, after 4 weeks exposure to SBF. As it is clearly observed, a dense Ca-P rich layer of between 15 and 20 μm was formed on the surface of the sample. The elemental X-ray maps of the cross section confirmed the presence of calcium and phosphorus in the layer (Fig. 7(B, C)). It was also noticed, from the line scans for these elements (Fig. 7(D, E)), that the count rates of the P and Ca elements in the surface layer matched the count rates for the same elements in the original sample, where the HAp filler was present. These results confirmed that the precipitated phase on the surface of the composite is a calcium phosphate phase of the apatite type.

The calcium phosphate phase formed on the surface showed a characteristic globular morphology. The globules formed a compact and continuous layer over the surface. In both cases, the globules were composed of many small particles. It was noticed that the surface globules of the composite containing treated HAp under non-polar conditions (Fig. 6(a)) were 2 to 3 times smaller than those of samples with treated HAp under polar conditions and subsequently washed with dichloromethane (Fig. 6(b)). Overall, the surface product formed on the composites with polar treated HAp and washed with DCM gave rise to a rougher finish than the one observed in the non-polar treated samples.

4. Discussion

Incorporation of filler particles into a resin matrix significantly improves the mechanical properties of the matrix material, if the filler particles are well bonded to the matrix. The filler-matrix bond can be achieved by treatment of the filler particles with a silane coupling agent. Dupraz *et al.* [9] treated HAp powder with five types of methoxysilane and reported that a chemical bond was developed between the HAp and the silane coupling agents. In the present study HAp powders were treated with γ-methacryloxypropyltrimethoxy-silane under different conditions of silanization. The silanization of HAp filler was confirmed by the presence of silane groups on the surface of HAp powders treated by both non-polar and polar methods (Fig. 2). It was assumed that bonding to the silica filler surface occurs by the reaction between silanol groups of the filler surface and hydrolyzed silane molecules [13]. In a similar way the silane molecule reacts with hydroxyl groups in the HAp molecule. The intensity decrease in the O-H stretching peak observed in treated HAp fillers in comparison with the untreated one indicates the silanization of the HAp by the coupling agent. TGA measurements also confirmed the presence of γ-MPS on the surface of the HAp filler particles (Fig. 3).

During the silanization process, multiple layers of silane molecules form a film around the filler particles, which is either physically or chemically attached to the filler particles [14]. HAp filler particles treated by both methods (nP and P) were washed with dichloromethane (nP1 and P1 methods) in order to remove the physically attached silane molecules which may weaken the composite due to reduced bonding of the filler to the matrix. Dichloromethane washing removed most of the loosely adsorbed silane as confirmed by the smaller weight loss obtained with the HAp powders treated by methods nP1 and P1 using TGA.

The incorporation of HAp filler, either untreated or treated, into the Bis-GMA base resin had an enhancing effect on the flexural strength and Young's modulus of the base resin. The flexural strength and Young's modulus values obtained for the composites investigated were in agreement with flexural strength and Young's modulus values of hybrid dental composites reported in the literature [15]. As far as the silane treatment is concerned, Labella *et al.* [4] reported that the flexural strength of Bis-GMA-based composites improved when a silanized HAp filler was used. However, in this study the mechanical properties of the filled resin were unaffected by the surface treatment of the HAp with the silane A-174. It appears that the solvent system or mode of application of the coupling agent had no influence on the effectiveness of the silanization as similar flexural strength and Young's modulus values were found for the composites incorporating treated HAp under different conditions. The difference in moduli between composites containing silanized HAp and those with silanized HAp washed with dichloromethane was small but significant. This can be attributed to the fact that the physically attached silane layers have been removed and hence a better bond between the filler and the matrix was achieved.

SEM examination of the fracture surface of the composites revealed that there was a better interaction between the surface treated HAp particles and the polymer matrix. The treated HAp particles were found to be more embedded in the polymer matrix. The presence of a noticeably larger proportion of HAp particles on the fracture surface of the composite containing untreated HAp filler suggests that poorer bonding between the filler and the polymer matrix exists.

These results suggest that a silane coating on the HAp particles was obtained with both methods of silanization, providing better bonding between the filler and matrix. The incorporation of higher proportions of treated filler in the polymer matrix indicates that the silane treatment also improves the wettability of the filler and hence its dispersion in the matrix. As the mechanical properties of the composites investigated in this work are not affected by the surface treatment of HAp filler, further investigation is being conducted in order to study the effect that the silane coating could have on the mechanical properties of the composites after immersion in water. Previous studies have shown that the hydrolytic stability of the silane coating on the silica filler is dependent upon the solvent system used in the process of silanization [11, 16]. Kikuchi *et al.* [16] also found that the removal of physisorbed silane products by washing with dichloromethane reduces the durability of the composite.

The filler loading and the HAp particle size seem to have an effect on the mechanical properties of the investigated composites. These composites showed greater Young's modulus values with higher filler concentration. In contrast, the lowest flexural strength was observed in the highly filled composite containing larger HAp particles. A possible explanation for this decrease in the flexural strength could be due to reduced wetting capacity of the resin matrix on the silanized filler particles, affecting the composite porosity. The HAp particle size had a significant effect on the flexural strength of composites containing similar percentage of filler while the modulus was not affected. Higher proportions of silane-treated HAp of smaller particle size could be incorporated into the monomer phase giving rise to composites of higher stiffness.

Bioactivity is defined as the ability of a material to generate a surface apatite layer that provides the bonding interface with tissues [17]. It is well known that, *in vitro*, ionic exchange at the interface between calcium phosphates and SBF leads to the formation of surface apatite. Miyaji *et al.* [18] studied the dependence on resin for apatite-forming ability in SBF for composites containing bioactive glass. They found that the BisGMA/TEGDMA-containing composite exhibited high apatite-formating ability due to the dissolution of these monomers from the composite surface into SBF, causing direct exposure of the glass grains to SBF. Our *in vitro* experiments performed on the Bis-GMA based composites formulated with silanized HAp filler revealed that the composite forms a calcium phosphate layer on its surface while exposed to an acellular simulated body fluid. Kokubo *et al.* [19] reported that composites consisting of HAp and Bis-GMA based resin formed apatite on the individual HAp grains after 1 week immersion in SBF. We found that after immersion in SBF for 4 weeks, the Ca-P rich layer formed on the composites surfaces was compact and continuous. This finding also demonstrates that the bioactivity of HAp is not affected by the silane coating. In fact, Dupraz *et al.* [9] reported that the presence of the γ-MPS coating on the HAp particles did not impair the tendency of the mineral to dissolve partly in water or to take up calcium and phosphate from SBF. These authors assumed that the applied silane coating was transparent for ionic transport in an aqueous environment.

Our study confirmed that the dichloromethane washing of the treated HAp filler removes the physisorbed silane molecules, resulting in a thinner silane coating on the filler surface. The reduced thickness of the coating encourages ionic transport and formation of larger surface globules and therefore rougher surface as observed in the composites containing filler washed with DCM (Fig. 6(b)).

5. Conclusion

This study found that flexural strength values showed a significant increase with the addition of HAp filler to the base resin, while Young's modulus values increased by a factor of three. Surface treatment of the HAp with the silane coupling agent is ineffective in augmenting the mechanical properties of the filled resin. The filler volume fraction and the particle size of hydroxyapatite have significant effects on the mechanical properties of the treated HAp-based composites. An *in vitro* study of the treated HAp-based composites showed that this material forms a calcium phosphate layer on its surface during exposure to a simulated body fluid.

Acknowledgments

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