doi:10.1111/j.1365-2591.2008.01478.x

Degradation properties and ion release characteristics of Resilon® and phosphate glass/polycaprolactone composites

P. Borbely[1], **K. Gulabivala**[1] & **J. C. Knowles**[2]

[1]Unit of Endodontology, Division of Restorative Dental Sciences; and [2]Division of Biomaterials Science & Tissue Engineering, UCL Eastman Dental Institute, University College London, London, UK

Abstract

Borbely P, Gulabivala K, Knowles JC. Degradation properties and ion release characteristics of Resilon® and phosphate glass/polycaprolactone composites. *International Endodontic Journal*, **41**, 1093–1100, 2008.

Aims To (i) characterise the degradation pattern and ion release characteristics of bioactive glass/polycaprolactone (PCL) composites; (ii) compare the degradation behaviour of composites containing phosphate glass to that of Resilon®; and (iii) determine the potential to control the degradation of the composites by modifying the iron content of phosphate glasses.

Methodology The degradation behaviour of the phosphate glass/PCL samples ($n = 3$) and that of Resilon® were determined in two aqueous solutions (buffered distilled water, Hanks Buffered Saline Solution) over a 7-day period using weight change measurement, ion chromatography, light microscopy (LM) and scanning electron microscopy (SEM).

Results An initial increase in weight of the samples was followed by loss. All phosphate glass/PCL composites released various amounts of iron, sodium, calcium and phosphate ions into solution. The Resilon® samples released only sodium and calcium ions. The visual methods (LM, SEM) showed distinct precipitate (calcium phosphate) formation on the surface of the phosphate glass/PCL composites but only a faint surface residue on the surface of Resilon®.

Conclusions The degradation pattern of the composites containing phosphate glass was different from that of Resilon®. The phosphate glass/PCL composites encouraged precipitate formation on the material surface, which may be advantageous in creating a marginal and apical seal.

Keywords: composites, ion release, phosphate glasses, polycaprolactone, Resilon®, root fillings.

Received 17 June 2008; accepted 9 August 2008

Introduction

Tissue engineering is a rapidly emerging discipline with the aim of favourably influencing regeneration of human tissues (Abou Neel *et al.* 2005). Bioactive and bio-resorbable materials that can stimulate specific and controlled cell responses are now of major interest for various medical applications. Whilst gutta-percha is known for its relative inertness, a newly marketed root filling material (Resilon®), composed of a biodegradable polymer and bioactive glass is potentially biologically active. The placement of Resilon® in contact with dental hard tissues, periapical tissues and potentially microorganisms could initiate a series of reactions resulting in changes in the material and its milieu (Tay *et al.* 2005a,b). These interactions may favourably or adversely affect the 'seal', the periapical healing process and any residual microflora. Currently, little is known about the degradation behaviour of Resilon®, or the nature of interaction between its components and the environment.

It may be possible to develop a new endodontic material that facilitates periapical healing as it

---

Correspondence: Professor J Knowles, Division of Biomaterials and Tissue Engineeering, UCL Eastman Dental Institute, 256 Grays Inn Road, London WC1X 8LD, UK (Tel.: 020 7915 1189; fax: 020 7915 1227; e-mail: j.knowles@eastman.ucl.ac.uk).

© 2008 International Endodontic Journal

degrades. Phosphate glasses (P-glasses) are a unique group of bioactive materials that offer great potential for hard and soft tissue engineering. This is because of their favourable dissolution properties (Kim *et al.* 2005) and customisability (Knowles 2003). The interaction between phosphate glasses and the biological environment is characterised by a hydration mechanism at the surface of the glass when placed in simulated body fluid (Clemént *et al.* 1999). Thus, totally hydrated phosphate chains disentangle and leach in to the surrounding solution; simultaneously, a hydrated layer forms on the surface of the glass. Raman spectroscopy and the X-ray analysis showed this layer to be composed of calcium orthophosphate groups (Clemént *et al.* 1999). The rate of the precipitate formation is determined by the characteristics of the surrounding medium and the solubility of the glass. Solubility measurements of phosphate glasses showed that there is compositional related nonlinearity in the dissolution of the glasses over time (Franks & Knowles 1999, Franks *et al.* 2000). Precipitation on a biomaterial surface may have significant biological consequences which include antimicrobial effects (Zehnder *et al.* 2004) and alteration of dentine permeability (Galvan *et al.* 1994).

Similar to other bioactive glasses and CaP ceramics, P-glass alone is highly brittle and has low fracture toughness. Composites of biodegradable polymers and glasses have been emerging as a possible way of creating a range of biomaterials that unite the advantageous processability of the polymer with the customizability of the phosphate glasses. The glass composite with polymers such as polycaprolactone (PCL) can enhance flexibility and mouldability of the glass (Prabhakar *et al.* 2005). Phosphate glass/PCL composites may have the potential to be used in endodontics. By modifying the glass composition, a wide range of composites with different degradation rates can be obtained (Franks *et al.* 2000, Salih *et al.* 2000). The degradation behaviour could be tailored to suit the end application.

The aim of the study was to characterise the degradation pattern and ion release characteristics of bioactive glass/PCL composites, to compare the degradation behaviour of composites containing phosphate glass to that of Resilon$^{\circledR}$, and to determine the potential to control the degradation of the composites by modifying the iron content of phosphate glasses.

Materials and methods

Composites of iron-phosphate glass and PCL were created using the solvent casting method (Prabhakar *et al.* 2005). The composites contained identical, 0.20 volume percentage of glass but the composition of the glass was systematically different for each composite. The $CaO–Na_2O–Fe_2O_3–P_2O_5$ glass system was used in this study. The compositions investigated were fixed at 50 mol% P_2O_5 and 40 mol% CaO. The Fe_2O_3 content was varied between 1 mol% and 5 mol%, substituting for the 10 mol% Na_2O. The glass compositions (Table 1) had been created for previous experiments (Prabhakar *et al.* 2005) at UCL Eastman Dental Institute, London, UK. The PCL was commercially available (Sigma Aldrich Chemical Company, Inc., Milwaukee, MI, USA) To produce the composites, the glasses were first milled using a Retsch$^{\circledR}$ MM301 ball mill (Retsch GmbH & Co., KG, Haan, Germany). Then, the PCL was weighed and added to chloroform, which was left to stir for 60 min or until the polymer had completely dissolved. The glass was added and left to stir for a further 5 min. This entire solution was rotary evaporated under vacuum conditions (Rotary Evaporator: RE 200, Bibby Sterilin, Staffordshire, UK; Vacuum Pump: Laboport, KNF, Neuberger, Germany) to produce a thin composite deposit film. The resulting composite film was left to dry overnight to allow evaporation of all traces of solvent. Uniform samples of size 24 × 3 × 0.5 mm (Fig. 1) were prepared using a heat press (Specac, Orpington, UK) and die cutter (H.W. Wallace, Croydon, UK). Epiphany$^{\text{TM}}$ points (Epiph-

Table 1 showing the test glass compositions

Glass code	Glass composition	CaO (mol%)	Na_2O (mol%)	Fe_2O_3 (mol%)	P_2O_5 (mol%)
64	$(CaO)_{0.40}–(Na_2O)_{0.09}–(Fe_2O_3)_{0.01}–(P_2O_5)_{0.50}$	40	9	1	50
66	$(CaO)_{0.40}–(Na_2O)_{0.09}–(Fe_2O_3)_{0.01}–(P_2O_5)_{0.50}$	40	9	1	50
74	$(CaO)_{0.40}–(Na_2O)_{0.08}–(Fe_2O_3)_{0.02}–(P_2O_5)_{0.50}$	40	8	2	50
76	$(CaO)_{0.40}–(Na_2O)_{0.08}–(Fe_2O_3)_{0.02}–(P_2O_5)_{0.50}$	40	8	2	50
83	$(CaO)_{0.40}–(Na_2O)_{0.07}–(Fe_2O_3)_{0.03}–(P_2O_5)_{0.50}$	40	7	3	50
93	$(CaO)_{0.40}–(Na_2O)_{0.06}–(Fe_2O_3)_{0.04}–(P_2O_5)_{0.50}$	40	6	4	50
96	$(CaO)_{0.40}–(Na_2O)_{0.05}–(Fe_2O_3)_{0.05}–(P_2O_5)_{0.50}$	40	5	5	50

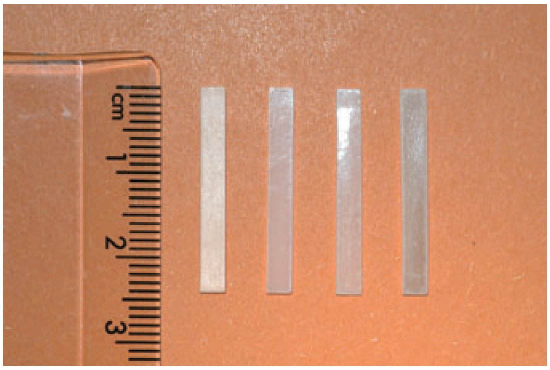

Figure 1 Showing the dimensions of the formed test composites.

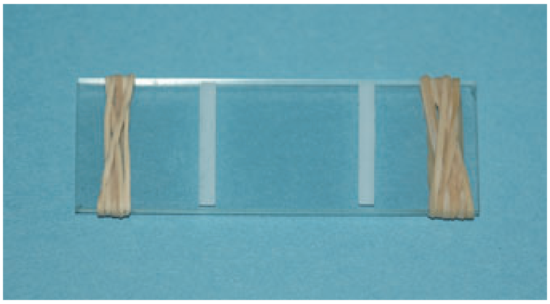

Figure 2 Creation of a semi-protected environment by inserting composites between glass slides.

any^{TM} Soft Resin Endodontic Obturation System, Pentron^{TM} Clinical Technologies, LLC, Wallingford, CT, USA) were heat pressed and cut using the same method to prepare Resilon® samples. A semi-protected environment model was designed to simulate the obturated root canal space. The semi-protected specimens were created by placing two of the same samples between microscope slides held together by elastic bands as shown in Fig. 2.

A short-term degradation study was carried out to monitor the *in vitro* behaviour of the samples in a semi-protected environment using two aqueous solutions, distilled water and Hanks Buffered Saline Solution (HBSS). Three samples of each of the five composites and three Resilon® samples were placed into pH adjusted distilled water and the same number of samples into HBSS solution (Gibco, Invitrogen, UK). Buffering of the water was achieved by adding a small amount of sodium bicarbonate to the water, and monitoring the pH closely until it reached 7. The samples were kept in an incubator at 37 °C. They were removed from the solution every 24 h for 7 days. After measurement, the samples were returned to fresh pH buffered distilled water or HBSS solution.

Weight change measurements

Weight change measurements (Prabhakar *et al.* 2005) were performed to evaluate the change in weight of glass/PCL composites in both solutions over a 7-day period. The samples were removed from the aqueous solution, blotted dry with tissue paper and weighed on a BDH analytical balance (Mettler Toledo AG204, San Ramon, CA, USA). The weight change was plotted as the initial weight minus the weight at time *t*, divided by the initial weight and the mean and standard deviation data were plotted against time.

Ion chromatography

Ion chromatography (Ahmed *et al.* 2005) was used to investigate the $Fe^{2+/3+}$, Na^{+}, Ca^{2+}, PO_4^{3-}, $P_2O_7^{4-}$, $P_3O_9^{3-}$, $P_3O_{10}^{5-}$ ion release from glass/PCL composites into buffered distilled water over a 7-day period. Iron release was measured using the Dionex ICS-2500 system (Dionex, Camberley, UK). An IonPacR CS5A column (Dionex) with attached CG5A guard column (Dionex) was used for the determination of Fe^{2+} and Fe^{3+} ions. The sodium and calcium ion release measurements were conducted on a Dionex ICS 1000 ion chromatography system (Dionex), with a mobile phase of 20 mmol L^{-1} methane-sulphonic acid solution in deionised water, with a flow rate of 1 mL per minute. A CS12A column (Dionex) and CAES suppressor (Dionex) was also used. Sodium and calcium standard solutions were used as calibrants. The release of phosphate groups, PO_4, P_2O_7, P_3O_9 and P_3O_{10} was measured using the ICS2500 system (Dionex). A mobile phase of deionised water was used and the flow rate was 1.5 mL per minute. An AS16A column (Dionex) was employed, mounted in an AS50 thermal compartment, along with an ASRS suppressor (Dionex). Blank samples were run to compensate for any background levels of ions although these were virtually immeasurable because of the ultrapure water used.

Scanning electron microscopy

Scanning electron microscopy (SEM) was applied to observe the surface morphology of glass/PCL composites after 4 weeks in HBSS solution in a free or semi-protected environment. In preparation for the SEM analysis, samples were coated with carbon (Quorum Technology, Newhaven, UK) and then examined in a Cambridge 90B SEM (Leo, Zeiss, Hertfordshire, UK) operating at 25 kV and a working distance of 27 mm. Elemental analysis was performed using an Oxford Instruments Inca 400 system (Oxford Instruments, Bucks, UK).

Light microscopy

Light microscopy was used to examine the changes in the sample-solution interface of all semi-protected specimens over a 7-day period in buffered distilled water or in HBSS solution. The interface was examined under LPL 40/0.60 160/1.5 magnification using CETI microscope (Medline Scientific Limited, Oxfordshire, UK) and digital photographic images were taken with a JVC Colour Video Camera (JVC, Fukuoka, Japan).

Results

Weight change measurements

An initial increase in weight of the samples up to 2 days was seen, followed by weight loss for all phosphate glass/PCL composites in distilled water (Fig. 3) as well as in HBSS (Fig. 4). However, the weight loss at the longer time points was lower in HBSS compared with those in distilled water. At the end of the study, the relative ranking of the samples, from the one with the largest net weight loss to that with the smallest was the following in both solutions: 2, 3, 4, 5 and 1 mol% Fe_2O_3–P-glass/PCL composite.

The weight of Resilon® remained relatively constant with minor changes.

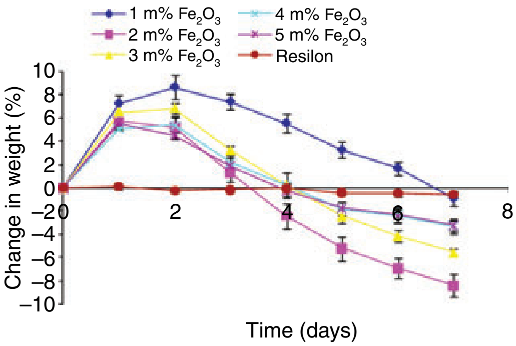

Figure 3 Change in weight of composite samples over 7 days in water.

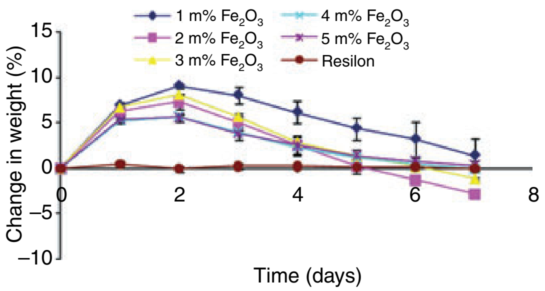

Figure 4 Change in weight of composite samples over 7 days in Hanks Buffered Saline Solution (HBSS).

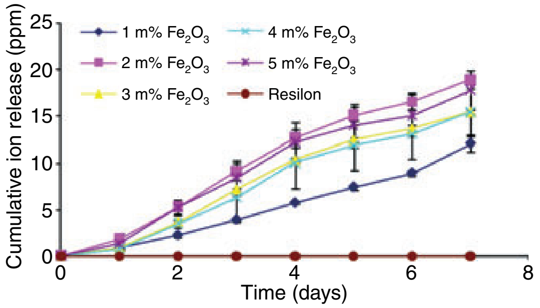

Figure 5 Iron release profiles of composites over 7 days.

Ion chromatography

The iron release from all samples except the control and Resilon showed a linear increase with time up to about 10–20 ppm in 7 days (Fig. 5). The sodium release tended to have a more exponential release profile, with the rate of sodium release slowing with time. The sodium release was inversely related to the amount of Fe_2O_3 in the glass i.e. the higher the Fe_2O_3 content the lower the levels of sodium release (Fig. 6).

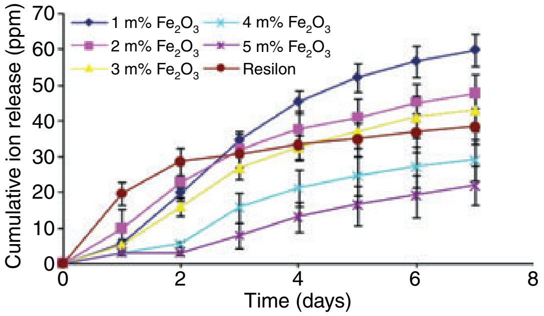

Figure 6 Sodium release profiles of composites over 7 days.

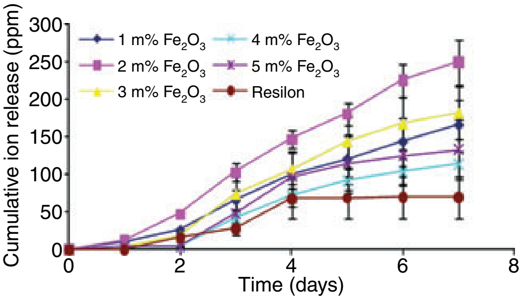

Figure 7 Calcium release profiles of composites over 7 days.

The calcium release appeared relatively linear with time up to 7 days (Fig. 7). The PO_4^{3-} release was very low up to day 1; this was then followed by a sharp increase which carried on at a relatively linear rate with time. This pattern of release was virtually identical for the $P_2O_7^{4-}$,$P_3O_9^{3-}$ and $P_3O_{10}^{5-}$. The Resilon showed no release of any phosphates (Figs 8–11).

Scanning electron microscopy

Examination of the semi-protected Resilon® specimen showed some minor but even residue of precipitation over almost the entire surface (Fig. 12), although it was slightly denser at the margins. In contrast, the precipitate on the semi-protected phosphate glass/PCL samples (Fig. 13) was confined to the free margins and was much more extensive with a width of 200 μm but could be up to 700 μm. The elemental analysis overlaid on Fig. 13 showed that the layer on the free edge consisted primarily of Ca, P and O i.e. a calcium phosphate. The curves of the ratios of the Ca to P gives a Ca : P ratio of approximately 0.66.

Discussion

Generally, the findings of the present study on the iron–phosphate glass/PCL composites are comparable with those of Prabhakar *et al.* (2005). It was anticipated that the degradation and ion release patterns would appear in a sequence that corresponded to the iron content. In reality, the sequence followed a different pattern as described below. The relatively inert nature of Resilon was anticipated as it is not marketed as a bioactive material. It was suspected that as Resilon contained silicate glass instead of phosphate glass, its degradation characteristics would be entirely different from that of P-glass/PCL composites.

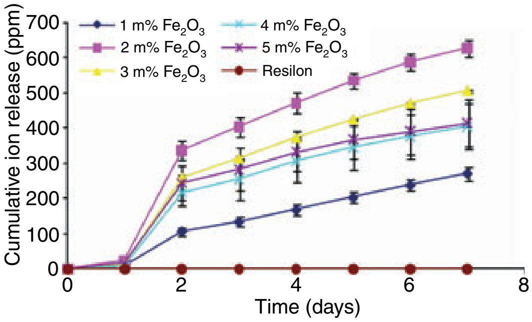

Figure 8 PO_4 release profiles of composites over 7 days.

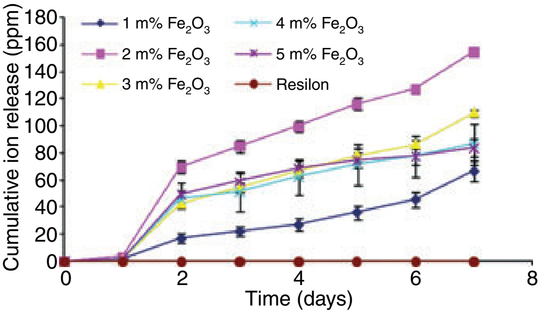

Figure 9 P_2O_7 release profiles of composites over 7 days.

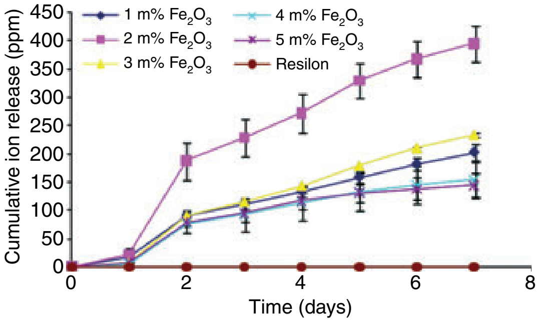

Figure 10 P_3O_9 release profiles of composites over 7 days.

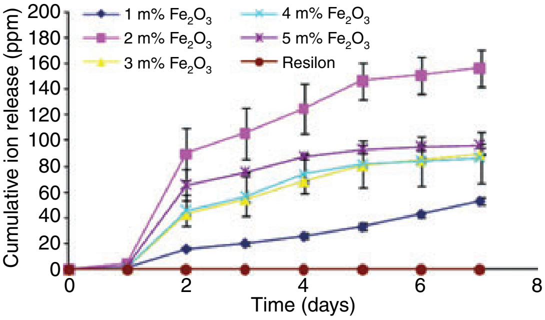

Figure 11 P_3O_{10} release profiles of composites over 7 days.

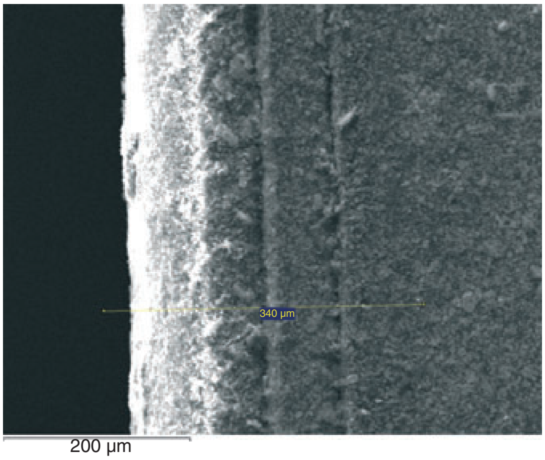

Figure 12 Scanning electron microscopy (SEM) view of Resilon showing an even residue of precipitate.

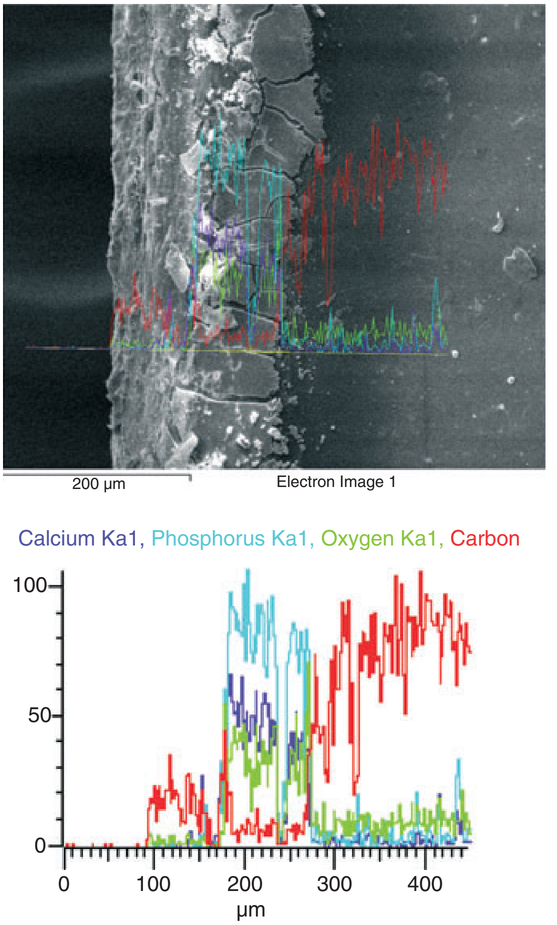

Figure 13 Scanning electron microscopy (SEM) view (upper) of precipitate at the exposed edge of a phosphate glass composite in a semi-protected environment and elemental analysis (lower) of precipitate showing calcium and phosphate components.

The weight change profile of the samples is the net result of two parallel processes: water uptake and material loss and this is controlled by two competing factors, the hydrophilic nature of the glass and the hydrophobic nature of the polymer. Initially water is taken up by the polymer, driven by the hydrophilic glass. However, on contact with moisture, the glass degrades and leaches out of the sample. This weight loss thus counteracts the weight increase because of water uptake. It should be noted that this water uptake and subsequent weight loss is much more controlled, compared with previous work (Prabhakar *et al.* 2005) and this is specifically related to the addition of iron to the glasses, which has a strong cross-linking effect on the glass structure.

The change in weight of Resilon remained within 0.5% over the 7-day period in both the aqueous solutions. The initial increase in weight caused by water uptake was subsequently compensated by the small weight loss of the samples.

All P-glass/PCL composites released calcium and phosphate ions into solution. The calcium release ranged between 110 and 250 ppm and the PO_4 release was between 270 and 630 ppm for the five composites that differed only in their iron content (1–5 mol% iron). In other words, minor difference in the composition of the materials resulted in more than twice as much calcium and phosphate release from the materials into solution. This means that it is possible to control the rate of calcium and phosphate release by modifying the composition of the glass component in the composite. The sodium and iron does not have a direct role in the precipitate formation. Sodium is normally present in the living tissues in relatively large quantities compared with the amount released from the composites. Iron is added to the glasses to reduce the rate of degradation of the composites.

The ion release data clearly showed an association with the weight loss. The PO_4 release, P_2O_7 release and P_3O_9 release of the P-glass/PCL composites seemed to be related to the change in weight. Specifically, at the end of day 7, the relative ranking of the samples from the one releasing the most PO_4 and P_2O_7 to that releasing the smallest quantity was the same as the ranking of the composites from the one with the greatest weight loss to the one with the smallest. The ranking of the samples was 2, 3, 4, 5

© 2008 International Endodontic Journal

and 1 mol%. On the P_3O_9 release graph, the ranking of the samples was the same except for the 1 mol% Fe_2O_3 composites which 'overtook' the 4 and 5 mol% samples.

Resilon did not release phosphate or iron ions most probably because it did not contain any. It did, however, release small amounts of sodium and calcium over the first 4 days of the study. The rate of ion release was close to zero after the fourth day. This was because the sodium and calcium had probably leached out from the surface of the sample as the remaining components of the material stayed attached, preventing further release of sodium and calcium ions from deeper layers.

The presence of a precipitate layer on the surface of the P-glass/PCL composites was obvious. Elemental analysis showed this layer to be composed of calcium and phosphorus (Ca/P = 0.66). It can be speculated that it is likely to have formed a hydrated calcium phosphate such as brushite (Ca/P = 1), a precursor to apatite formation. Such a layer did not appear to have been deposited on the Resilon® samples. Given that Resilon® is intended to be used with an adhesive or conventional sealer, this is probably a positive feature in that context. If, however, the material/dentine interface were devoid of such material, it cannot be relied upon to develop a seal, through exposure to moisture.

The changes in surface morphology of the samples corresponded to the characteristics of degradation predicted from the weight change and ion chromatography methods. A precipitate layer formed on the surface of P-glass/PCL composites but not on the surface of Resilon®.

All P-glass/PCL composites released calcium and phosphate ions into solution. The clinical significance of this is that it causes high calcium and phosphate concentrations in the surrounding tissues and subsequent re-precipitation of the ions on the material surface. The CaP precipitate will eventually be transformed into mineralized tissue mainly by chemical processes. The exact mechanism for this is currently unknown. It is very likely, however, that the larger the calcium and phosphate concentration in the tissues, the quicker the re-precipitation occurs. The rapid precipitation may be advantageous if a seal at the apical foramen is to be created. This study and that of Prabhakar *et al.* (2005) showed that by modifying the composition of the material, the calcium and phosphate ion release could be controlled. However, this study differs in that the specimens are placed in solution in a 'semi-protected' environment. The rationale behind this has been driven by previous composite work wherein no precipitate was found on the surface when placed in an open environment. Considering the high levels of calcium and phosphate released this was unexpected. The use of the semi-protected environment inhibits diffusion and drives the localized concentration of ions, which has been confirmed in this study. This has implications for the use in the root canal system, where the material enters a relatively quiescent chemical environment and thus favours ion concentration and subsequent precipitation, when exposed to moisture. This implies that a material could be created that degrades and releases ions at a rate favourable for precipitate formation on the material surface in contact with tissue fluids inside the root canal space and at the apical foramen. This may facilitate periapical healing in one of several ways: (i) formation of a seal between the residually contaminated root canal space (Nair *et al.* 2005) and the periapical tissues; (ii) killing of residual bacteria by the antimicrobial effect created by the material in the environment; (iii) encouragement of bone deposition within the periapical lesion. The release of ions from the material may lead to change in weight or volume of the material; however, this is unlikely to interfere with the healing process. The investigation of such materials for endodontic application may include a reconsideration and probably modification of the principles and techniques of root canal preparation and filing.

The results obtained and discussed have provided a snapshot of the initial breakdown behaviour of the iron-phosphate glass/PCL composites *ex vivo*. Further studies need to be carried out to investigate the degradation behaviour of these materials using more clinically relevant models and over a longer period of time to establish their viability as a biomaterial and to assess their potential to be used in endodontics.

Conclusions

Within the limitations of the study, it may be concluded that:

1. The degradation pattern of the composites containing phosphate glass was different from that of Resilon®.

2. The phosphate glass/PCL composites encouraged precipitate formation on the material surface, particularly when semi-protected.

3. Phosphate glass/PCL composites may have potential as bioactive root fillings.

References

Abou Neel EA, Ahmed I, Blaker JJ *et al.* (2005) Effect of iron on the surface, degradation and ion release properties of phosphate-based glass fibres. *Acta Biomaterilia* **1**, 553–63.

Ahmed I, Lewis MP, Nazhat SN, Knowles JC (2005) Quantification of anion and cation release from a range of ternary phosphate-based glasses with fixed 45 mol% P_2O_5. *Journal of Biomaterials Applications* **20**, 65–80.

Clemént J, Manero JM, Planell JA, Avila G, Martinez S (1999) Analysis of the structural changes of a phosphate glass during its dissolution in simulated body fluid. *Journal of Materials Science. Materials in Medicine* **10**, 729–32.

Franks K, Knowles JC (1999) Non-linear solubility behaviour in a ternary phosphate based glass system. *Journal of Dental Research* **78**, 1038–45.

Franks K, Abrahams I, Knowles JC (2000) Development of soluble glasses for biomedical use Part I: in vitro solubility measurement. *Journal of Materials Science. Materials in Medicine* **11**, 609–14.

Galvan DA, Ciarlone AE, Pashley DH, Kulild JC, Primack PD, Simpson MD (1994) Effect of smear layer removal on the diffusion permeability of human roots. *Journal of Endodontics* **20**, 83–6.

Kim HW, Lee EJ, Jun IK, Kim HE, Knowles JC (2005) Degradation and drug release of phosphate glass/polycaprolactone biological composites for hard-tissue regeneration. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **75B**, 34–41.

Knowles JC (2003) Phosphate based glasses for biomedical applications. *Journal of Materials Chemistry* **13**, 2395–401.

Nair PN, Henry S, Cano V, Vera J (2005) Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after ''one-visit''endodontic treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **99**, 231–52.

Prabhakar RL, Brocchini S, Knowles JC (2005) Effect of glass composition on the degradation properties and ion release characteristics of phosphate glass–polycaprolactone composites. *Biomaterials* **26**, 2209–18.

Salih V, Franks K, James M, Hastings GW, Knowles JC, Olsen I (2000) Development of soluble glasses for biomedical use Part II: the biological response of human osteoblast cell lines to phosphate-based soluble glasses. *Journal of Materials Science. Materials in Medicine* **11**, 615–20.

Tay FR, Pashley DH, Williams MC *et al.* (2005a) Susceptibility of a polycaprolactone-based root canal filling material to degradation. I. Alkaline hydrolysis. *Journal of Endodontics* **31**, 593–8.

Tay FR, Pashley DH, Yiu CK *et al.* (2005b) Susceptibility of a polycaprolactone-based root canal filling material to degradation. II. Gravimetric evaluation of enzymatic hydrolysis. *Journal of Endodontics* **31**, 737–41.

Zehnder M, Soderling E, Salonen J, Waltimo T (2004) Preliminary evaluation of bioactive glass S53P4 as an endodontic medication in vitro. *Journal of Endodontics* **30**, 220–4.

© 2008 International Endodontic Journal

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.