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The processing, mechanical properties and bioactivity of strontium based glass polyalkenoate cements

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Abstract The suitability of zinc-based glass polyalkenoate cements (GPCs) for use in orthopaedics can be improved by the substitution of strontium into the glass phase which should impart improved radiopacity and bone forming properties to the cements without retarding strength. The purpose of this research was to produce novel GPCs based on calcium–strontium–zinc-silicate glasses and to evaluate their mechanical properties and biocompatibility with the ultimate objective of developing a new range of cements for skeletal applications. Three glass compositions, based on incremental substitutions of strontium for calcium, were synthesized; BT100 (0.16CaO, 0.36ZnO, 0.48SiO_2), BT101 (0.04SrO, 0.12CaO, 0.36ZnO, 0.48SiO_2) and BT102 (0.08SrO 0.08CaO, 0.36ZnO, 0.48SiO_2). Each glass was then mixed with varying concentrations and molecular weights of polyacrylic acids in order to determine the working times, setting times, compressive strengths and biaxial flexural strengths of the novel cements. The maximum working time and setting time achieved was 29 and 110 s respectively; which, at present is inadequate for current clinical procedures. However, the optimum compressive and biaxial flexural strengths were up to 75 and 34 MPa respectively indicating that these formulations have potential in load bearing applications. Importantly, the substitution of Ca with Sr in the glasses did not have a deleterious effect on strengths or working times. Finally, the bioactivity of the best performing cements was determined in vitro using simulated body fluid. It was found that all cements facilitate the formation of an amorphous calcium phosphate at their surface which increases in density and coverage with time, indicating that these cement will bond directly to bone in vivo.

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1 Introduction

Polymethylmethacrylate (PMMA) is the primary bone cement used in prosthetic stabilization [1, 2] and in spinal corrective surgeries such as vertebroplasty and kyphoplasty [3]. However, there are concerns relating to the use of these cements in vivo. PMMA has been shown to cause bone necrosis due to both the exothermic setting reaction and through the leaching of unreacted monomer from the cement mantle [4]. Additional concerns with PMMA cements include a lack of chemical bonding to bone [5], modulus mismatch [5] and volumetric shrinkage (7%) [6, 7]. Consequently, focus has turned to the development of alternative bone cements. A new synthetic bone void filler based on resin composite chemistry, Cortoss® (Orthovita, Malvern, PA, USA), exhibits a lower setting exotherm than PMMA, but research has shown that leakage cement occurs upon implantation. Studies by Palussiere et al. [8] concluded that out of 91 vertebrae augmented with Cortoss, 70% experienced leakage [8] which may result in serious systemic effects [9].

Glass polyalkenoate cements (GPCs) were developed in the early 1970s [10] and are used in luting and restorative dental applications [11]. However, due to their excellent biocompatibility [12], their ability to adhere to surgical metals and bone, and their lack of volumetric shrinkage and heat evolution [13], they have been considered for the purposes of skeletal cementation. GPCs set *via* an acid/base reaction between an ion leachable glass, and a polyalkenoic

acid, usually polyacrylic acid (PAA). The acid degrades the glass structure releasing metal cations into the polysalt matrix. The released ions form chelates with the carboxylate groups of the polymer. The metal cations serve to crosslink the polyacrylate chains resulting in a cement consisting of residual glass particles with a surrounding siliceous hydrogel embedded in a polysalt matrix [14, 15]. GPCs can also be formulated to release beneficial ions [16], such as zinc, which assists in healthy bone metabolism [17].

One of the primary concerns with the use of conventional GPCs in orthopaedics is the presence of the trivalent aluminium ion (Al^{3+}) in the glass phase. Aluminium (Al) has been implicated in the pathogenesis of the degenerative neurological disorder Alzheimer's disease [18], and was identified as the cause of one patient's death during oto-neurosurgery[19]. However, the authors have previously shown that it is possible to remove Al from the glass structure in these novel GPCs and replace it with zinc (Zn) [13]. Zn^{2+} acts as both a network modifier and an intermediate oxide (in a similar fashion to Al^{3+}) whilst having both a beneficial effect on bone metabolism [17] and imparting an antibacterial nature to the cement [20]. The authors are now developing a second generation of these cements where strontium (Sr) is incorporated into the glass network to improve both radiopacity and bioactivity of the resultant GPCs.

Sr is known to behave in a similar fashion to Ca, where it is re-circulated through the bloodstream and re-used by growing bone or excreted from the body [21]. Sr^{2+} and Ca^{2+} are interchangeable in the crystal lattice of HA, due to the ions having similar ionic size and polarity [22]. The role of Sr in bone metabolism has been well documented. Strontium ranelate (SR), marketed as Protelos®, (Servier Laboroatories, Dun Laoghaire, Ireland) is marketed as an anti-osteoporotic drug that has anti-resorptive and bone forming effects which can consequently decrease the risk of fracture in postmenopausal women. Protelos®, is the only drug that has a dual effect on bone remodelling [23, 24]. In vitro studies have shown that Protelos® can induce bone cell replication and collagen synthesis, while also inhibiting the activity of osteoclasts [23, 24]. Both processes are crucial to the formation of new healthy bone. These effects are specific to Sr since neither Ca-ranelate nor Na-ranelate have the same effect. In vivo studies also concluded that Protelos® can promote bone formation and inhibit bone resorption in animals with bone remodelling [23, 24].

Bioactivity is important when considering a material for implantation into the body. Bioactive materials can bond directly to living bone through the formation of an apatite layer on the surface [25]. In order to reproduce the formation of apatite layers on potential bioactive materials in vitro, Kokubo et al. [25] developed acellular simulated body fluid (SBF) that has inorganic ion concentrations similar to human body fluid. In a study by Kamitakahara et al. [26], the suitability of GPCs for bone cementation was questioned as the authors found that it was not possible for apatite to form on conventional, Al-containing, GPCs after immersion in SBF. The study concluded that the presence of small quantities of PAA released from the GPCs inhibits apatite formation. However, in a related study to the work contained herein, Boyd and Towler [13] undertook SBF studies of the Zn-based GPCs and showed that an amorphous layer of calcium phosphate does indeed nucleate and grow on the surface of these cements, should the cement release less than a threshold level of Zn^{2+}.

This study will build on the previous work [13] and examine the effect of PAA molecular weight, concentration and maturation time on the mechanical properties, working and setting times of GPCs formulated from a range of ionomeric strontium–calcium–zinc-silicate glasses that contain increasing amounts of Sr in place of Ca. SBF studies will subsequently show that these cements exhibit bioactivity in vitro in SBF.

2 Material and methods

2.1 Glass synthesis

Three glass formulations were synthesized. Glasses BT 100, BT 101 and BT 102. BT 100 contains only calcium, zinc and silica, while BT 101 and BT 102 contain increasing quantities of strontium at the expense of calcium. Table 1 illustrates these glass compositions, expressed as mole fractions.

Glasses were prepared by weighing out appropriate amounts of analytical grade reagents (Sigma-Aldrich, Dublin, Ireland) and ball milling for 1 h. The mixes were subsequently dried in an oven (100 °C, 1 h). Compositions were then fired (1,580 °C, 1 h) in mullite crucibles and shock quenched into water. The resulting frit was dried, ground and sieved to retrieve a glass powder with a maximum particle size of <45 μm.

Table 1 Glass compositions (mole fraction)

Glass	SrO	CaO	ZnO	SiO_2
BT 100	0	0.16	0.36	0.48
BT 101	0.04	0.12	0.36	0.48
BT 102	0.08	0.08	0.36	0.48

2.2 Polyacrylic acid (PAA)

The PAAs used in this study were supplied by Advanced Healthcare Limited (Kent, UK) and were coded E8 (Mw, 50,000) and E9 (Mw, 80,800). Each acid was freeze dried, ground and sieved to retrieve <45 μm acid particles.

2.3 Cement preparation

Cements were prepared by thoroughly mixing the glass powders (<45 μm) with the PAA and distilled water on a glass plate. Complete mixing was undertaken within 30 s. About 18 formulations were used to make cements (*i.e.* three glasses mixed with two PAAs at three concentrations; 40, 45 and 50 wt%) (Table 2).

2.4 Determination of working and setting times

The working time (W_t) was defined as the period of time from the start of mixing during which it was possible to manipulate the material without having an adverse effect on its properties. The setting times (S_t) of the cement series were tested in accordance with ISO9917E [27].

2.5 Determination of compressive strength

The compressive strengths of the cements were evaluated in accordance with ISO9917E. Cylindrical samples were tested after 1, 7, 30 and 90 days. Testing was undertaken on an Instron 4082 (Bucks, UK) using a 5 kN load cell at a crosshead speed of 1 mm/min^{-1}.

2.6 Determination of biaxial flexural strength

The flexural strengths of the cements were evaluated by a method described by Williams et al. [28]. Cement discs were tested after 1, 7, 30 and 90 days. Testing was undertaken on an Instron 4082 (Bucks, UK) using a 5 kN load cell at a crosshead speed of 1 mm/min^{-1}.

Table 2 Cement formulations

Wt%	Glass (g)	PAA (g)	H_2O (ml)
40	1	0.3	0.45
45	1	0.33	0.41
50	1	0.37	0.37

2.7 Simulated body fluid trial

Simulated body fluid (SBF) was produced in accordance with the procedure outlined by Kokubo et al. [25]. The composition of SBF is outlined in Table 3. The ionic concentrations (mM) of SBF and human blood plasma are compared in Table 4. The reagents were dissolved in order, from reagent 1–9, in 500 ml of purified water using a magnetic stirrer. The solution was maintained at 36.5 °C. 1 M-HCl was titrated to adjust the pH of the SBF to 7.4. Purified water was then used to adjust to volume of the solution up to 1 l. The SBF was stored in a refrigerator and any that formed precipitates was discarded. Each glass formulation was mixed with E9 PAA at a concentration of 50 wt% to produced samples of cement cements for immersion in SBF. These cement discs (n = 2) were produced in an identical fashion to the flexural samples and were subsequently stored in SBF for 1, 7, 30 and 90 days in an incubator at 37 °C. A Phillips Xpert MPD Pro 3040/60 X-ray Diffraction (XRD) Unit (Phillips, Netherlands) was used to perform glancing angle (G-XRD) on the surface of the cements exposed to SBF. A JOEL JSM-840 scanning electron microscope equipped with a Princeton Gamma Tech (PGT) Energy Dispersive X-ray (EDX) system was used to obtain secondary electron images and carry out chemical analysis of the surface of cement discs. All EDX

Table 3 Composition of SBF

Order	Reagent	Required amount
1	NaCl	7.996 g
2	$NaHCO_3$	0.35 g
3	KCl	0.224 g
4	$K_2HPO_4 \cdot 3H_2O$	0.228 g
5	$MgCl_2 \cdot 6H_2O$	0.305 g
6	1 M-HCl	40 ml
7	$CaCl_2$	0.278 g
8	Na_2SO_4	0.071g
9	$NH_2C(CH_2OH)_3$	6.057 g

Table 4 Ionic concentration (mM) of SBF and human blood plasma

Ion	Reagent	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

spectra were collected at 20 kV, using a beam current of 0.26 nA. Quantitative EDX converted the collected spectra into concentration data by using standard reference spectra obtained from pure elements under similar operating parameters.

3 Results

3.1 Working and setting times

The working time (W_t) was defined as the period of time from the start of mixing during which it was possible to manipulate the material without having an adverse effect on its properties. The S_t of the cement series were tested in accordance with ISO9917E. Table 5.

3.2 Compressive and flexural strength testing

Figures 1–4 illustrate the effect of maturation time and PAA concentration and molecular weight on the compressive and biaxial flexural strength of the GPCs. Increasing PAA concentration and molecular weight result in cements with higher strengths. However, strength did not increase with maturation. This result is in contrast to the behaviour of conventional GPCs. The strongest cements were produced by mixing glasses with the higher molecular weight acid at the highest concentration (*i.e.* E9 at 50 wt% concentration).

Table 5 Working and setting times of cement series

	Working times (s)				Setting times (s)		
	Wt %	40% Wt	45% Wt	50% Wt	40% St	45% St	50% St
E8	BT 100	29	28	27	65	61	58
	BT 101	26	25	24	48	45	44
	BT 102	23	23	23	34	34	32
E9	BT 100	24	22	20	105	75	55
	BT 101	21	20	19	110	65	55
	BT 102	19	17	16	50	55	60

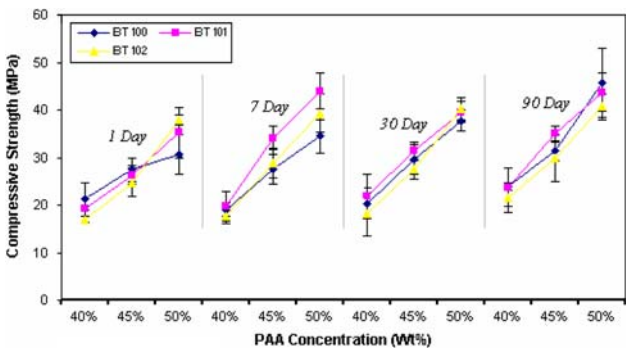

Fig. 1 E8 Compressive strength with respect to PAA concentration and maturation

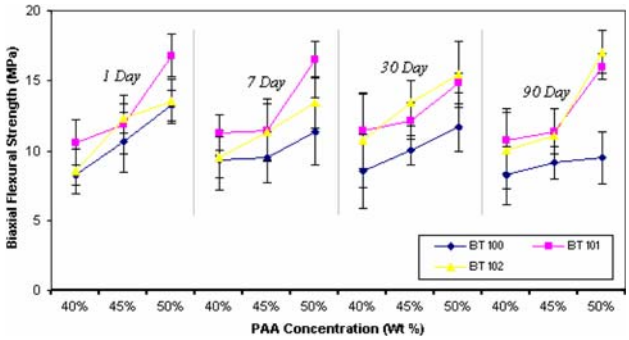

Fig. 2 E8 Biaxial flexural strength with respect to PAA concentration and maturation

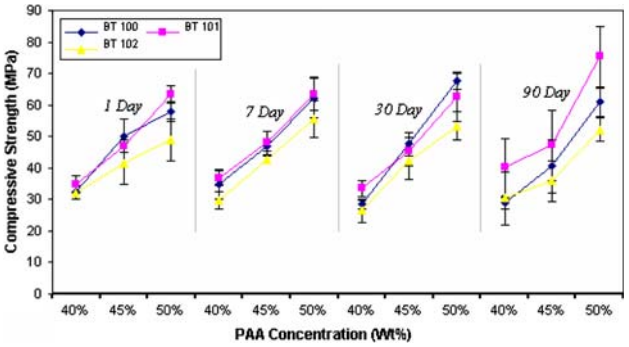

Fig. 3 E9 Compressive strength with respect to PAA concentration and maturation

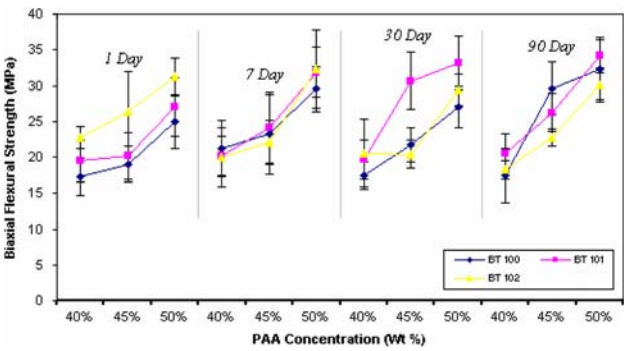

Fig. 4 E9 Biaxial flexural strength with respect to PAA concentration and maturation

3.3 Simulated body fluid trial

The cements chosen for the SBF studies were those that exhibited the highest strengths, *i.e.* all three glasses mixed

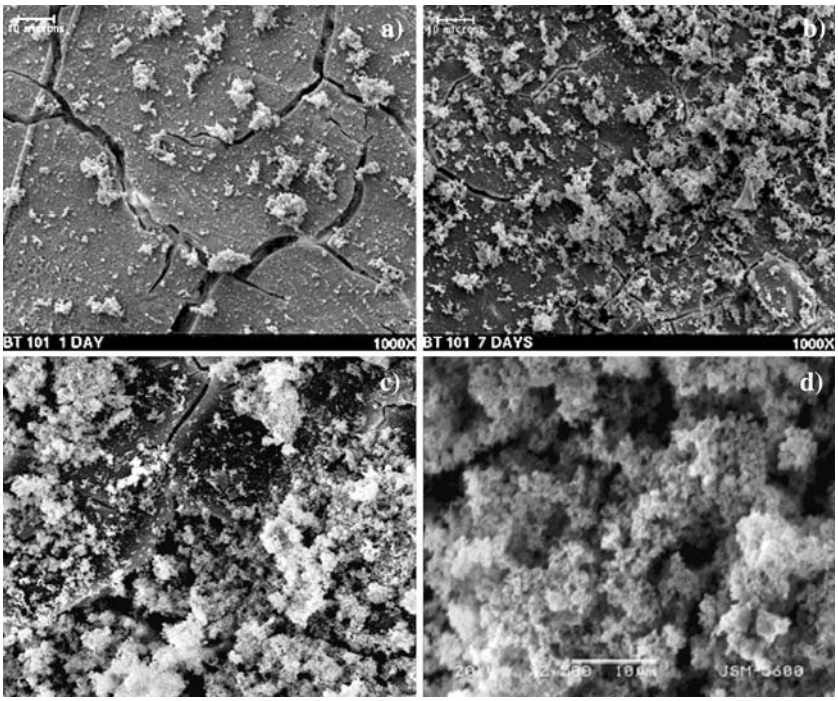

Fig. 5 SEM image of BT 101at 1,000× after (**a**) 1 day, (**b**) 7 days, (**c**) 30 days and (**d**) 90 days

with E9 at 50 wt% concentration. Each GPC exhibited similar bioactivity, with a Ca/P layer growing on the surface of each disc. Figure 5 is a selection of SEM images of BT 101 over four time frames (1, 7, 30 and 90 days). Glancing angle X-Ray diffraction (G-XRD) was subsequently performed on the best performing cement (based on BT101 glass) to qualify the presence of a Ca/P layer.

4 Discussion

The cements examined herein demonstrate the typical working and setting time characteristics of conventional GPCs; specifically, the W_t and S_t of the experimental cements decrease with increasing molecular weight and/or concentration of PAA. This effect, previously identified in the literature [29], is due to a number of factors including an increase in the amount of carboxylate groups present that can form a chelates with released metal ions during setting [30], and increased entanglement, leading to increased viscosity associated with longer PAA chains [29]. The longest W_t attained was associated with the cement formulated from BT 100/E8 at 40 wt% (29 s), while the shortest W_t was attained from a cement formulated from BT 102/E9 at 50 wt% (16 s). Concurrent with the evaluation of W_t, the S_t of the various cement formulations were also determined. As expected, it was observed that the S_t of the cements decreased with an increase in PAA concentration. However, cements formulated with E9 (M_w = 80,800), yielded cements with a longer setting time than cements formulated with E8 (M_w = 50,000). The cement that yielded the longest S_t was formulated from BT 101/E9 at 40 wt%, while the shortest S_t was obtained from BT 102/E8 at 50 wt%. This result is anomalous, and the reason for this divergence remains unclear. However, a combination of particle size effects and slight variations between production glass batches may account for this discrepency.

An additional characteristic noted with respect the W_t and S_t of the cements was, that as Ca was substituted with Sr in the glass network, a concomitant decrease in W_t and S_t was observed; *i.e.* BT 100 was the least reactive glass, whilst BT 102 was the most reactive of all glasses. This increasing reactivity, associated with increasing Sr substitutions for Ca in Ca–Sr–Zn–SiO_2 glass networks has been observed elsewhere, [31] with the augmented reactivity being attributed to the increased basicity of SrO as compared with CaO in the glass network. Regardless of the trends discussed, both the working and setting times of the experimental cements examined herein are too rapid for clinical deployment. However, recent advances [32] indicate that the early setting reaction of these cements may be altered without adversely affecting the strengths of the materials.

In addition to W_t and S_t being evaluated, the CS and BFS for each cement formulation was determined. The results indicate, as expected [14], that the CS and BFS of the novel GPCs increase with increasing PAA molecular weight and concentration. However, in direct contrast to the behavior of conventional [14] GPCs the strengths of the

experimental materials did not increase with maturation time. Rather, these cement series' obtained the majority of their strength within the first 24 hours after setting, in a similar fashion to zinc polycarboxylate cements [14]. Importantly however, the cements yielded CS values of up to 75 MPa (BT101/E9 at 50 wt%). Such CS values are in accordance with the clinical requirements as stated in the literature [27, 33] and clearly indicate the suitability of these materials for various load bearing applications.

However, given the accepted limitations of the compressive strength test modality, the biaxial flexural strength of the materials was also determined. The BFS test exhibits a number of advantages over other strength tests such as uniaxial compression testing; primary amongst these is the absence of intersecting planes of shear, and elimination of specimen edge defects [28]. Additionally, the test also ensures the application of a pure tensile stress across the lower face of the specimen. Complementary to these features there exists a state of biaxial stress when materials are loaded in vivo therefore the BFS test mimics more closely the in vivo loading situation for a biomaterial [34], thus providing essential information on materials performance. The cements herein exhibit BFS values up to 34 MPa, which is comparable to commercially available load bearing GPCs [35], and further reinforces the potential load bearing ability of the novel GPCs.

To gain insight into the possible bioactivity of the novel GPCs, specimens of each formulation were incubated in SBF (37 °C) for periods up to 90 days. It is the accepted philosophy that materials capable of forming an apatite layer in SBF bond directly to bone upon implantation in living bone [25].

All specimens immersed in SBF facilitated the deposition of a layer at their surfaces, which increased in coverage and density with immersion time (Fig. 5). EDX analysis of these surface layers indicate that they are primarily composed of calcium and phosphorus; the principle components of hydroxyapatite (HA). However, quantitative EDX analysis (Table 6) suggests that the Ca:P ratio of the layers is significantly dissimilar to HA (Ca:P of 1.67). After 1 day, the Ca:P ratio (3.07) is uncharacteristic of an apatite layer. However, this is likely due to an increased Ca signature from the cement, which has not yet been masked by the deposition of a dense apatite layer. After 7 days

Table 6 Normal wt% of Ca and P at the surface of cement based on BT101, E9 50 wt% after SBF immersion

Norm wt%	1 day	7 days	30 days	90 days
Ca	14.3	12.51	21.24	16.38
P	4.66	11.91	22.98	15.13
Ca/P	3.07	1.05	0.92	1.08

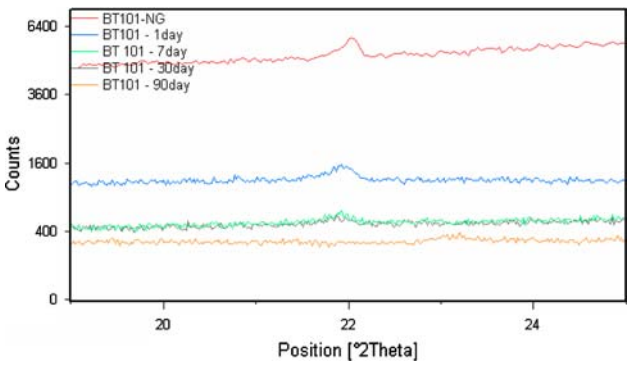

Fig. 6 G-XRD of BT 101 with control (BT1010-NG) and immersed cements with respect to time

immersion, when the coverage of the layer appears more dense, the Ca:P ratio stabilises (1.05) and varies only marginally up to 30 days (1.08). Such Ca:P ratios are characteristic of calcium phosphates like Dicalcium Phosphate Dihydrate (DCPD)[36] which are regarded as precursors to HA. G-XRD indicated that the calcium phosphate surface layers were amorphous (Fig. 6), with the lack of crystallinity due to the inhibitory effects of zinc ions on the crystallization kinetics calcium phosphates [37]. However, GPCs based on BT101 exhibit a single crystalline peak; reflective of residual crystallinity in the glass phase of BT101, from which the cement is formulated. This peak, present at 2θ equal to 22° reduces in height with respect to immersion time, due to the masking effects of the increasingly dense Ca/P layer growing on the surface of the cement. However, the principal finding is that the cements examined herein are capable of forming a calcium phosphate layer at their surface and based on current opinion this indicates that a direct bond with living bone tissue will be possible in vivo.

5 Conclusions

The working times and setting times of the GPCs examined in this paper are, at present, too fast for clinical use. However, with manipulation of the cement formulation it is expected that these properties can be adjusted without adverse effects on the mechanical properties. Additionally, the compressive strengths, biaxial flexural strengths and in vitro response indicate that the materials are suitable for load bearing applications and will likely bond to living bone tissue in vivo. Finally, the incorporation of Sr into the glass phase of the cements did not have a deleterious effect on the examined properties of the GPCs. An in-vivo study is now required to determine whether the presence and release of Sr imparts a bone building quality to the cements.

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