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Fluoride-containing bioactive glasses and Bioglass[®] 45S5 form apatite in low pH cell culture medium



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ABSTRACT

The dissolution re-precipitation behaviour of a series of fluoride-containing bioactive glasses was investigated under physiologically relevant conditions of low pH. All formulations including the classic fluoride-free 45S5 Bioglass[®] dissolved in acetate-buffered MEM, and formed apatite despite the pH remaining acidic throughout. Fluoride incorporation into the glasses and subsequent fluoride release resulted in the formation of an apatite (fluorapatite) of higher crystallinity; however, with increasing fluoride content in the glass, fluorite was formed at the expense of apatite. Results suggest that low fluoride content BG form apatite well at low pH conditions, representative of those present during the inflammation phase of normal bone healing or within the oral environment during caries or an acid-erosive challenge.

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1. Introduction

Physiological pH is widely regarded as being pH 7.4; however, under several ‘normal’ conditions commonly encountered at healing wound sites or in the oral environment, the pH is known to become acidic. Infection and bacterial growth at a fracture site produce persistent acidic local pH [1,2], while uninfected tissue pH is also lower than normal serum pH during early healing [3]. Furthermore, in the oral environment, the pH fluctuates to below 5.5 due to caries activity [4], or during consumption of acidic beverages. Osteoclasts reduce the local pH to 4.5 in order to demineralise/resorb bone tissue [5]. Local acidosis affects physiological mechanisms, for instance, bone nodule mineralisation [6], and VEGF expression from osteoblasts [7].

Despite this, *in vitro* tests on bioactive glasses (BG) and other biomaterials are usually performed in test solutions such as SBF or cell culture media, at pH 7.4. As acidic pH conditions prolong the inflammatory process and delay tissue healing, BG compositions that perform well in such conditions could find novel applications in orthopaedics [8], and as remineralising additives for toothpastes.

Here, a series of fluoride-containing BG and Bioglass[®] 45S5 are evaluated by *in vitro* dissolution tests using Eagle’s Minimal

Essential Medium with Earle’s Salts at pH 4.8. The study aims to understand how acidic pH conditions affect bioactivity in association with BG fluoride content.

2. Materials and methods

Three fluoride-containing BG compositions were prepared by melt-quench route (Table 1), as previously described [9]. Bioglass[®] 45S5 was prepared as a control. The dissolution medium (A-MEM) was based on Eagle’s Minimal Essential Medium with Earle’s salts (Sigma Aldrich, UK). Briefly, MEM powder was dissolved in deionised water, sodium acetate buffer solution (pH 4.6) and sodium azide were added and the pH adjusted to 4.80. Dissolution experiments, elemental analysis and powder characterisation were carried out as previously described [10].

One-way analysis of variance (ANOVA) with *post hoc* Bonferroni analysis was used for statistical analysis; *p* values < 0.05 were considered significant. Mean values ± standard deviations are presented.

3. Results and discussion

BG immersion in low pH A-MEM resulted in the typical pH increase; however, pH remained below 6 for all BG (Fig. 1a). F17 gave the lowest pH at both time points (*p* < 0.05), but the pH continued to rise between days 3 and 7 (*p* < 0.05), while it stayed constant for F4 and F9 (*p* > 0.05). Control 45S5 also gave a pH increase throughout the study (*p* < 0.05), but absolute values

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were comparable to F4 and F9. This pH rise commonly observed for BG is caused by ion exchange, where modifier cations released from the glass are exchanged for protons from the surrounding solution [12]. Fluoride-containing BG demonstrate a less pronounced pH rise [13], and this effect might partially be caused by exchange of F⁻ from the glass for OH⁻ from the solution, reciprocal to the cation exchange [14]. The pH trends suggest that ion exchange continues for longer (at least over 7 days) in F17 and 45S5 than for F4 and F9, presumably caused by differences in surface layer formation.

Alkaline pH conditions generally favour apatite formation; however, apatite is the most stable calcium phosphate between pH 4.5 and 10 [15], and thus pH values around 5 should not impede apatite precipitation. Despite the low pH testing conditions, all BG formed apatite within 7 days or less. FTIR spectra (Fig. 2) show a split band at 560–610 cm⁻¹ and a sharp band at 1040 cm⁻¹ at day 3, both of which indicate the presence of apatite or other crystalline calcium orthophosphates.

XRD supports this by showing characteristic reflections for apatites, including FAp and carbonated apatites, in the 30–35° 2θ range (Fig. 3). There are, however, distinct differences with glass composition. 45S5 exhibits peak broadening on XRD, which is typical for apatite formed by BG, as the crystallites are usually in the nanometre size range [16] and of poor crystallinity owing to

substitutions into the apatite lattice [13]. The reflections for F4, by contrast, are of higher intensity and much more clearly defined, suggesting that the presence of F⁻ (Fig. 1e) either causes faster

Table 1
Nominal glass compositions (mol%); network connectivity [11] of all glasses is 2.1.

Glass	SiO ₂	P ₂ O ₅	CaO	Na ₂ O	CaF ₂
F4	47.12	1.02	21.98	25.13	4.75
F9	44.88	0.97	20.94	23.93	9.28
F17	40.68	0.88	18.98	21.69	17.76
Bioglass [®] 45S5	46.1	2.6	26.9	24.3	–

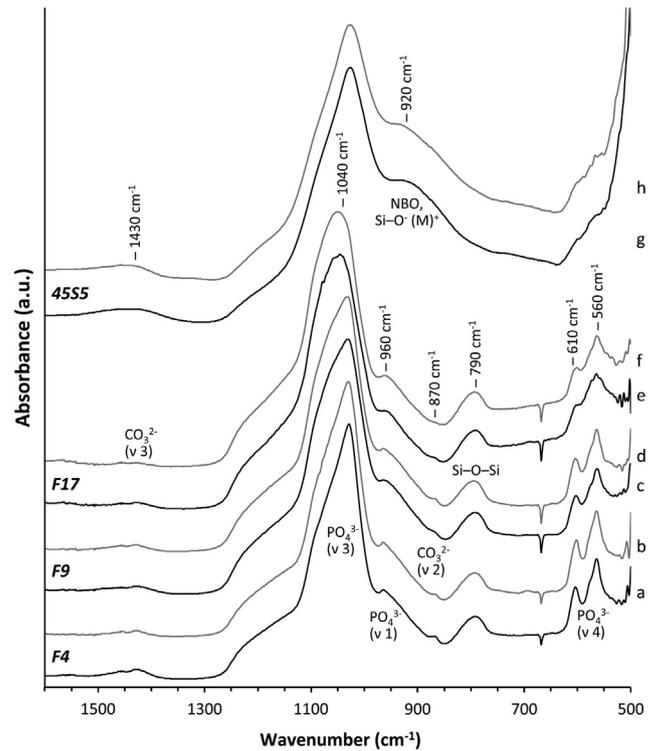


Fig. 2. FTIR spectra of F4, F9, F17, and 45S5 (a, c, e, and g, respectively) after 3 days and F4, F9, F17 and 45S5 (b, d, f, and h, respectively) after 7 days immersion in A-MEM.

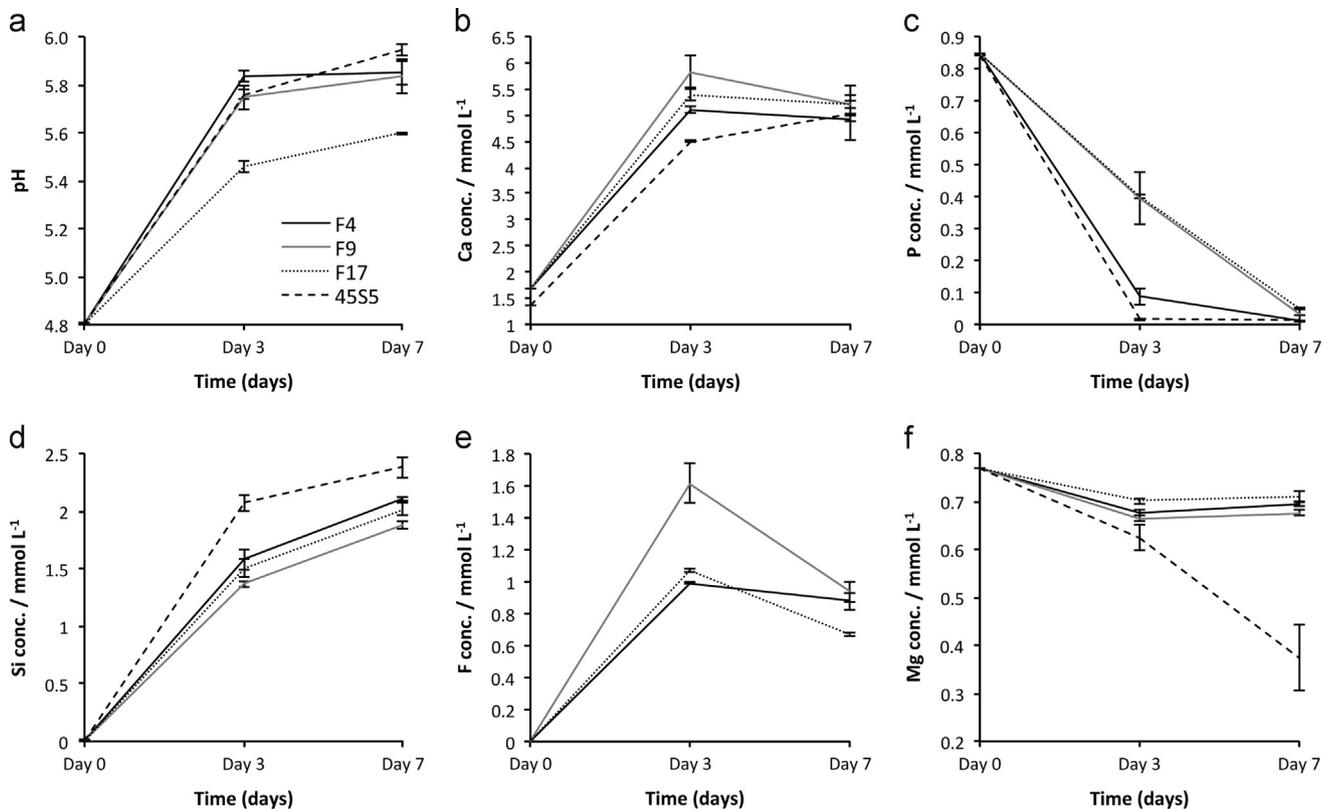


Fig. 1. pH changes and concentrations (± SD) of Ca²⁺, PO₄³⁻, Si⁴⁺, F⁻, and Mg²⁺ at day 0 (as-prepared), 3 and 7. (Lines are visual guides only).

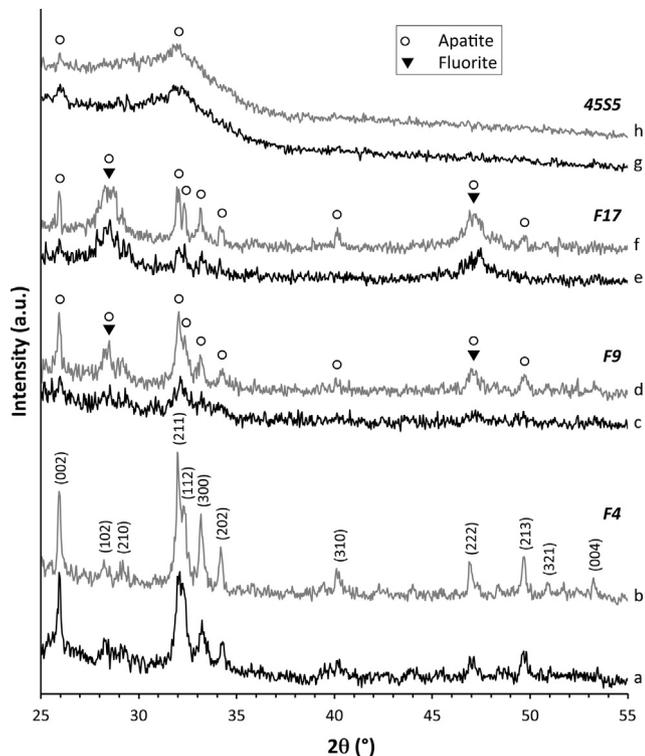


Fig. 3. XRD patterns of F4, F9, F17, and 45S5 (a, c, e, and g, respectively) after 3 days and F4, F9, F17 and 45S5 (b, d, f, and h, respectively) after 7 days immersion in A-MEM.

apatite formation and/or improved apatite crystallinity, most likely through formation of FAp rather than HAp or HCA [17]. And, indeed, FAp is known to exhibit lower solubility than HAp or HCA [18], and should be expected to form preferentially at low pH if fluoride ions are present.

While fluoride clearly seems to have beneficial effects on apatite formation, more fluoride does not necessarily enhance apatite formation, since apatite reflections on XRD show a pronounced decrease in intensity with increasing glass fluoride content. These observations are confirmed by FTIR, where apatitic phosphate bands are much more clearly defined for F4 than 45S5 and decrease in intensity in the order $F4 > F9 > F17$. This has been explained by the formation of fluorite (CaF_2) in addition to apatite [13], as confirmed by the typical reflections corresponding to fluorite for glasses F17 (days 3 and 7) and F9 (day 7) on XRD.

SEM (Fig. 4) shows the presence of typical apatite needle-like structure. The crystals are smaller, more numerous, and develop a higher aspect ratio with increasing glass fluoride content and reaction time. 45S5 is known to form smaller, less-distinct crystallites (Supplementary data), considered to (partially) be octacalcium phosphate (OCP) before transforming to HCA, as shown previously by ^1H MAS-NMR spectroscopy [19]. Fluoride in the glass results in direct apatite formation without going through OCP and the needle-like habit is observed. There is evidence of some small cubic crystals with F17 and F9 at day 7, which probably correspond to the typical cubic crystal habit of fluorite.

Apatite formation can be followed by measuring P (or PO_4^{3-}) concentrations in solution, which decrease significantly between days 0 and 3 (Fig. 1c). Again, differences are observed with glass composition. F4 and 45S5 solutions are depleted by day 3, while

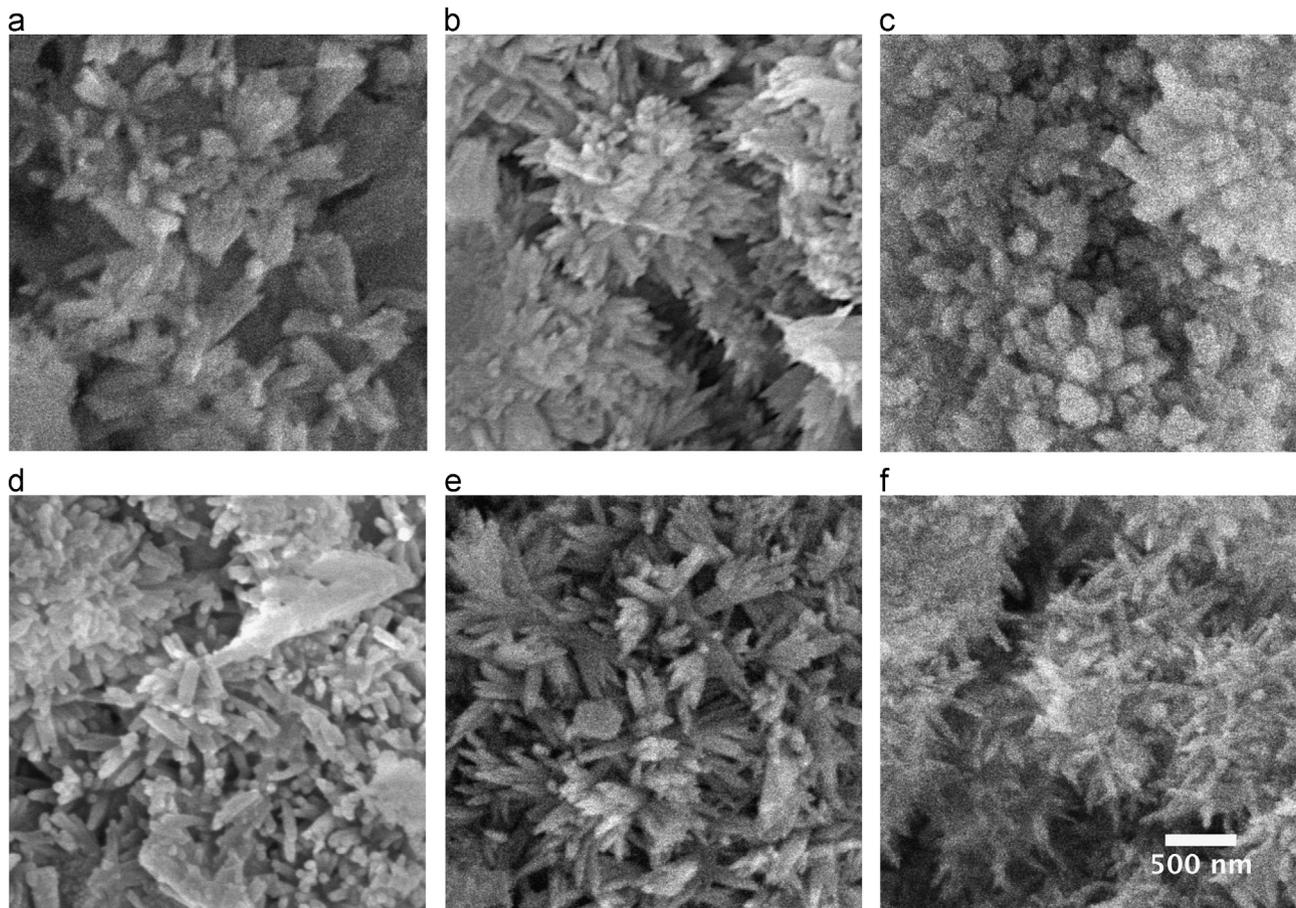


Fig. 4. SEM images showing representative regions of F4, F9, and F17, respectively after immersion for 3 days (a–c) and 7 days (d–f) in A-MEM.

relatively more remains in F9 and F17 solutions, but continues to decrease between days 3 and 7. This suggests that fluorite formation consumes Ca^{2+} and F^- , thus disfavours apatite precipitation. Interestingly, for both F4 and 45S5 all available phosphate is consumed during the first 3 days, indicating that apatite formation for 45S5 is not slower than for F4 as suggested by FTIR and XRD. The poor intensity of apatite reflections on XRD for 45S5 is therefore likely to be caused by differences in crystallinity (or crystallite size) compared to F4. Phosphate depletion shows that the presence of phosphate is the limiting factor for apatite formation, as Ca^{2+} (Fig. 1b) and F^- (Fig. 1e) which are released from the glass, increase between days 0 and 3 and subsequently either stay constant or decrease slightly between days 3 and 7. In test solutions of higher phosphate concentration, apatite formation should be expected to increase and fluorite formation to decrease [20].

Fluoride ions originate from glass dissolution only, as the culture medium is fluoride-free. Reduction of fluoride concentrations in solution suggests the possibility of fluoride-substituted (rather than fluoride-free) apatite, as previously shown by ^{19}F MAS-NMR spectroscopy [13,17].

The presence of Si^{4+} ions in solution (Fig. 1d) shows that glass dissolution not only occurs by ion exchange but also by dissolution of the silicate network, through a combination of Si–O–Si bond cleavage and solubilisation of entire chains [11]. The solubility of Si^{4+} increases with reducing pH, which explains the high Si^{4+} concentrations found particularly at 7 days.

Mg^{2+} ions originate from the culture medium only. Interestingly, the concentrations decrease dramatically between days 0 and 3, after which they remained relatively constant (Fig. 1f), suggesting that Mg^{2+} is incorporated into the apatite lattice. Mg^{2+} ions retard apatite formation [21] by adsorption onto crystal surfaces and blocking active growth sites [22], irrespective of whether Mg^{2+} ions originate from test media or from the glass [23]. Here, the presence of Mg^{2+} did not impede apatite formation; however, Mg^{2+} substitution into the apatite lattice reportedly results in higher solubility than stoichiometric apatite and lower crystallinity (smaller crystallite size), an effect comparable to CO_3^{2-} and in contrast to F^- . Increasing F^- content reduces the solubility of magnesium-containing fluorapatite at low pH [24], an effect most likely to have happened here which probably partly explains the greater apatite formation seen for the fluoride-containing glasses.

4. Conclusions

Bioactive glasses partially dissolved in acetate-buffered MEM and formed apatite despite the pH remaining below 6 throughout the study. Fluoride incorporation into the glasses and subsequent fluoride release resulted in the formation of an apatite (fluorapatite) of higher crystallinity; however, with increasing fluoride content in the glass, fluorite was formed at the expense of apatite. These results suggest that low-fluoride content BG forms apatite well at low pH conditions, for example during inflammation or even in the mouth during a caries or acid erosion challenge.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.matlet.2013.12.102>.

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