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The effect of the incorporation of fluoride into strontium containing bioactive glasses

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ABSTRACT

Recently, the porous bioactive glasses have attracted a lot of attention for use as scaffolds for tissue engineering bone; such glasses include the high phosphate, strontium containing glass (Stronbone P). However, the previous studies suggest that strontium can have a detrimental effect on the ability of apatite-like phase formation of the glass. The previously studied high phosphate all Sr glass showed an unidentified phase rather than an apatite-like phase upon immersion. Octa-calcium phosphate (OCP) is believed to be a precursor phase to apatite, however octa-strontium phosphate does not exist. Fluoride is known to knock out the OCP formation and promotes fluorapatite formation. This work presents the incorporation of a small amount of fluoride into calcium/strontium bioactive glasses. Differential scanning calorimetry was used to estimate the glass thermal properties. All of the studied glass compositions were subjected to bioactivity studies in Tris buffer (pH = 7.4) for up to 21 days. The initial glasses and the resultant precipitates were analysed using Fourier transform infrared spectroscopy, X-ray diffraction and magic angle spinning-nuclear magnetic resonance. The findings showed that all the fluoride containing glasses were amorphous and there was a marked increase in the rate of apatite formation in vitro compared to the equivalent fluoride free glasses, particularly for the all strontium containing glass. This indicates that the presence of fluoride affects the pathway of apatite formation, forming fluorapatite directly instead of via the transformation from OCP to hydroxyapatite. Therefore, fluoride may have potential future clinical applications as an additive to increase apatite formation.

1. Introduction

There is considerable interest in developing porous bioactive glasses for the use as scaffolds for tissue engineering bone. A glass suitable for the use as a scaffold should fulfill the following criteria:

1. It should be capable of being sintered by a viscous flow mechanism without crystallisation to give a three dimensional open porous structure with an interconnect size of at least 100 μm to facilitate infiltration by both blood vessels and osteoblasts.
2. It should form an apatite-like layer on its surface.
3. It should not result in a too high pH rise in vivo.
4. It should actively stimulate the formation of new bone tissue and should osseointegrate.
5. It should promote angiogenesis.

The strontium containing bioactive glass (SP-Ca/Sr, Stronbone P) given in Table 1 exhibits these properties and shows rapid osseointegration and new bone formation in vivo [1]. Strontium is known to up-regulate osteoblasts and down-regulate osteoclasts [2]. Strontium is also slightly bactericidal [3] [4] and also of relatively high atomic number enabling the placement and resorption of strontium based biomaterials to be visualised using standard X-ray methods. Silicon released from bioactive glass has been shown to stimulate angiogenesis [3]. Glass SP-Ca/Sr exhibits a large processing window defined as the onset temperature of crystallisation minus the glass transition temperature ($T_{conset} - T_g$). This large processing window of over 200 °C enables the glass particles to be viscous flow sintered without crystallisation occurring. This enables the glass to be formed into porous structures by a variety of routes. The low alkali metal content and high phosphate content reduces the pH rise, while the high phosphate content is known to increase the amount of apatite formed and reduce the time to form apatite [5] [6]. However, Sriranganathan et al. [7] found that the introduction of strontium has a detrimental effect on the apatite-like phase formation ability of the glasses.

Sriranganathan et al. investigated the in vitro bioactivity of the glass given in Table 1 and the equivalent glasses containing only calcium and no strontium and only strontium and no calcium [7]. The all strontium

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Table 1
Glass composition (mol%), network connectivity (NC) and glass firing temperature (°C).

Glass code	SP-Ca/Sr[a]	SP-Ca[b]	SP-Sr[c]	SPF-Ca/Sr[d]	SPF-Ca[e]	SPF-Sr[f]
SiO_2	44.5	44.5	44.5	43.63	43.63	43.63
P_2O_5	4.5	4.5	4.5	4.41	4.41	4.41
Na_2O	4	4	4	3.92	3.92	3.92
K_2O	4	4	4	3.92	3.92	3.92
CaO	17.8	35.6	0	17.45	34.90	0
SrO	17.8	0	35.6	17.45	0	34.90
MgO	7.5	7.5	7.5	7.35	7.35	7.35
CaF_2	0	0	0	1.96	1.96	1.96
NC	2.31	2.31	2.31	2.2	2.2	2.2
T_{firing}	1460	1450	1470	1450	1440	1460

[a] SP-Ca/Sr represents Stronbone P composition.
[b] SP-Ca represents the derived Stronbone P composition where all Sr has been replaced by Ca.
[c] SP-Sr represents the derived Stronbone P composition in which all Ca has been replaced by Sr.
[d] SPF-Ca/Sr represents the derived Stronbone P composition where CaF_2 is added to Stronbone P with the ratios of other components kept constant.
[e] SPF-Ca represents the derived SP-Ca composition in which CaF_2 is added to SP-Ca with the ratios of other components kept constant.
[f] SPF-Sr represents the derived SP-Sr composition where CaF_2 is added to SP-Sr with the ratios of other components kept constant.

high phosphate bioactive glass showed the glass to form a new unidentified phase upon immersion in Tris buffer. This new phase is not a strontium apatite ($Sr_{10}(PO_4)_6F_2$) or Collins salt ($Sr_6H_3(PO_4)_5 \cdot 2H_2O$) and has not been found previously. This phase seems to be a strontium orthophosphate, based on FTIR and ^{31}P MAS-NMR data. We have termed this new unidentified phase as "X-phase". In contrast, the related calcium containing glass and mixed calcium/strontium glass form an apatite-like phase in both Tris buffer and simulated body fluid (SBF). The all strontium glass also forms an apatite-like phase in SBF though its identification is far from conclusive. It is thought that apatite formation goes via the formation of an octa-calcium phosphate precursor phase ($Ca_8(PO_4)_6H_2 \cdot 5H_2O$) if the pH < 9 [8] [9]. However, octa-strontium phosphate ($Sr_8(PO_4)_6H_2 \cdot 5H_2O$) does not exist [7]. Consequently with the strontium bioactive glass it is thought that because the precursor phase cannot form, that the formation of an apatite phase is not possible and the X-phase forms instead. In SBF, which contains calcium ions, the strontium containing glass degraded and resulted in octa-calcium phosphate formation with possibly a small amount of strontium substituted for calcium in the octa-calcium phosphate lattice. Fluoride is known to knock out the OCP precursor phase and form an apatite directly [10]. Brauer et al. [11] and Mneimne et al. [12] showed that fluoride containing bioactive glasses formed fluorapatite and that apatite formation was enhanced by the presence of fluoride. Their results [11] [12] can be explained by fluoride knocking out the precursor OCP phase and forming an apatite directly. The fluoride ion is smaller than hydroxyl ion and therefore the fluoride ion can fit into the space at the center of the Ca(II) triangle in the apatite crystal lattice and form a more stable fluorapatite compared with hydroxyapatite. There is little direct conclusive evidence for OCP formation with bioactive glasses. This is in part due to OCP and hydroxyapatite being very difficult to distinguish by Fourier transform infrared spectroscopy or X-ray powder diffraction. OCP exhibits one characteristic diffraction line at 4.68° two theta with copper K-α X-rays. Most studies in the literature do not access this very low two theta range. Furthermore, OCP transforms readily to hydroxycarbonated apatite and the 4.68° two theta spacing corresponds to the large >2 nm water layer which is very prone to structural disorder and Scherrer line broadening. Fluoride is also known to knock out the formation of Collin's salt and also cause the direct formation of apatite [10]. Fluoride has been shown to not only knock out OCP as a precursor phase to apatite, but also inhibits the formation of Collin's salt from strontium and phosphate containing solutions at pH = 7.0, resulting in direct formation of a strontium fluoridated apatite [13]. Feenstra et al. [13] also found that the fluoridated apatite formed exhibited very strong heterogeneous nucleation and its formation exhibited a maximum with fluoride concentration in solution.

Apart from promoting rapid fluorapatite formation, it is also shown that fluoride regulates bone-forming cell activities, promotes osteoblasts-like cell pre-osteogenic, pro-angiogenic responses and bone resorption in vitro [14] [15]. Moreover, it is also found that fluoride enables the stimulation of osteoblast mitosis and bone formation [16]. Those benefits are favourable in the applications of bone substitutes.

Based on these factors it was chosen to investigate bioactive glasses with small fluoride additions and evaluate their degradation and apatite formation abilities in Tris buffer. The hypothesis being that fluoride release from the bioactive glasses will prevent the formation of OCP and other phases like the Collin's salt, the X-phase and form a fluoridated apatite directly, competing with the normal hydroxyapatite formation pathway [17].

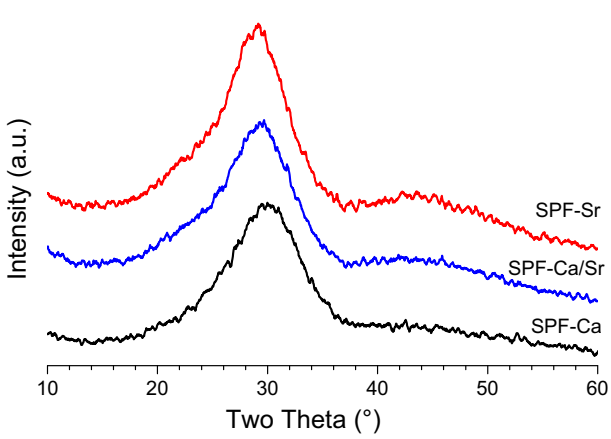

Fig. 1. XRD patterns of glasses SPF-Ca, SPF-Ca/Sr and SPF-Sr.

2. Materials and methods

The studied glasses were produced by a melt-quenched method in a similar way to a previous paper published by this research group, Sriranganathan et al. [7]. The relevant oxides and fluorides were mixed and melted at the appropriate firing temperature shown in Table 1 for 1 h in a platinum crucible. Different temperatures were used to account for the contrasting effects of strontium and fluorine on the melting temperature of the glasses [18]. After 1 h melting, deionised water was used to quench the glass and the resultant coarse powder glass was collected, dried overnight and ground into powder using a Gy-Ro Mill (Glen Creston Ltd., Twickenham UK). An Endecotts EFL 2000/1 automated sieve shaker was used to separate the powder into a fine (<38 μm) and coarse powder. A Stanton Redcroft DSC 1500 (Rheometric Scientific, Epsom, UK) was then used to analyse the fine and coarse powder to determine the glass transition temperature (T_g), crystallisation onset temperature (T_{conset}), processing window (defined

Table 2
T_g values, onset temperatures for crystallisation (T_{conset}) and crystallisation temperatures T_{c1} and T_{c2} with an accuracy of ±5 °C for glasses.

	T_g (°C)	T_{conset} (°C)	T_{c1} (°C)	T_{c2} (°C)	$T_{conset} - T_g$ (°C)
SPF-Ca <38 μm[a]	596	717	801	874	121
SPF-Ca Frit[b]	591	782	819	926	191
SPF-Ca/Sr < 38 μm	570	687	770	899	117
SPF-Ca/Sr Frit	569	771	811	914 weak	202
SPF-Sr < 38 μm	562	703	740	824	141
SPF-Sr Frit	559	708	743	906	184

[a] <38 μm represents fine powder with particle size smaller than 38 μm.
[b] Frit represents the collected glass particle with particle size about 1–2 mm.