

## Fluoride-Containing Bioactive Glasses

Delia S. Brauer<sup>1,a</sup>, Natalia Karpukhina<sup>1,b</sup>, Daphne Seah<sup>1</sup>, Robert V. Law<sup>2</sup>  
and Robert G. Hill<sup>1,c</sup>

<sup>1</sup>Imperial College, Department of Materials, Exhibition Road, London SW7 2AZ, UK

<sup>2</sup>Imperial College, Department of Chemistry, Exhibition Road, London SW7 2AY, UK

<sup>a</sup> d.brauer@imperial.ac.uk, <sup>b</sup> n.karpukhina@imperial.ac.uk, <sup>c</sup> r.hill@imperial.ac.uk

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**Abstract.** Fluoride is an important mineral for hard tissues in the body and appropriate fluoride exposure and usage are beneficial to bone and tooth integrity. Fluoride increases both bone density and bone mass due to stimulation of bone formation and it is used as a treatment for osteoporosis. Bioactive glasses have the capacity to form an intimate bond with living bone tissue due to formation of a mixed hydroxycarbonate apatite layer (HCA) in vitro and in vivo. This makes fluoride-containing bioactive glasses attractive biomaterials. In order to design fluoride-containing bioactive glasses, we need to understand the role of fluorine within the glass structure.

A series of bioactive glasses with increasing fluoride content was prepared by a melt-quench route. Characterisation included differential scanning calorimetry (DSC), density measurements, MAS-NMR spectroscopy and studies in simulated body fluid (SBF).

DSC results showed a linear decrease in glass transition temperature ( $T_g$ ) with increasing amounts of fluoride. Density of the glasses increased with increasing amounts of fluoride. This may indicate an expansion of the silicate glass network accompanying incorporation of  $\text{CaF}_2$ . <sup>19</sup>F MAS-NMR spectroscopy showed broad peaks at chemical shifts between -135 and -120 ppm. As sodium fluoride gives a chemical shift of -223 ppm and calcium fluoride of -108 ppm, this indicated possible formation of mixed calcium sodium fluoride species. HCA and calcium phosphate layers were found on the glasses after one week of immersion in SBF showing the bioactivity of the glass series.

## Introduction

Bioactive glasses are known to form an intimate bond to bone due to formation of a hydroxycarbonate apatite (HCA) layer on the surface when in contact with body fluids [1]. This is due to dissolution processes on the glass surface. Their bone bonding ability makes bioactive glasses of interest for use as implant materials, especially for use as a reconstructive material for hard tissue such as bone. Fluorine is an important element for mineralised tissue in the body and appropriate fluoride exposure and usage are beneficial to bone and tooth integrity. While the use of high doses of fluoride for prevention of osteoporosis is considered experimental at this point, it may have enormous potential [2].

Silicate glasses can be regarded as inorganic polymers of oxygen crosslinked by silicon atoms. The properties of such glasses may be explained on the basis of network connectivity (NC) [3]. NC is the number of bridging oxygen atoms (BO) per network forming element. Thus a glass with a NC of 2 consists of linear Si-O-Si chains while a pure silica glass has a NC of 4. NC can be used to predict glass surface reactivity, solubility or the likelihood of undergoing glass-in-glass phase separation. For example, the lower the network connectivity of a glass, the lower its glass transition temperature and the greater its reactivity and solubility. In general, reactivity and solubility change dramatically at a network connectivity of 2, which is the point where the glass structure changes from a crosslinked network to linear chains of decreasing molar mass. Thus NC is a helpful tool when designing new bioactive glass compositions.

Glass structure can be investigated using solid state nuclear magnetic resonance (NMR). NMR is element specific and highly sensitive to the local chemical environment of atoms containing magnetically active nuclei. Placing the sample in a strong magnetic field aligns the magnetic moments and splits the energy levels (Zeeman effect). The sample is pulsed at  $90^\circ$  to the applied field with radio frequency radiation and the transition from lower to higher energy states can be measured. To average out dipolar interactions of nuclei with the magnetic field a magic angle spinning (MAS) NMR technique is routinely used. During measurements the sample is rotated about an axis tilted at  $54.7^\circ$  from the vertical axis of the magnetic field inside the spectrometer.  $^{19}\text{F}$  and  $^{29}\text{Si}$  are typical spin  $\frac{1}{2}$  nuclei used for structural studies by solid state NMR. In fluoride-containing silicate glasses  $^{19}\text{F}$  and  $^{29}\text{Si}$  NMR can provide information on the linkages formed by fluorine and the  $\text{Q}^n$  structure of the silicate matrix, respectively.

The purpose of this study was to examine the effects of incorporating fluoride into bioactive glasses. Especially when designing new bioactive glass compositions, it is important to know the glass structure and how it is affected by fluoride incorporation. The aim was to determine the possible structural species formed as a result of fluoride incorporation into the bioactive glasses and how these species affect the physical properties (glass transition temperature,  $T_g$ , and density) and bioactivity of the glasses.

## Materials and Methods

**Glass Synthesis.** Glasses in the system  $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-Na}_2\text{O-CaF}_2$  were produced using a melt-quench route.  $\text{CaF}_2$  was added in increasing amounts while the ratio of the other components was kept constant. Glass composition is shown in Table 1. Mixtures of  $\text{SiO}_2$ ,  $\text{P}_2\text{O}_5$ ,  $\text{CaCO}_3$ ,  $\text{Na}_2\text{CO}_3$  and  $\text{CaF}_2$  with addition of  $<0.1$  wt%  $\text{CoCO}_3$  (to accelerate spin-lattice relaxation for  $^{29}\text{Si}$  NMR measurements) were melted in platinum crucibles for 1 hour at  $1430^\circ\text{C}$ . A batch size of approximately 100 g was used. After melting, glasses were rapidly quenched in water to prevent crystallisation. Glass transition temperature of the glasses was determined using differential scanning calorimetry (DSC). 50 mg of glass frit were analysed in a platinum crucible using analytical grade alumina powder as reference with a heating rate of 10 K/min.

Glass	$\text{SiO}_2$	$\text{P}_2\text{O}_5$	$\text{CaO}$	$\text{Na}_2\text{O}$	$\text{CaF}_2$	$\text{NC}_1$	$\text{NC}_2$	Table 1. Synthetic glass composition in mol% and theoretical network connectivity.
A	49.47	1.07	23.08	26.38		2.13	2.13	
B	47.00	1.02	21.93	25.06	5.00	2.13	1.49	
C	44.52	0.96	20.77	23.74	10.00	2.13	0.78	
D	42.05	0.91	19.62	22.42	15.00	2.13	-0.01	
E	39.58	0.86	18.46	21.10	20.00	2.13	-0.90	

$\text{NC}_1$  calculated assuming fluorine complexes calcium

$\text{NC}_2$  calculated assuming fluorine forms non-bridging fluorines attached to silicon

**Density.** A batch of glass frit of each composition was re-melted for an hour to ensure that no bubbles were formed. The melt was poured into a pre-heated rod shaped graphite mould (diameter 10 mm) and annealed. A Buehler slow speed diamond saw with coolant was used to section the glass into rods of 25 mm length. The rods were surface-finished by polishing with SiC paper. Density of the samples was measured at room temperature using Archimedes's principle with distilled water as the immersion fluid and a Stanton Unimatic laboratory balance (accuracy  $\pm 0.001$  g). The sample weight in air and when completely submerged in water was determined using a copper wire assumed to be of negligible weight compared to the glass. Density of the sample was calculated from

$$\text{density} = \frac{\text{weight of glass in air} \times \rho_{\text{water}}}{\text{weight of glass in air} - \text{weight of glass in water}} \quad (1)$$

where  $\rho_{\text{water}} = 0.997298 \text{ g/cm}^3$  at the recorded temperature.

**Bioactivity.** Bioactivity of the glasses was tested by investigating the formation of a HCA layer on the glass in simulated body fluid (SBF) [4]. SBF has ion concentrations similar to those of human blood plasma. 75 mg of glass powder (grain size < 38  $\mu\text{m}$ ) were immersed in 50 mL of SBF at pH 7.25 and placed in a shaker at 37  $^{\circ}\text{C}$  for various time periods. The solution was then filtered and the glass powder analysed using Fourier transform infrared spectroscopy (FTIR).

**MAS-NMR.** For solid state MAS-NMR experiments, the glass was ground using a Glen Creston Gyro Mill for 7 min. Glass structure was analysed using  $^{19}\text{F}$  and  $^{29}\text{Si}$  MAS-NMR. Analyses were performed using a Bruker 200 MHz spectrometer (4.7 T).  $^{19}\text{F}$  NMR data was collected at a Larmor frequency of 188.2 MHz under spinning conditions of 12.5 kHz.  $^{29}\text{Si}$  MAS NMR experiments were carried out at a Larmor frequency of 39.7 MHz and spinning speed of 4.5 kHz. To avoid the ringing effect of the probe on the measurements the Hahn-echo pulse sequence  $90^{\circ}-\tau-180^{\circ}$  was applied with the echo delay  $\tau$  76.5  $\mu\text{s}$ . The  $90^{\circ}$  pulses of 2.35 and 5.25  $\mu\text{s}$  were applied for the  $^{19}\text{F}$  and  $^{29}\text{Si}$  NMR experiments correspondingly. A recycle delay of 10 s was used for  $^{19}\text{F}$  NMR measurements, with 16 dummy scans performed before counting.  $^{19}\text{F}$  NMR background signal thoroughly acquired on the fluorine free glass of this series was subtracted from the spectra of the fluoride-containing glasses.  $^{29}\text{Si}$  NMR was set up using a 3 min recycle delay.  $^{19}\text{F}$  chemical shift scale was referenced using the -223 ppm peak of crystalline NaF. The -1.5 ppm peak of tetrakis(trimethylsilyl)methane was used for reference in  $^{29}\text{Si}$  NMR.

## Results and discussion

$T_g$  of the glasses decreased linearly with increasing  $\text{CaF}_2$  concentration (Fig. 1), while glass density increased with increasing amounts of  $\text{CaF}_2$  (Fig. 2). The linear trend in  $T_g$  of the glasses indicates that fluoride was not lost in disproportional amounts. Detailed investigations of fluorine concentration will be subject of future studies.

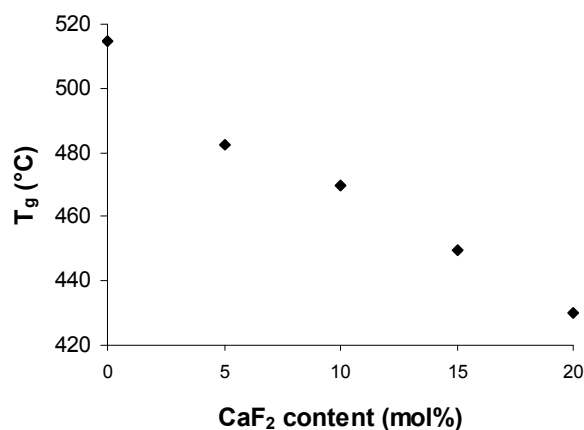


Fig. 1: Glass transition temperature ( $T_g$ ) vs.  $\text{CaF}_2$  content.

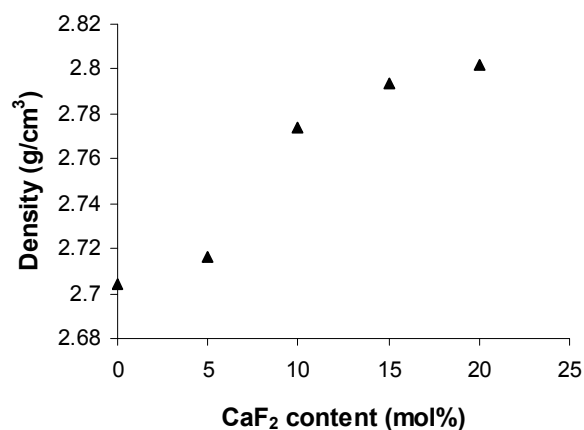


Fig. 2: Experimental density vs.  $\text{CaF}_2$  content.

Bioactivity of the glass series was investigated by immersion in SBF [4] and subsequent FTIR analysis. Formation of a HCA layer indicates bioactivity of the material. Fig. 3 shows the results for glass B. Comparing the spectrum of the untreated glass (day 0) with the immersed glass samples showed that the original broad peaks located at approximately  $1035\text{ cm}^{-1}$ ,  $925\text{ cm}^{-1}$  and  $513\text{ cm}^{-1}$  decomposed into several narrower peaks. After one week a new peak is emerging at  $570\text{ cm}^{-1}$ , which can be attributed to the formation of amorphous calcium phosphate. The formation of a Si-O-Si (stretch) peak at  $1230\text{ cm}^{-1}$  and the sharpening of the Si-O-Si (bend) peak at  $474\text{ cm}^{-1}$  indicate the formation of a silica gel layer. The peak at  $800\text{ cm}^{-1}$  can be attributed to Si-O-Si (tetra) vibrations between two neighbouring  $\text{SiO}_4$  tetrahedra. Furthermore, the Si-O peak at  $925\text{ cm}^{-1}$  which can be seen in the spectrum of the untreated glass disappeared after SBF treatment. This is due to

condensation and repolymerisation of a  $\text{SiO}_2$  rich layer on the surface, which is depleted in alkali and alkaline earth cations. The peak at  $570 \text{ cm}^{-1}$  indicates the formation of an amorphous calcium phosphate layer. This peak then splits into two peaks at  $613 \text{ cm}^{-1}$  and  $574 \text{ cm}^{-1}$  which can be explained by crystallisation of calcium phosphate, thus indicating formation of an apatite crystalline phase. The formation of crystalline HCA also causes a P-O (stretch) peak to occur at  $1076 \text{ cm}^{-1}$ . One week after immersion in SBF a carbonate peak emerges at  $1450 \text{ cm}^{-1}$ . Another carbonate peak is observed at  $950 \text{ cm}^{-1}$  which is characteristic for crystalline hydroxycarbonate apatite.

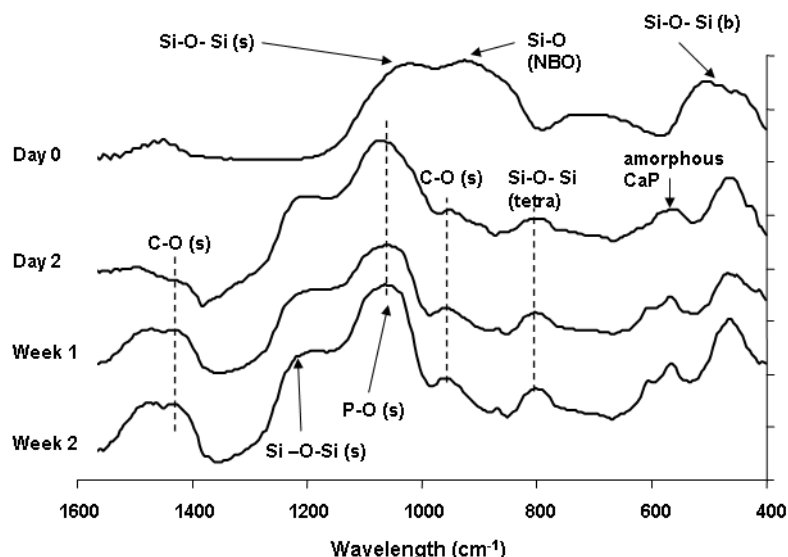


Fig. 3: FTIR spectra of glass B before and after immersion in SBF for 2 days, 1 week and 2 weeks.

Species	Chemical Shift [ppm]
F-Ca(4)	-108 [5]
F-Ca(3)Na(1)	-137 (calculated)
F-Ca(2)Na(2)	-166 (calculated)
F-Ca(1)Na(3)	-194 (calculated)
F-Na(6)	-223 [6]
Si-F-Ca(n)	-135; -129; -123 [6]
Si-F-Na(2)	-152 [6]

Table 2. Chemical shift values.

$^{19}\text{F}$  NMR spectra show a peak in the range of -135 ppm (glass B) to -120 ppm (glass E) (Fig. 4). Glass B shows two additional peaks at -220 ppm and -160 ppm, while glasses C to E show a shoulder at about -180 ppm. As sodium fluoride and calcium fluoride give peaks at -223 ppm and -108 [5], respectively, these peaks can be attributed to mixed calcium sodium fluoride species (Table 2). According to Kiczinski and Stebbins [6], Si-F-Ca(n) species show chemical shift values between and -135 ppm and -123 ppm while Si-F-Na(2) gives a chemical shift at -152 ppm. Hence some of the peaks could also be attributed to Si-F species. *E.g.* the main peak between -130 and -120 ppm in all  $^{19}\text{F}$  spectra could indicate the formation of Si-F-Ca(n) species. To investigate possible structural changes in the silicate matrix  $^{29}\text{Si}$  NMR was performed. No significant changes in  $^{29}\text{Si}$  NMR spectra are observed with the addition of  $\text{CaF}_2$  to the glass composition (from glass A to glasses B-E, Fig. 5). The Silicon spectra would be expected to move to less negative chemical shift if there was the formation of S-F bonds in the glass which does not occur. We can conclude therefore that there is no evidence for Si-F bonds in these glasses. This indicates that  $\text{CaF}_2$  addition does not cause changes in NC in this glass system with a constant  $\text{SiO}_2/\text{CaO}$  ratio. All spectra show a peak at -80 ppm, which is slightly skewed. Glasses C to E clearly show a shoulder at -92 ppm. The peak at -80 ppm corresponds to  $\text{Q}^2$  units [5], while the asymmetry for glasses A and B and the shoulder for glasses C to E indicate the presence of a small amount of  $\text{Q}^3$  units. This is in good agreement with NC calculations assuming fluorine is binding calcium and thus reducing the availability of calcium for forming NBO ( $\text{NC}_1$ , Table 1). Results give a NC of 2.13 for the glasses.

If the network connectivity is calculated assuming fluorine forms non-bridging fluorines attached to Si ( $NC_2$ , Table 1), the values are far too low to be credible.

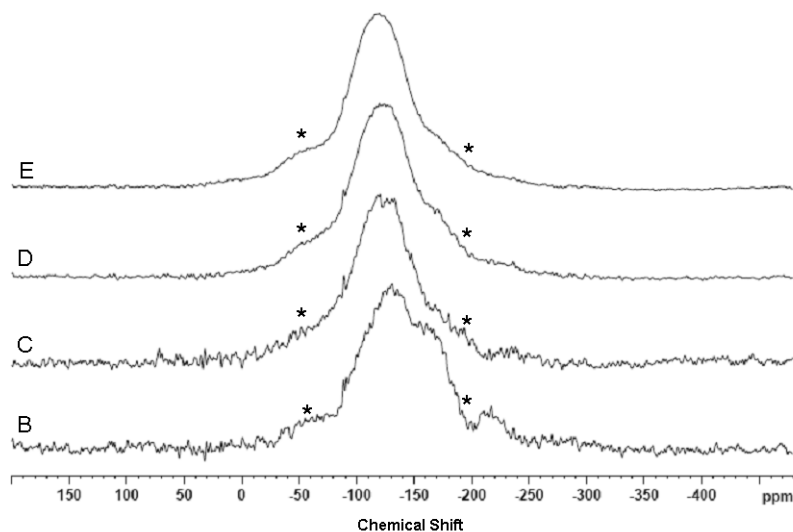


Fig. 4:  $^{19}\text{F}$  MAS NMR spectra of the glasses. Spinning side bands are marked by an asterisk.

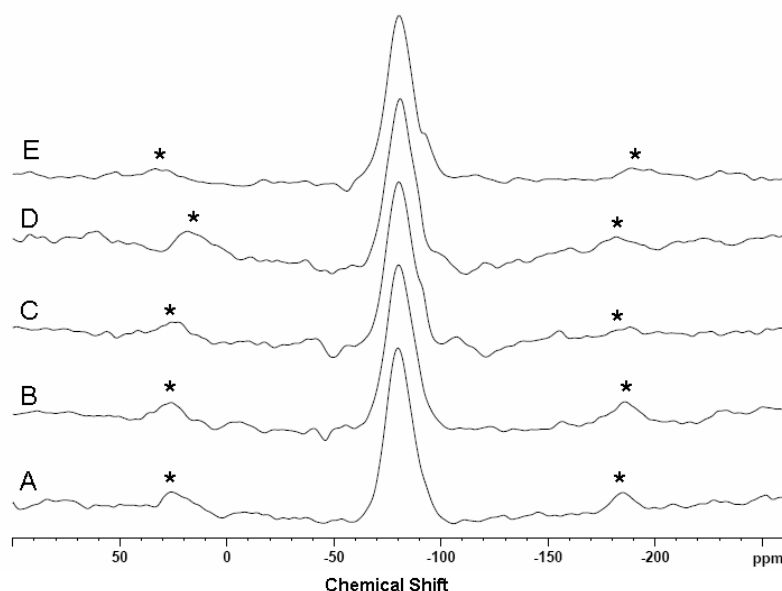


Fig. 5:  $^{29}\text{Si}$  MAS NMR spectra of the glasses. Spinning side bands are marked by an asterisk.

The structure of silicate glasses containing  $\text{CaF}_2$  has been discussed in literature. However, the conclusions vary and views range from predominant formation of Si-F bonds to  $\text{F}^-$  being coordinated with  $\text{Ca}^{2+}$  without the breakage of Si-O bonds [7-9]. Our results show good agreements with the findings by Hayashi *et al.* [7] who investigated the effect of fluorine addition on the network connectivity of glasses in the system  $\text{SiO}_2\text{-CaO-CaF}_2$  using X-ray photoelectron spectroscopy (XPS). According to their results fluorine is predominantly coordinated with  $\text{Ca}^{2+}$  and does not affect the network connectivity or the amount of non-bridging oxygen atoms (NBO). This was confirmed by  $^{19}\text{F}$  NMR investigations by Watanabe *et al.* [10].  $^{19}\text{F}$  NMR investigations on  $\text{SiO}_2\text{-CaO-CaF}_2\text{-Na}_2\text{O}$  glasses by Hayashi *et al.* showed that fluorine is coordinated with  $\text{Ca}^{2+}$  and  $\text{Na}^+$  [11]. In glasses with a network connectivity around 2, presence of Si-F bonds is unlikely since there is a large concentration of NBO in the glasses and  $\text{Si}^{4+}$  has a higher affinity for  $\text{O}^{2-}$  ions than for  $\text{F}^-$  ions [5]. Thus, fluorine preferentially binds to calcium.

The decrease in  $T_g$  and increase in density can also be explained by the fact that fluorine is complexing calcium. In fluorine-free glass A, divalent calcium ions bind together silicate anions by electrostatic forces and the calcium ions effectively act as ionic bridges between two NBO. When  $\text{CaF}_2$  is added (Fig. 6),  $\text{CaF}^+$  ion pairs are added to the silicate ions which reduces the electrostatic forces between non bridging oxygens considerable [7] and results in a decrease in  $T_g$ . Density

however increases as a result of adding  $\text{CaF}_2$  which exist in a fluorite like environment.  $\text{CaF}_2$  has a density of  $3.18 \text{ g/cm}^3$  which is much higher than that for silicate glasses.

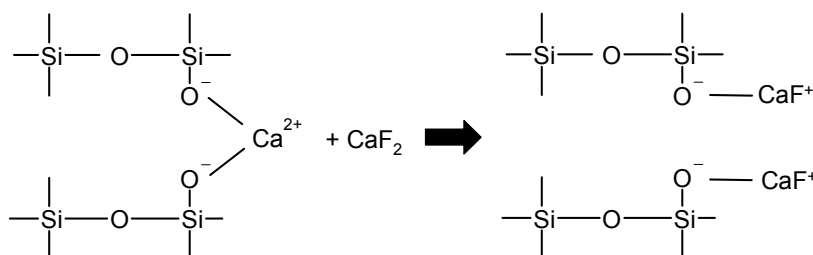


Fig. 6: Hypothetical effect of  $\text{CaF}_2$  addition on silicate network [7].

## Summary

In the fluoride-containing bioactive glasses studied fluorine is present principally as mixed calcium sodium fluoride species with no evidence of Si-F species. Thus there is no evidence for non-bridging fluorines. Network connectivity calculations assuming fluorine complexes calcium give a network connectivity of about two for the glasses studied.

$T_g$  decreased with increasing amounts of  $\text{CaF}_2$  in the glass composition, while glass density increased. Both can be explained on the basis of formation of  $\text{CaF}^+$  species. Calcium ions ( $\text{Ca}^{2+}$ ) act as ionic bridges between NBO. If  $\text{Ca}^{2+}$  is replaced by two  $\text{CaF}^+$  species, electrostatic forces are reduced, which results in the glass structure effectively being more disrupted.

The glasses showed formation of hydroxycarbonate apatite after one week of immersion in SBF. This indicates bioactivity of the glasses. The results of this study will be valuable in designing and producing new fluoride-containing bioactive glasses which are of interest for use as reconstructive material for bone and other hard tissue.

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