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The Atomic-Scale Interaction of Bioactive Glasses With Simulated Body Fluid

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Abstract. The formation of a carbonate-containing hydroxyapatite, HCAp, layer on bioactive calcium silicate sol-gel glass of the formula $(CaO)_{0.3}(SiO_2)_{0.7}$ has been studied *in-vitro* in Simulated Body Fluid (SBF). Extended X-ray Absorption Fine Structure (EXAFS), X-ray Absorption Near Edge Structure (XANES), X-ray diffraction (XRD), and solid state nuclear magnetic resonance (NMR) measurements have been performed with results showing the formation of a significantly amorphous HCAp layer after less than 5 hours in solution.

Introduction.

Most implants in use today in the field of tissue replacement continue to suffer from problems which include interfacial stability with the host tissues and the biomechanical mismatch of elastic moduli: the use of implants made from biomaterials represents a significant advance. Simple binary CaO-SiO_2 gel-glasses exhibit *Class A* bioactivity, due to the activation of seven families of gene that are present in human bone cells, by the release of soluble silica and calcium ions in very specific concentrations.[1,2]

The mechanism of the bond formation between bioactive glass and living tissue is a complex one, involving cellular processes.[3] It is well documented [3-5] that when immersing a bioactive glass in body fluid or simulated physiological solutions, an exchange of alkali ions such as $Ca^{2+}$from the glass with H_3O^+ from the fluid rapidly occurs, along with network dissolution through attack of the silica structure by hydroxyl ions. As a consequence, a layer of silanol (Si-OH) groups are formed on the glass surface and a series of polycondensation reactions form siloxane bonds (Si-O-Si). This process forms a silica gel layer with high surface area, which promotes apatite formation, through which Ca^{2+} and PO_4^{-3} ions migrate from surrounding fluids to form an amorphous CaO-P_2O_5 –rich film. This finally crystallises to carbonate-containing hydroxyapatite, HCAp, by the incorporation of OH^- and CO_3^{-2} from the surrounding tissues. This mechanism can be studied using solutions with ion concentrations similar to those of human blood plasma, such as SBF (Simulated Body Fluid).[5]

We seek to understand the process of HACp (hydroxy carbonate apatite) formation on the surface of $(CaO)_{0.3}(SiO_2)_{0.7}$ after immersion in SBF solution by looking at one of the stages in the reaction pathway, calcium sorption (Stage 2) [6] and at the increasing HCAp-like character of the gel-glass after 30 days in an SBF solution. We are using a broad-based characterisation approach, combining X-ray and neutron diffraction, X-ray absorption spectroscopy, FTIR, UV and ^{29}Si, ^{17}O and ^{43}Ca MAS NMR and here report findings on XRD, NMR, XANES and EXAFS measurements.

Experimental.

Sample Preparation. Bioglass samples were synthesised in a controlled manner following well-documented procedures [7]. The $(CaO)_{0.3}(SiO_2)_{0.7}$ sample was immersed in a Simulated Bodily

Fluid solution, equivalent to that used by Hench and co-workers [7]. Incubation periods were varied from 1 min to 30 days. After immersion, specimens were removed from SBF and washed with water and then acetone and dried at 100°C.

X-ray Diffraction Data Collection. X-ray diffraction data were collected on station 9.1 at the SRS Daresbury Laboratory, UK, using a two-circle diffractometer. The wavelength was set to 0.4858 Å and calibrated using the K edge of a silver foil; this wavelength was low enough to provide data to a high value of Q, where $Q = 4\pi\sin\theta / \lambda$, of ~22 $Å^{-1}$, which ensures good resolution in real space. The sample was packed into the hole in the centre of a circular plate, 15mm in diameter and 1mm depth and covered with polyimide windows. Data were collected in $\theta / 2\theta$ scans from $2\theta = 1.6$ to 125 degrees in steps of 0.2 degrees at ambient temperature. XRD data was also collected on a Bruker D8 instrument in $\theta / 2\theta$ scans from $2\theta = 10$ to 70 degrees at ambient temperature.

Extended X-Ray Absorption Fine Structure and X-Ray Absorption Near Edge Structure (EXAFS and XANES) Data Collection. For general information regarding EXAFS and XANES techniques we refer the reader to the text of Koningsberger and Prins [8]. The EXAFS data were collected on Station 7.1 at the SRS, UK, and on Station E4 at the Hasylab, Hamburg, Germany. In both cases absorption spectra were recorded in transmission mode, with a third ion chamber used to verify the stability of the monochromator. At the SRS, the X-ray energy incident on the sample was defined using a Si(111) reflection from a double crystal that allowed the beam to be sagitally focussed. In order to improve the energy resolution only the central segment of the focussing crystal was used, giving an overall energy resolution of 1eV at the Ca edge. The monochromator crystals were offset to provide a harmonic rejection of 80%. The edge region was scanned in monochromator steps of 1 mdeg, and the associated incident X-ray energy was calibrated using a Ti foil placed downstream in the post-sample transmission detector, and before the third ion chamber. At the Hasylab, the X-ray beam was monochromated by two independently driven Si(111) crystals. The instrument was evacuated to ~10^{-5} Pa in order to reduce X-ray losses due to attenuation in the air. An overall energy resolution of 0.8 eV at the Ca edge was achieved. The edge region was scanned in 0.1 eV steps, and the energy calibrated using a calibrant of CaF_2 placed between the transmission and third ion chamber.

EXAFS and XANES Data Reduction. Data reduction was performed using the standard programs, EXCALIB and EXBACK [9]. SRS data was summed and converted from a monochromator angle to the equivalent energy scale using EXCALIB; Hasylab data was output directly as energy data. All spectra were isolated from the background absorption profile using EXBACK to fit polynomials to the pre- and post-edge regions, with the edge step being normalised to 1.

For EXAFS analysis EXCURV 98 [9] was used to fit using curved wave theory using the parameters of the radial shells of atoms surrounding the central atoms as variables. XANES analysis was carried out by the procedure described by Sowrey et al [10].

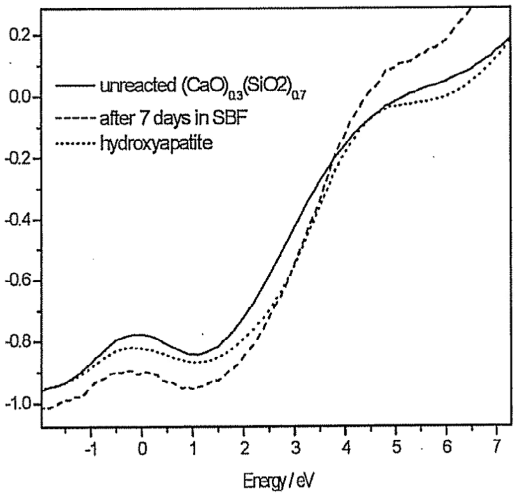
^{31}P Magic Angle Spinning NMR Data Collection. NMR experiments were carried out on a Varian CMX 360 (8.45 T) spectrometer operating at 145.8 (^{31}P) MHz, with a Bruker 4mm probe spinning at 6 kHz with recycle delay 1-2s, and referenced to $NH_4H_2PO_4$ solution at 0 ppm.

Results and Discussion.

The results derived from fitting to the EXAFS data are shown in Table 1. The Ca-O bond distances and coordination numbers agree well with the values reported in the literature (typically 2.3Å and ~7) for the calcium sorption stage, i.e. at ~5 hours immersion in SBF [6]. The apatite structure consists of Ca^{2+} ions in different sites; Ca(1) occupy the columnar site and are connected to the neighbouring Ca^{2+} ions above and below by three shared oxygen atoms and three further oxygen atoms at a further distance, to give a nine-fold coordination of oxygen. The remaining one third of the Ca^{2+} ions, Ca(2), are located in the channels between the three-dimensional network of PO_4 tetrahedra and columnar Ca^{2+} ions, and are seven-fold coordinated [11]. The values for samples after 30 days immersion in SBF agree with the radial distribution function of HCAp; Ca-O bond distances of 2.4Å and an increase in coordination numbers are observed [11].

Time (hr)	0	1	5	10	20	30	48	72	168	720
R [±0.02Å]	2.32	2.37	2.38	2.35	2.32	2.33	2.35	2.37	2.37	2.38
N[±20%]	5.2	7.0	7.3	5.0	6.4	6.4	6.3	6.7	7.6	6.4
A [$Å^2$]	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.04	0.03

Table 1. First shell Ca-O structural parameters for $(CaO)_{0.3}(SiO_2)_{0.7}$ sol-gels before and after reaction with SBF obtained from EXAFS fitting.



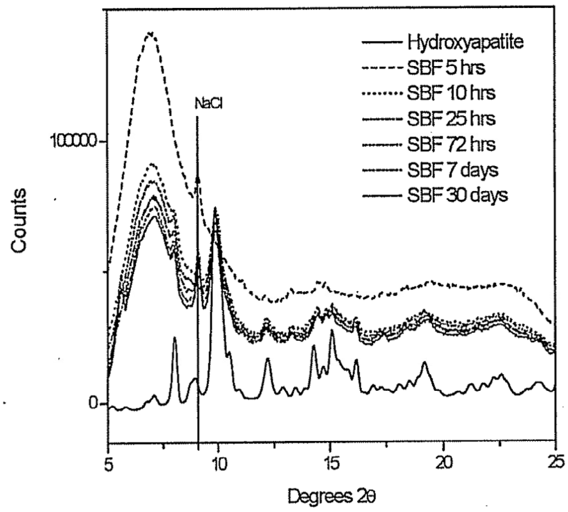


Figure 1 (left). XANES data for unreacted $(CaO)_{0.3}(SiO_2)_{0.7}$, after immersion in SBF for 7 days and for hydroxyapatite. The energy scale is relative to the calcium K-edge (4038 eV).

Figure 2 (right). XRD data for $(CaO)_{0.3}(SiO_2)_{0.7}$ sol-gel glass after reaction with SBF, and hydroxyapatite. (note that the Bragg feature labelled "NaCl" is associated with a small residue of SBF dried onto the surface of the solid left in the solid after the reaction had been terminated with acetone).

In the XANES the Ca K-edge absorption position correlates with the Ca-O coordination, a shift in edge position of 1eV indicating the change in coordination from 6 to 8 [10]. We observe a shift in edge position of ~0.5eV (Fig. 1). For the unreacted sol-gel sample, the edge position fits well to that of reference calcium compounds with 6-fold Ca-O coordination, and a shift of 0.5eV indicates an increase in coordination towards 7 on exposure to SBF.

From X-ray diffraction it can be seen that there is an HCAp layer formed after 5 hours in solution, which becomes more crystalline with immersion time (Fig. 2).

Hydroxyapatite can be annealed at temperatures exceeding 600°C to give a more crystalline structure. A sample of the 7 day SBF treated gel-glass was annealed at 650°C overnight, and XRD results confirm that the HCAp layer forming on the surface of the gel-glass is substantially amorphous in character (Fig. 3).

The 31P NMR spectra show the formation of a hydroxyapatite layer in the sol-gel glass samples, including one taken after only 0.5 hours reaction time with SBF (Fig. 4).

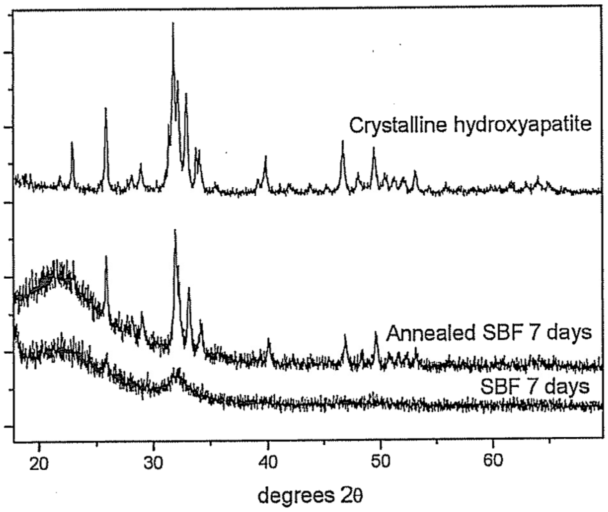
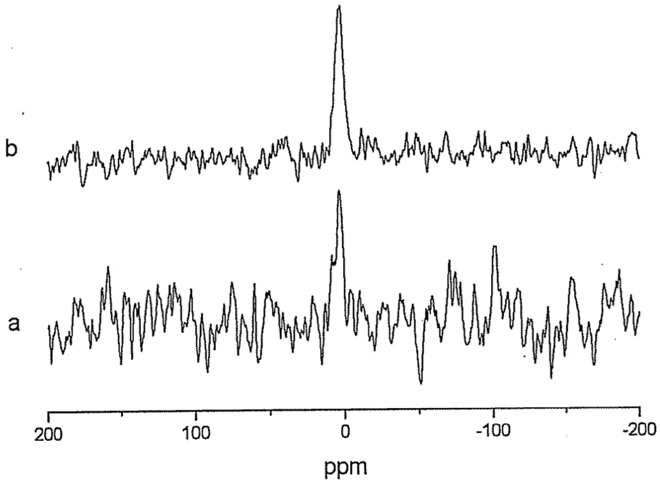


Figure 3 (above). XRD data for $(CaO)_{0.3}(SiO_2)_{0.7}$ sol-gel glass after reaction with SBF for 7 days, after annealing the sample at 650°C overnight, and crystalline hydroxyapatite.

Figure 4 (right). ^{31}P MAS NMR spectra of $(CaO)_{0.3}(SiO_2)_{0.7}$ sol-gel glass after reaction with SBF (a) 0.5 hour and (b) 24 hours.



Conclusions.

By using a coherent suite of complementary experimental techniques to study changes in the composition of calcia:silica sol-gel glass it is possible to observe changes in the atomic scale structure of the starting material, the $(CaO)_{0.3}(SiO_2)_{0.7}$ gel-glass, on reaction with SBF. The calcium initially resides in a six-fold coordinated state with oxygen at a Ca-O distance of 2.3Å; after the calcium sorption stage of the reaction with SBF, around 5 hours in solution, the coordination becomes closer to seven-fold; and after 30 days in an SBF solution, the calcium is in an environment more like HCAp with an increase in coordination number and Ca-O bond distances of 2.4Å. Amorphous hydroxyapatite begins to form after 0.5 hours in SBF solution, and this becomes more crystalline as the immersion periods increase.

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