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Interfacial reactions of glasses for biomedical application by scanning transmission electron microscopy and microanalysis

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Abstract

Short-term physico-chemical reactions at the interface between bioactive glass particles and biological fluids are studied for three glasses with different bioactive properties; these glasses are in the SiO_2–Na_2O–CaO–P_2O_5–K_2O–Al_2O_3–MgO system. Our aim is to show the difference between the mechanisms of their surface reactions. The relation between the composition and the bioactive properties of these glasses is also discussed. The elemental analysis is performed at the submicrometer scale by scanning transmission electron microscopy associated with energy-dispersive X-ray spectroscopy and electron energy loss spectroscopy. After different immersion times (ranging from 0 to 96 h) of bioactive glass particles in a simulated biological solution, results show the formation of different surface layers at the glass periphery in the case of two bioactive glasses (A9 and BVA). For the third glass (BVH) we do not observe any surface layer formation or any modification of the glass composition. For the two other glasses (A9 and BVA), we observe the presence of different layers: an already observed (Si, O, Al) rich layer at the periphery, a previously demonstrated thin (Si, O) layer formed on top of the (Si, O, Al) layer and a (Ca, P) layer. We determine the different steps of the mechanisms of the surface reactions, which appear to be similar in these glasses, and compare the physico-chemical reactions and kinetics using the different immersion times. The A9 glass permits the observation of all important steps of the surface reactions which lead to bioactivity. This study shows the important relationship between composition and bioactivity which can determine the medical applicability of the glass.

Keywords: EDXS; STEM; EELS; Bioactive glass; Apatite precipitation

1. Introduction

When exposed to biological fluids, bioactive glasses undergo a series of physico-chemical reactions at the periphery, resulting in the formation of a silica gel and a calcium phosphate layer on their surfaces [1–3]. This property is known as bioactivity and is strongly dependent on the exact glass composition [4]. Bioactive materials can be used as coatings for metallic prostheses or as bone fillers [5]. Composition has to be optimized to give a suitable compromise between bioactivity and solubility [6].

However, the understanding of the formation of these layers is not straightforward because of the complexity of short-term events that happen at the interface. In a previous paper, we presented the different steps of the mechanisms of surface reactions of bioactive glass particles (called A9, the SiO_2–Na_2O–CaO–P_2O_5–K_2O–Al_2O_3–MgO system) in a biological fluid (Dulbecco's modified Eagle's medium, DMEM) [7]. We demonstrated for the first time the presence at the periphery of a temporary thin (Si, O) layer, characterized by a high O/Si atomic ratio, on top of a (Si, O, Al) layer. This thin layer could be characterized

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by the presence of $Si(OH)_4$ groups. The formation of this layer leads to the formation of a (Ca–P) layer on top, due to the ability of the calcium ion to create a bond with the $Si(OH)_4$ group. Measurement of the O/Si atomic ratio is the only way to prove the existence of this temporary layer. The thin (Si, O) layer disappears as the (Ca, P) layer becomes thicker. These results were obtained by the measurement of the elemental distribution at the bioactive glass periphery, which is an important parameter in understanding the physico-chemical mechanisms involved in the formation of the Si gel and the (Ca, P) layer. This requires analysis at the submicrometer scale. This study was performed by scanning transmission electron microscopy (STEM) associated with energy-dispersive X-ray spectroscopy (EDXS) and with electron energy loss spectroscopy (EELS) using a dedicated light elements quantification process [8].

For a better understanding of the relation between the surface reaction mechanisms and the composition of the glass, we studied in the same way two other glasses with different degrees of bioactivity: a glass with greater bioactivity and a glass with less bioactivity. In this paper, we want to compare the mechanisms associated with these different glasses in order to improve our understanding of the short-term physico-reactions that happen at the interface between glasses and biological fluids. The three glasses are in the SiO_2–Na_2O–CaO–P_2O_5–K_2O–Al_2O_3–MgO system. We performed, by means of X-ray microanalysis, elemental weight concentration profiles across the periphery of each glass sample immersed for different immersion times (0, 12, 24, 48 and 96 h) in a simulated biological fluid (DMEM). These measurements permit the determination locally of the O/Si atomic ratio.

2. Materials and methods

2.1. Composition of the glasses

The compositions of the glasses (referred to as BVA, A9 and BVH) are given in Table 1. The compositions of A9 and BVA seem similar but the A9 glass contains more Al_2O_3 than the BVA glass. The composition of the BVH glass is different than the two other glasses, notably having a high concentration of SiO_2 and an absence of P_2O_5. This glass also contains 2% Al_2O_3 like A9 glass. Addition of Al_2O_3 may be used to control the solubility of the glass, but this addition may inhibit the bone bonding [6,9]. Greenspan and Hench showed that 3% Al_2O_3 added to a glass inhibits bone bonding [10].

Table 1
Composition of the three glasses studied

	Oxide	SiO_2	Na_2O	CaO	P_2O_5	K_2O	Al_2O_3	MgO
BVA	% weight	47	19	20	7	5	1	1
A9	% weight	50	20	16	6	5	2	1
BVH	% weight	71	14	9	–	1	2	3

2.2. Samples preparation

The glasses were obtained by melting the components at 1350 °C. Then, the glasses were cast, crushed, and transformed into powders of grain sizes under 40 μm in diameter. The glass powders (2 mg) were immersed at 37 °C for different times (0, 12, 24 and 96 h) in 1 mL of a standard DMEM [11]. DMEM is a simulated body fluid and contains the following ingredients (mg/L): 6400NaCl, 400KCl, 200$CaCl_2$, 200$MgSO_4 \cdot 7H_2O$, 124NaH_2PO_4 and 3700$NaHCO_3$. Then the glass powders were embedded in resin (Agar, Essex, UK). Thin sections of 90 nm nominal thickness were prepared by means of an FC 4E Reichert Young ultramicrotome. The sections were placed on a copper grid (200 mesh). Sections were coated with a conductive layer of carbon in a sputter coater to avoid charging effects.

2.3. Analysis materials

Our STEM experiments were carried out using a Philips CM30 microscope. The microscope was fitted with an energy dispersive X-ray spectrometer (EDAX 30 mm^2 Si(Li) R-SUTW detector) and with a parallel electron energy loss spectrometer (Gatan model 666) placed under the STEM column. Analyses were carried out using a beryllium specimen holder with a 30°-tilt. The electron probe diameter was around 13 nm. EDXS spectra were acquired at an accelerating voltage of 100 kV, with an energy resolution of 10 eV per channel and an electron dose of approximately 3×10^6 e^-/nm^2. EELS experiments were performed at an accelerating voltage of 250 kV with an energy dispersion of 0.1 eV per channel.

2.4. Analysis methods

Recent developments of thin-window or windowless detectors allow the detection of low atomic number (Z) elements (like boron, carbon, nitrogen, oxygen, fluorine) [12]. Nevertheless, quantitative analysis of these elements is problematic due to the absorption of the low energy X-rays in the sample itself [13]. Using the classical quantification method (the ratio method also known as the Cliff and Lorimer method) [14] without taking into account absorption phenomena leads to an underestimate of the concentrations of the light elements. This quantification ratio method requires the detection of all elements of the sample and dictates that the sum of all concentrations is equal to unity.

For an element x, the expression of the weight concentration C_x can be written

$$C_x = \frac{1}{\sum_i \frac{I_i}{I_x} \cdot K_{i,x}}, \quad (1)$$

where I_x is the characteristic X-ray peak intensity of an element x and $K_{i,x}$ is a factor usually called the K-factor and which has already been determined. Moreover with this method the concentrations are related to each other. Thus,

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