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In vitro and in vivo behaviour of zinc-doped phosphosilicate glasses

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Abstract

The aim of this work was to study the behaviour of zinc-doped phosphosilicate glasses based on Bioglass® 45S5. In vitro (in simulated body fluid), the reactivity was analysed by means of inductively coupled plasma spectrometry, environmental scanning electron microscopy–energy-dispersive spectroscopy (ESEM–EDS) and X-ray diffraction. In vivo (a rat implanted with glass), the reactivity and the tissue behaviour were analysed by conventional histology, histochemistry, microradiography and ESEM–EDS. The in vivo behaviour matches that in vitro perfectly; they show comparable glass degradation processes and rates, ruled by the amount of zinc in the glass. The reaction mechanism for the formation of a polymerized silica layer superimposed with a peripheral calcium phosphate layer is clearly substantiated by ESEM–EDS investigations. The crystallization of a biologically active hydroxyapatite (HA) layer is observed in both cases; the in vitro experiment shows the presence of HA after 4 days.

Keywords: Bioactive glasses; Zinc; In vitro test; In vivo test; Environmental scanning electron microscopy (ESEM)

1. Introduction

In the early 1970s, the concept of bioactive material was formulated by Hench et al. [1]. Since then, the field of bioactive ceramics has expanded to include a great variety of compositions [1–4], and various kind of glasses and glass-ceramics have been found to bond to living bone [5]. The common characteristic of bioactive ceramics is a time-dependent, kinetic modification on the surface that occurs upon implantation. When exposed to physiological solutions, they form an amorphous calcium phosphate layer on their surface; this layer crystallizes into a biologically active hydroxycarbonate apatite layer [1–3] within a few days. This phase is chemically and structurally equivalent to the mineral phase in bone and is responsible for the interfacial bonding.

The first and best-studied bioactive glass is Bioglass® 45S5 [1], of molar composition $1Na_2O$–$1.1CaO$–$0.1P_2O_5$–$1.9SiO_2$. The development of Bioglass 45S5 enabled the requirements for a silicate glass to be considered a bioglass to be defined: optimized surface reactivity and the formation of a stable chemical bond between the tissues and the glass. These requirements can be summarized as a content of SiO_2 less than 60% (molar composition), a high Ca/P ratio and a high percentage of alkaline/alkaline earth oxides.

Glasses of very different compositions have been studied to evaluate their mechanical strength up to a level comparable to cortical bone, thus overcoming the most severe limiting factor in medical use [6–8]. Kokubo et al. [9,10] also revealed that the same kind of biologically active apatite layer can be formed on the surfaces of bioactive glasses and glass-ceramics even in an acellular simulated body fluid (SBF), with ion concentrations and pH nearly equal to those of human blood plasma [11,12].

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By varying the chemical nature and the concentration of the glass constituents [13], new important biological properties (bacteriostatic, cariostatic, etc.) can also be added and the glass can be tailored to specific clinical applications. For example, it is known [13] that the addition of zinc to silicate and borosilicate glasses improves the thermal and mechanical properties, and in the case of phosphate glasses improves the chemical durability in water. It has been also demonstrated that zinc, an essential trace element, manifests stimulatory effects on bone formation both in vitro and in vivo; in fact, the slow release of zinc incorporated into an implanted material promotes bone formation around the implant and accelerates the patient's recovery [14,15]. The partial replacement of sodium with zinc in bioactive glass materials has been proposed to stimulate bone cells proliferation and differentiation, and to improve the bone-bonding ability of bioglass [16].

Our previous studies [13], based on experimental and computational approaches (molecular dynamics simulations), revealed that, in phosphosilicate glasses, zinc adopts a tetrahedral coordination irrespective of its concentration and copolymerizes with the silicon tetrahedral, causing an overall increment of glass reticulation with respect to Bioglass 45S5. This is expressed by the Q^n species distribution, which is defined as the number of bridging oxygens surrounding a network formed of ions such as silicon or phosphorous. This behaviour explains the increment in the chemical durability (tests in water) of zinc-bearing glasses.

Moreover, our studies based on cell culture tests (MC-3T3 osteoblast cells) and related cytotoxicity tests [17] allowed the development of a zinc-bearing glass (hereafter HZ5) with an optimal Zn/P ratio for the maintenance of cell adhesion and cell growth, comparable to the Bioglass 45S5.

Conventionally, the in vivo bioactivity of materials is evaluated using animal experiments, including the bonding strength of bone to the material or the bone ingrowth ratio among the material particles [18]. In the present work, we investigated the reactivity of phosphosilicate glasses based on Bioglass 45S5 and doped with different amount of zinc oxide by means of in vitro and in vivo tests. In the first case, the glasses were tested in simulated body fluid (SBF) and their reactivity analysed by means of inductively coupled plasma spectrometry (ICP), environmental scanning electron microscopy–energy-dispersive spectroscopy (ESEM–EDS) and X-ray diffraction (XRD). In the second case, glasses were implanted into the dorsal muscles or inside a calvaria pocket in rats (without press-fit fixation to bone); their reactivity and the response of host tissues were analysed by means of conventional histology, histochemistry, microradiography and ESEM–EDS.

2. Materials and methods

2.1. Specimen preparation

Three different types (Table 1) of doped glasses were prepared with the composition $1Na_2O$–$1.1CaO$–$0.1P_2O_5$–$1.9SiO_2$–xZnO and, as a reference, a glass with the composition corresponding to that of Bioglass® 45S5 (hereafter H glass) was also prepared.

Table 1
Batch composition (mol.%) from the general glass formula $1Na_2O$–$1.1CaO$–$0.1P_2O_5$–$1.9SiO_2$–xZnO

Glass	x	SiO_2	Na_2O	CaO	P_2O_5	ZnO
H	0	46.2	24.3	26.9	2.6	–
HZ5	0.16	44.4	23.4	25.9	2.5	3.8
HZ10	0.32	42.5	22.5	24.8	2.4	7.8
HZ20	0.78	38.8	20.5	22.6	2.2	15.9

About 100 g of batch was obtained by mixing reagent grade SiO_2, Na_2CO_3, $CaCO_3$, $Na_3PO_4{\cdot}12H_2O$ and $ZnCO_3$ raw materials in a sealed polyethylene bottle for 1 h. Premixed batches were put into a 50 ml platinum crucible and melted in an electric oven for 2 h at 1550 °C.

The melts were poured into graphite moulds of different dimensions in order to obtain rectangular specimens ($10 \times 10 \times 1$ mm^3 in size) and cylinders (Ø 3 mm, length 6 mm) for in vitro and in vivo study, respectively; some glasses were also ball milled in agate jars and sieved to produce particles in the size range of 600–1000 μm for in vivo testing.

2.2. In vitro bioactivity assay in SBF

The rectangular glass specimens were washed with pure acetone and immersed in 24 ml of SBF, prepared according to Kokubo et al. [12]. A constant solid/liquid ratio was maintained in all cases; the SBF soaking was carried out at 37 °C and for various times (1, 2 and 10 h, and 4, 15, 30 and 60 days).

2.3. In vivo experiments

Experiments were performed in compliance with the Italian laws on animal experimentation. The animal research protocol was approved by the Ethical Committee of the University of Modena and Reggio Emilia as required by Italian law.

Adult male Sprague–Dawley rats ($n = 16$, Charles River Italia, Italy), with an average body weight of 600 g (range 550–650 g, 180 days of age), were used for the glass implants. The rats were kept in stainless-steel cages supplied with a self-wash system, air conditioning and lighting, in agreement with Italian guidelines on housing of laboratory animals. They were given at a daily "good laboratory practice" diet (4RF21, Charles River Italia) and water ad libitum, and checked with regard to health status and body weight. The glass insertion was performed under general anesthesia induced by an intramuscular injection of a mixture of 30 mg per kg body wt. ketamine (Ketavet 100, Intervet prod. S.r.l., Italy), 0.75 mg kg^{-1} acepromazine (Prequillan, Fatro, Italy) and 4 mg kg^{-1} xylazine (Rompun, Bayer, Germany).

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