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Development and in vitro characterization of sol–gel derived $CaO–P_2O_5–SiO_2–ZnO$ bioglass

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Abstract

A $CaO–P_2O_5–SiO_2–ZnO$ bioglass was formed by the sol–gel technique and characterized by Raman spectroscopy, X-ray diffraction, energy dispersive X-ray analysis (EDXA) and scanning electron microscopy (SEM). The surface reactivity of the resultant glass-ceramic specimens was analyzed by immersion studies in simulated body fluid (SBF). SEM–EDXS and inductively coupled plasma atomic emission spectrometry techniques were used to monitor changes in the glass surface and SBF composition. Osteoblast cell culture experiments were performed to assess the biocompatibility and the alkaline phosphatase activity. Cell counts of the osteoblasts cultured on the bioglass samples were studied and compared with the polystyrene plates. The cells cultured on the bioglass disks consistently showed a higher alkaline phosphatase activity and cell counts compared to cells cultured on either polystyrene plates or the base $CaO–P_2O_5–SiO_2$ bioglass. This was due to cell proliferation and differentiation promoted by the zinc-substituted bioglass.

Keywords: Sol–gel; Bioglass; SBF; Osteoblasts; Biocompatibility

1. Introduction

Bioactive glasses and glass-ceramics have been extensively investigated as bone grafts or fillers owing to their ability to form a direct bond to living bone [1–3]. Recent years have witnessed the use of several types of bioceramics to repair damaged and diseased bones. These bioceramics are generally classified as bioinert (e.g. Al_2O_3 and ZrO_2), bioactive (e.g. bioglass and hydroxyapatite (HAP)) or biodegredable ceramics (e.g. tricalcium phosphate and bone cement). Bioglasses and glass-ceramics have received special interest due to their unique characteristics, including (i) a rapid rate of surface reaction that leads to their direct attachment to bone via a chemical bond [4]; (ii) their relatively low softening temperature that can be used as a sintering aid to bond the ceramic particles and fill the micropores during the sintering process; (iii) the ease of compositional design with properties specific to particular clinical applications; and (iv) their excellent controllability over a wide range of chemical properties and rate of bonding with tissues [5,6].

In general, bioglasses can be formed by the traditional melting method, which is regarded as simple and suitable for mass production [7–9]. However, this method is limitated by the evaporation of the volatile component P_2O_5 during high-temperature processing. The sol–gel technique is an alternative approach to fabricating bioglasses that has been widely studied in recent years [10,11]. The advantages of the sol–gel process are well known: the process takes place at low temperatures, and gives homogeneous mixtures in the final glass composition. It has been proven that commercially available glass compositions, e.g. 45S5, 58 S and 64 S, can be synthesized by the sol–gel method [12–15]. A variety of sol–gel-derived bioglass materials

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incorporating modifiers such as magnesium, cerium, boron, strontium and calcium have been reported [12–15] with the aim of improving specific characteristics. The possibility of incorporating zinc into bioglasses has received special interest in recent years due to the potential benefits of such a glass [16–18]. However, the formation of zinc-containing bioglasses and their properties and characteristics does not seem to have been well explored. Zinc as an essential trace element is known to have stimulatory effects on bone formation in vitro and in vivo [19–21]. The beneficial features of zinc have stimulated our current interest in zinc-containing bioglasses described herein. The synthesized bioglass was characterized and bioactivity studies were performed to analyze its feasibility as an implant material.

2. Materials and methods

2.1. Preparation and characterization

The sol–gel synthesis of the glass of composition SiO_2–P_2O_5–CaO–ZnO (64% SiO_2, 26% CaO, 5% P_2O_5, 5% ZnO, mol%) was performed as follows: Initially, tetraethoxysilane (TEOS; Aldrich) was added to 0.1 M nitric acid and the mixture was allowed to react for 60 min for the acid hydrolysis of TEOS. Then a series of reagents was added in the following sequence, allowing 45 min for each reagent to react completely: triethylphosphate (TEP; Aldrich), calcium nitrate tetrahydrate (Aldrich), and zinc nitrate hexahydrate (Aldrich). After the final addition, mixing was continued for 1 h to allow the completion of hydrolysis. The Zn-free bioglass SiO_2–P_2O_5–CaO (64% SiO_2, 26% CaO, 10% P_2O_5, mol%) was also synthesized by the same procedure to compare its bioactivity with that of the Zn-substituted bioglass. The resultant solution was kept in a sealed Teflon container for 10 days at ambient temperature to allow gelation to occur. The resultant gel was kept in a sealed container and heated at 70 °C for an additional 3 days. The water was removed and a small hole was inserted in the lid to allow the leakage of gases while the gel was heated to 120 °C for 2 days to remove all the free water. The dried gel was then heated for 24 h at 700 °C to stabilize the glass and eliminate residual nitrates.

The characterization of the bioglass samples was carried out using confocal Raman microscopy (Lab Ram, Horiba, Jobin-Yvon, France), X-ray diffraction (XRD) analysis (Rigaku D/max-IIB), energy dispersive X-ray analysis (EDXA; 30 mm^2 Si (Li) R-RSUTW detector), scanning electron microscopy (SEM; JEOL 5610 and LEO 1525 FEG) and inductively coupled plasma atomic emission spectrometry (ICP-AES; J.Y. Jobian Yvon Horiba Ultima 2000).

2.2. In vitro analysis

2.2.1. Immersion in SBF

The HAP-forming ability of the bioglass disks was analyzed by immersing the samples in simulated body fluid (SBF) for various periods of time. Each specimen was immersed in Tris-buffered SBF solution with ion concentrations and pH nearly equal to that of human blood plasma (Table 1) at 37 ± 0.5 °C, for 7, 14 and 21 days. A surface area to volume ratio of 0.1 cm^{-1} was maintained for all immersions, and the SBF solutions were not exchanged during the experiments. The SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 in deionized water. The solution was buffered to pH 7.4 with Tris–(hydroxymethyl)-aminomethane ($(CH_2OH)_3CNH_3$) and hydrochloric acid [22]. The samples were removed from the incubator, rinsed gently, first with pure ethanol and then using deionized water, and left to dry at ambient temperature in a desiccator for 3 h. All the samples were analyzed by SEM–EDXA for elemental composition and morphology; ICP–AES was used to analyze the ionic concentration of the SBF after immersion of the glass samples.

2.2.2. Cell culture studies

The stabilized glass powder was finely ground and sieved through a 60-mesh wire and then compacted into disks (15 mm diameter, 3 mm thickness) for the cell culture analysis. Prior to cell culture studies, the glass disks were sterilized with UV light in a tissue culture laminar flow-hood for 20 min on each side. The osteoblasts were isolated by sequential trypsin–collagenase digestion on calvaria of neonatal (<2 days old) Sprague–Dawley rats as described elsewhere [23]. The isolates were washed in PBS and dissected into about 1 mm^3 fragments, which were treated with 0.25% collagenase (type I; Sigma, St. Louis, MO) for 2 h at 37 °C. The collagenase-treated bone fragments were washed extensively with Dulbecco's modified Eagle's medium (DMEM) supplemented with glutamine (292 mg l^{-1}), 10% heat-inactivated fetal calf serum (FCS) and 1% antibiotics (100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin). After several washes, the cells were collected by centrifugation, suspended in DMEM, plated in 25 cm^2 flasks and cultured in DMEM supplemented with 10% FCS at 37 °C. At confluence, the cells were detached with

Table 1
Ionic concentration of simulated body fluid

Description	Ionic concentration (mM)							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO^{3-}	HPO_4^{2-}	SO_4^{2-}
Plasma (human)	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

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