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Influence of Cu doping in borosilicate bioactive glass and the properties of its derived scaffolds

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ABSTRACT

Copper doped borosilicate glasses (BG–Cu) were studied by means of FT-IR, Raman, UV–vis and NMR spectroscopies to investigate the changes that appeared in the structure of borosilicate glass matrix by doping copper ions. Micro-fil and immunohistochemistry analysis were applied to study the angiogenesis of its derived scaffolds in vivo. Results indicated that the Cu ions significantly increased the B–O bond of BO_4 groups at 980 cm^{-1}, while they decrease that of BO_2O^- groups at 1440–1470 cm^{-1} as shown by Raman spectra. A negative shift was observed from ^{11}B and ^{29}Si NMR spectra. The ^{11}B NMR spectra exhibited a clear transformation from BO_3 into BO_4 groups, caused by the agglutination effect of the Cu ions and the charge balance of the agglomerate in the glass network, leading to a more stable glass network and lower ions release rate in the degradation process. Furthermore, the BG–Cu scaffolds significantly enhanced blood vessel formation in rat calvarial defects at 8 weeks post-implantation. Generally, it suggested that the introduction of Cu into borosilicate glass endowed glass and its derived scaffolds with good properties, and the cooperation of Cu with bioactive glass may pave a new way for tissue engineering.

1. Introduction

Bioactive glasses used in bone repair applications have been developed for tissue engineering applications. They are well known for their excellent biodegradability by conversion to hydroxyapatite (HA) and proven to have good osteoconductivity and ability to form a strong bond between bone and soft tissues [1]. An extensive amount of research work has been carried out to develop silicate, borate and borosilicate bioactive glasses for biomedical and technological applications in tissue engineering [25–27]. The silicate bioactive glasses such as 45S5 Bioglass® and 1393 have received considerable interest for years. More recently developed borate-based bioactive glasses with more controllable degradation rates to form HA have also been attracting interest [28–30]. The replacement of varying amounts of SiO_2 in 45S5 Bioglass® or 13–93 glass has resulted in the formation of borate-based bioactive glasses [2]. Previous studies have shown that the borate-based bioactive glass scaffolds have a better capacity to stimulate bone regeneration in osseous defects than that of the silicate bioactive glasses [29,31].

Bioactive glasses are also famous for their favorable bioactivity and released nutrition ions, such as Si, Ca ions, for bone regeneration [3]. Simultaneously, the compositional flexibility of glass can also be used to deliver trace quantities of elements (such Sr, Cu and Ag), that are known to endow glass with multifunction such as osteogenesis, angiogenesis and antibacterial activity for successful clinical application of engineered bone constructs [4–8]. Trace elements of stable inorganic compound, such as Cu, Zn ions, are also known as stimulation cell signaling pathways towards tissue equilibrium by acting as enzyme cofactors [9,10].

Copper ions are particularly involved in the activity of several transcription factors and bind to cell membrane releasing complex [11–13], facilitating release of vascular endothelial growth factor (VEGF) and cytokines from producing cells [14]. Moreover, copper has been reported to enhance angiogenesis in vitro and stimulate endothelial cell proliferation [15]. In reverse, lack of copper could suppress vascular formation for several inactive copper-binding proteins [16,17]. Because of the benefit of copper, different Cu-containing biomaterials have been studied and developed in recent years [18]. And many different approaches have been approached to incorporate Cu into a scaffold material, such as, Cu doping into calcium phosphates [19], into silicate glasses [20], into phosphate based glasses [21], and incorporate with polymer coatings in bioactive glass scaffolds [22]. Hopper [23] has reported that the dopant copper

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could lead some changes on the glass physics properties and bioactivity. Wu [20] has studied copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity.

However, the incorporation of copper ions into borosilicate bioactive glass and the influence on the derived scaffolds have not been investigated until now. Understanding the effect of this dopant, Cu, on the properties of the devices fabricated from biomaterials is significant for improving their applications. In addition, the biodegradation behavior of materials is the key to controlling the biological performance of bioactive glass and derived scaffolds to elucidating the mechanism of interaction between the released ions and cells. Thus, the objective of our study was to investigate the effect of copper dopant on the structural properties of the borosilicate glass network and the changes of reactivity degradation behavior of the derived glass scaffolds.

In view of the properties of borosilicate bioactive glass and Cu ions described above, this study was undertaken to create Cu doped borosilicate bioactive glass and derived scaffolds. The effects of copper doping on the physicochemical property of glass and properties biodegradation of the derived scaffolds were investigated. And their angiogenesis of derived scaffolds was evaluated in a rat calvarial defect model. In particular, ^{11}B and ^{29}Si nuclear magnetic resonance (NMR) spectroscopy were employed to acquire the short-range structure of Cu doping borosilicate glasses, which provided accurate quantitative data on the coordination state of boron and silicate [24,25], giving detailed insight into the evolution of copper involved in borosilicate glass. Fourier transform infrared (FT-IR) and Raman spectroscopy will also be applied to study the group changes in the doped glass. As to the fabricated scaffold, biodegradation was investigated by field emission scanning electron microscopy (FESEM), inductively-coupled plasma atomic emission spectroscopy (ICP) and X-ray diffraction (XRD). Micro-fil and immunohistochemistry staining were used to evaluate the angiogenesis of derived scaffolds.

2. Materials and methods

2.1. Fabrication of Cu doping borosilicate bioactive glass

A borosilicate bioactive glass with varying Cu contents (0, 1 and 3 wt.%, defined as BG, BG–1Cu and BG–3Cu, respectively) with the composition $6Na_2O$, $8K_2O$, 8MgO, 22CaO, $36B_2O_3$, $18SiO_2$, and $2P_2O_3$ (mol%) was prepared by melting the requisite amounts of Na_2CO_3, K_2CO_3, $MgCO_3$, $CaCO_3$, SiO_2, H_3BO_3, and NaH_2PO_4 (analytical grade; Sinopharm Chemical Reagent Co., Ltd. China) a platinum crucible for 2 h at 1150 °C and quenching the melt between cold steel plates.

2.2. Fabrication of borosilicate bioactive glass scaffolds

Borosilicate bioactive glass scaffold samples were produced using the foam replica technique as described elsewhere [26]. The fabricated glass was crushed, ground, and sieved to give particles of size < 30 μm. Then a slurry was prepared by mixing 52.6 wt.% bioactive glass particles, 3.5 wt.% ethyl cellulose (analytical grade, Sinopharm Chemical Reagent Co., Ltd., China) and 43.9 wt.% anhydrous ethanol. A polyurethane foam (Shanghai No. 6 Plastic Co., Ltd, China) was immersed in the slurry to coat the surface of the foam with the slurry. After drying for 24 h at room temperature (RT), the as-coated foams were heated for 2 h at 470 °C (heating rate = 1.5 °C min^{-1}) to decompose the foam and then for 2 h at 570 °C (heating rate = 2.5 °C min^{-1}) to densify the glass particles into a strong network.

2.3. Structural characterization of BG–Cu glass

2.3.1. UV–vis absorption spectral measurements

The optical absorption spectra of BG–Cu and BG glass were measured at RT in the range from 200 to 2000 nm using a computerized recording spectrophotometer (type JASCO corp., V-570, Rel-00, Japan). Polished samples of equal thickness (2 ± 0.1 mm) were used in these measurements.

2.3.2. FT-IR and Raman spectral measurements

Compositional group analysis of scaffold changes was performed by Fourier transform infrared (FT-IR) spectroscopy (Bruker EQUINOX SS/HYPERION2000; Germany). In the FT-IR analysis, 2 mg of the powder was mixed with 100 mg KBr, pressed to form pellets (diameter = 25 mm) and analyzed in transmittance mode in the wavenumber range 4000–400 cm^{-1}.

Raman studies were carried out using Horriba Yvon Jobin Lab-RAM HR micro-Raman spectrometer equipped with a CCD detector. Excitation wavelength of 532 nm was used and beam intensity was about 10 mW. Acquisition time was set to 30 s. The spectra were recorded in the 1400–200 cm^{-1} range of Raman shifts at 1.3 cm^{-1} spectral resolution.

2.3.3. NMR spectroscopy

Structural examinations of the glasses were based on nuclear magnetic resonance (NMR) method. The ^{29}Si and ^{11}B NMR investigations were carried out at RT, using CMV-400 Chemagnetics pulse spectrometer. The spectra were recorded on a VNMRS-300 (Varian, US) spectrometer operating at a resonant frequency of 59.56 MHz. Spectra were obtained by applying a 90° pulse of 5 μs every 8 s with proton decoupling with typically 2000 accumulations for each spectrum.

2.4. Biodegradation of BG–Cu glass scaffolds in vitro

The porosity of the as-prepared scaffolds was measured according to Archimedes' principle. Degradation and conversion of the as-fabricated scaffolds were evaluated as a function of immersion time in simulated body fluid (SBF), which was prepared according to Kokubo's method [27]. The biodegradation and bioactivity of scaffolds were investigated by immersed scaffolds in SBF (1 g to 100 ml) for 90 days at 37 °C [28]. The morphology, microstructure and surface changes of as-fabricated scaffolds in degradation process were observed by field emission scanning electron microscopy (FESEM, Hitachi S-4700; Tokyo, Japan). Samples were fixed on a FESEM sample holder, air dried under vacuum and coated with a thin layer of gold.

The weight loss was measured after removing, washing and drying the scaffolds. The pH change of the immersion solution was measured by a pH meter (FE20; Mettler Toledo) directly. The released ions in the SBF, resulting from the degradation of scaffolds, were detected by inductively-coupled plasma atomic emission spectroscopy (ICP-AES; Optima 2100 DV; USA). After the immersion, the scaffolds were ground into powder to detect the phase transformations by X-ray diffraction (XRD, D/max 2550 V, Rigaku, Cu-Kα, Japan).

2.5. Angiogenesis of BG–Cu scaffolds in vivo

2.5.1. Animals and surgical procedure

All animal surgical procedures were approved by the Animal Research Committee of the Sixth People's Hospital, Shanghai Jiao Tong University School of Medicine. 12 male Sprague–Dawley rats (12 weeks old; body weight 250–300 g) were used for the present experiments. Based on cell culture results, the BG–3Cu scaffolds were selected for evaluation and the BG scaffolds served as control.

The rats were anesthetized with pentobarbital (Nembutal 3.5 mg/100 g). With sterile instruments and aseptic techniques, a 1.0–1.5 cm sagittal incision was made on the scalp. A critical-size defect (5 mm in diameter) was created in the central area of each parietal bone by a 5 mm electric trephine (Nouvag AG, Goldach, Switzerland) under constant irrigation with sterile 0.9% saline. Then BG and BG–3Cu scaffolds (n = 12) were implanted in these defects. After that, the soft tissues were repositioned and sutured. Each animal was given an intramuscular injection of antibiotics post-surgery and was allowed