Research Article

Hierarchical Structures and Shaped Particles of Bioactive Glass and Its *In Vitro* Bioactivity

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In this study, bioactive glass particles with controllable structure and porosity were prepared using dual-templating methods. Block copolymers used as one template component produced mesopores in the calcined samples. Polymer colloidal crystals as the other template component yielded either three-dimensionally ordered macroporous (3DOM) products or shaped bioactive glass nanoparticles. The *in vitro* bioactivity of these bioactive glasses was studied by soaking the samples in simulated body fluid (SBF) at body temperature (37°C) for varying lengths of time and monitoring the formation of bone-like apatite on the surface of the bioactive glass. A considerable bioactivity was found that all of bioactive glass samples have the ability to induce the formation of an apatite layer on its surface when in contact with SBF. The development of bone-like apatite is faster for 3DOM bioactive glasses than for nanoparticles.

1. Introduction

During the last decade, the use of mesoporous materials, which have pores ranging in size from 2 to 50 nm, was proposed in tissue engineering because their large surface area and pore volume may enhance their bioactive behavior and allow them to be loaded with the osteogenic agents used to promote new bone formation [1–4]. Mesoporous bioactive glasses (MBGs) have attracted much attention in many potential applications, such as catalysis, adsorption/separation, synthesis of nanomaterials [5–9], and, recently, also in the field of biomaterial science as bone scaffolds [10, 11], bone filler [12], and drug delivery systems [11, 13–15].

Although all of the reported MBGs show favorable bioactivity, they are difficult to use as scaffolds for the regeneration of bone tissues at this stage because their mesosized pores are too small to promote cell growth. To overcome this pore size limitation, our group successfully prepared hierarchically structured three-dimensionally ordered macroporous (3DOM) by the sol-gel method using a block-copolymer and polymer colloidal crystals as dual templates, which can generate either three-dimensionally ordered macroporous structures or shaped bioactive glass nanoparticles.

2. Experimental

2.1. General. Calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), the surfactant Brij 56 ($\text{C}_{16}\text{H}_{33}(\text{OCH}_2\text{CH}_2)_n\text{OH}$, $n \sim 10$), and polymethylmethacrylate (PMMA) colloidal crystals with 400 nm size were prepared by a published method [17].

2.2. Preparation of Bioactive Glasses. MBGs were synthesized by a sol-gel method. In a typical synthesis of bioactive glass nanoparticles M58S (M58SP), Brij 56 was used as a structure-directing agent for the mesostructure [18] and tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were the sources of Si, P, and Ca, respectively ($\text{Si}/\text{Ca}/\text{P} = 60:36:4$, molar ratio). Amounts of 0.850 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 0.096 g of citric acid were dissolved in 2 mL of 1 M HNO_3 , and 0.073 g of TEP was added into the solution while stirring at room temperature. A solution containing 1.250 g of TEOS and 0.340 g of Brij 56 was then added to this mixture. The combined mixture was stirred at room temperature for 30 min or until the solution became clear. A monolithic piece of the PMMA colloidal

FIGURE 1: Wide angle XRD patterns of (a) M80S, (b) M70S, and (c) M58S (symbol ● calcium phosphate and ◇ calcium phosphate silicate).

FIGURE 2: EDS spectrum and the element ratio of (a) M80S, (b) M70S, and (c) M58S and (d) the percentage of element of MBGs.

crystal template was immersed into this clear solution. After complete infiltration, the samples were aged at 45°C for 48 h and dried at 45°C for 24 h in air. The as-synthesized samples were calcined at 600°C in air for 6 h in order to remove the template completely. The heating rate for the calcination was fixed at 2°C/min. In the case of M80S, M70S, and M58S, bioactive glass materials were synthesized by the same method, without ageing at 45°C. The nominal compositions of the MBGs under study are listed in Table 1. The structure of the bioactive glasses was analyzed in detail

by X-ray diffraction (XRD), energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR).

2.3. Study of Bioactivity. The *in vitro* bioactivity study was performed by soaking the MBGs in simulated body fluid (SBF) prepared by the method of Kokubo et al. [19] at 37°C for 3, 6, 12, 24, and 48 h, and the surface of the samples was characterized by FTIR, SEM, and XRD.

FIGURE 3: SEM and TEM images of (a) M58S and (b) M58SP.

FIGURE 4: Schematic of the formation of nanoparticles through the disassembly of 3DOM structure. Copyright 2007, Wiley-VCH [16].

TABLE 1: Chemical compositions of MBGs (mol%).

Samples	SiO ₂	CaO	P ₂ O ₅
M80S	80	20	—
M70S	70	30	—
M58S, M58SP	60	36	4

3. Results and Discussion

3.1. Sample Characterization. Figure 1 shows the XRD patterns of MBGs after calcination at 600°C with a ramp rate 2°C/min in air. All samples show a broad peak in the 2θ range 15–35°, which suggests that all MBGs with different chemical compositions exist as amorphous phases. The pattern of the M58S sample contained additional reflections assigned to calcium phosphate silicate and calcium phosphate (JCPDS-00-050-0905 and 00-044-0752). The EDS spectra of all MBGs showed peaks of carbon, oxygen, silicon, phosphate, and calcium with relative amounts indicated in Figure 2.

Figure 3 shows the representative SEM images of (a) M58S and (b) M58SP. Bioactive glass with hierarchical porosity was first formed through the PMMA and Brij 56 dual-templating system. Without of aging step at 45°C, no structure transformation occurred and the 3DOM structure was produced (Figure 3(a)) due to no shrinkage process between the ageing step [16, 20]. After a sufficient time of aging (at least 48 h), no 3DOM structure remained and, nanocube with ~100 nm edge lengths were obtained (Figure 3(b)). Typically, an fcc array of spheres, octahedral (O_h), and tetrahedral (T_d) voids exist between the spheres. The bioactive glass precursor infiltrated these voids and formed an inverse replica of the template. So correspondingly, the 3DOM structure can be considered to be built up from these two basic units (O_h and T_d) which are interconnected through narrow necks. The disassembly occurs first by shrinkage in a polycondensation reaction during aging process until the silanol groups are no longer close enough to react with each other [16, 20]. Follow with the syneresis is defined as “spontaneous shrinkage of the gel and the resulting expulsion of liquid from the pores” [20].

FIGURE 5: FTIR spectra of MBGs with different chemical compositions before and after soaking in SBF for 12 h.

FIGURE 6: SEM images of (a) M80S, (b) M70S, (c) M58S, and (d) M58SP after soaking in SBF for different times (0, 3, 6, 12, and 24 h).

By complete disconnection of the skeleton at the narrowest connection points. Hence, the nanocubes produced from the octahedral holes. This formation mechanism is known as disassembly and occurs in order to stabilize the structure of the 3DOM material (Figure 4) [16].

3.2. Study of Bioactivity. Figure 5 shows the FTIR spectra of MBGs with different chemical compositions before and after soaking in SBF for 12 h. The FTIR spectra of series

M80S, M70S, and M58S before soaking in SBF, all spectra show the characteristic absorption bands of the Si-O-Si asymmetric stretching and symmetric bending mode at 1060 cm^{-1} , 800 cm^{-1} , and 478 cm^{-1} , respectively, [21, 22]. The shoulder around 950 cm^{-1} is related to the Si-O-Ca vibration mode [21]. Spectra of M70S, M58S, and M58SP, after samples were soaked in SBF for 12 h, a doublet at 568 cm^{-1} and 607 cm^{-1} is shown, which is associated with the P-O bending vibration and at 1040 cm^{-1} and 1090 cm^{-1} can be attributed to the stretching PO_4 vibration [21, 23].

FIGURE 7: Wide angle XRD patterns of (a) M80S, (b) M70S, (c) M58S, and (d) 58SP before and after soaking in SBF with different times (symbol $\blacklozenge$ brushite, $\diamond$ calcium phosphate silicate, $\bullet$ calcium phosphate, and black star hydroxyapatite).

Additionally, SEM micrographs of the sample surfaces before and after soaking in SBF for different times are shown in Figure 6. For M80S, M70S, and M58SP after 6 h of soaking, small needle-like crystal agglomerates were observed on the surface. In contrast, the surfaces of the M58S samples were needle-like after soaking in SBF within 3 h. All samples were completely covered by a needle-like crystal layer in 24 h. XRD patterns (Figure 7) confirmed that the needle-like crystal can be assigned to the bone-like apatite phase. Furthermore, the *in vitro* bioactivity of MBGs is dependent on the Si/Ca ratio in the network which, as a result of the higher calcium content exhibits the best *in vitro* bioactivity [2]. It is clear that M58S shows faster bioactivity than M70S and M80S, and the 3DOM structure of M58S shows higher bioactivity than M58SP.

4. Conclusion

The bioactive glass skeleton with hierarchical porosity was first formed via a surfactant and polymer sphere dual-templating system, and then the three-dimensionally ordered structure was disassembled to obtain bioactive nanocubes. In addition, the study of *in vitro* bioactivity found that all

of bioactive glass samples have the ability to induce the formation of a bone-like apatite layer on its surface when in contact with SBF within 6 h and the transformation to bone-like apatite was more efficient for M58S than for M80S and M70S. Furthermore, the 3DOM structure of M58S shows higher bioactivity than M58SP.

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