Novel Mesoporous CaO–B_2O_3–SiO_2 Glasses with In Vitro Bioactivity

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Wormhole-like-ordered mesoporous CaO–B_2O_3–SiO_2 (MCBS) glasses, prepared by a combination of surfactant-templating, sol–gel method and evaporation-induced self-assembly (EISA) processes, show good in vitro bioactivity, demonstrated by final formation of plate-like-carbonated calcium-deficient hydroxyapatite (d-HAp) on the surface after soaking in simulated body fluid (SBF) for a short period of time.

Bioactive glasses and glass-ceramics have attracted much attention since the pioneering work by Hench et al. in 1972.[1] The prerequisite for glasses and glass ceramics to bond to living bone is the formation of a layer of biologically active apatite on the surface of these biomaterials, without inducing toxic, inflammatory, or immune responses, when they are exposed to physiological fluids.[1,2] The behavior that apatite is formed on biomaterials in SBF is known as the in vitro bioactivity, an important evaluation for the in vivo bioactivity of such bone-forming biomaterials.[3]

CaO and SiO_2 are reported to be two important compositions in bioactive materials.[2] The introduction of B_2O_3 into the CaO–SiO_2 system is expected to enhance the bioactivity, as more soluble boric compounds increase the supersaturation of Ca ions in the SBF solution and water-corrosive borosilicate glass forms Si–OH groups that act as nucleation sites for the apatite layer.[4] Meanwhile, increasing the specific surface area and pore volume of bioactive glasses greatly accelerates the apatite formation and, therefore, enhances the bioactive behavior.[5] Wormhole-like mesoporous materials have high specific surface areas, uniform channel diameters, and short-range hexagonal-like-packed channels.[6,7] Furthermore, channel branching within the framework greatly facilitates access to reactive sites on the framework walls. In this sense, wormhole-like mesoporous materials provide very promising possibilities in the field of bone tissue regeneration.

Herein, we report the first preparation of wormhole-like mesoporous CaO–B_2O_3–SiO_2 glasses. The in vitro bioactivity of the glasses was also investigated, demonstrated by formation of initially worm-like and finally plate-like carbonated calcium-deficient hydroxyapatite crystals after soaking the glasses in static SBF for a short period of time.

Wormhole-like-mesostructured MCBS glasses with different chemical compositions were obtained by a combination of surfactant ($EO_{20}PO_{70}EO_{20}$, P123)-templating, sol–gel method and EISA processes (see Electronic Supporting Information, ESI).[16]

Evidence for the formation of borosiloxane bonds (Si–O–B) is provided by FT-IR spectra (ESI).[15] The absorption bands at ≈920 and ≈670 cm^{-1} confirm the formation of Si–O–B linkage.[8] Nitrogen sorption isotherms confirm the existence of mesopores (Figure 1). The N_2 adsorption–desorption isotherm of MC15BS80 (C15 and S80 represents the molar fraction of CaO and SiO_2 in the initial composition, respectively) is a type IV isotherm, typical of mesoporous materials.[9] The BET surface area, pore volume, and pore size were calculated to be 284 $m^2\,g^{-1}$, 0.331 $cm^3\,g^{-1}$, and 4.2 nm, respectively.

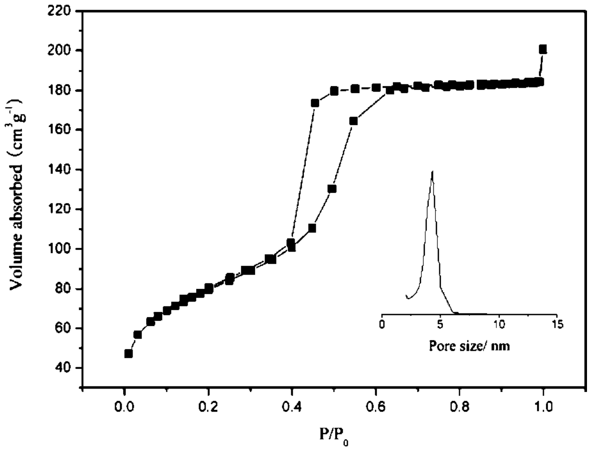

Figure 1. Nitrogen adsorption–desorption isotherm and pore size distribution (inset) of MC15BS80.

The small-angle powder X-ray diffraction (XRD) pattern (Figure 2) of the calcined MC15BS80 shows one strong diffraction peak in the small-angle regime, indicative of wormhole-like mesoporous channel packing order. Wide-angle XRD (ESI)[16] study confirms that the calcined glasses exist in an amorphous form, as no diffraction peaks can be observed except a broad band between 15 and 30° (2θ). Arrangements of the pores can be clearly seen in the high-resolution transmission electron microscope (HRTEM) image of calcined MC15BS80 (inset of

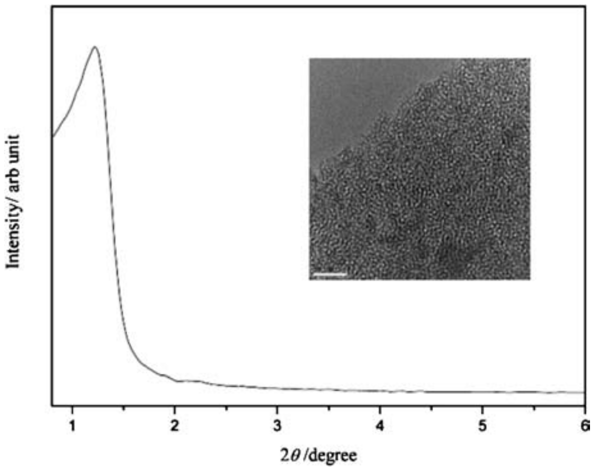

Figure 2. Small-angle XRD patterns and HRTEM (inset) of MC15BS80 (The scale bar in the inset is 50 nm).

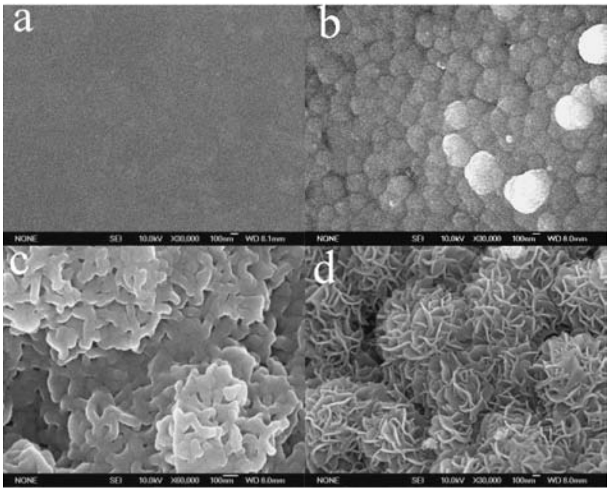

Figure 3. SEM micrographs obtained from the MC15BS80 after soaking in SBF for different time (a) 0 h, (b) 6 h, (c) 24 h, (d) 120 h (The scar bars are 100 nm for all the pictures.).

Figure 2). The glass contains a large number of channels. The cylindrically to hexagonally shaped channels are regular in diameter and show short-range hexagonal-like packing order, although they lack the discernible long-range packing order.

The bioactivity of MCBS in vitro was tested in static SBF to monitor the formation of hydroxyapatite (HAp) on the surface of MCBS over time. The FT-IR spectra (ESI)[16] of MC15BS80, after soaking in SBF for 6 h, demonstrates the presence of amorphous phosphate group with an absorption band at 566 cm^{-1}.[10] After 24 h, the band at 566 cm^{-1} begins to split into a doublet at 566 and 601 cm^{-1}, characteristic of the phosphate group in the crystalline HAp phase.[11,12] However, after 120 h, new absorption bands at 879, 1430, and 1483 cm^{-1} due to the carbonate group are also observed, indicating that the HAp phase is a carbonate-containing HAp similar to the apatite on the surface of bones in the body.[11,12]

SEM image of MC15BS80 before soaking (Figure 3a) shows a smooth and homogenous surface. After soaking in SBF for 6 h (Figure 3b), some sphere-like agglomerates begin spreading over the glass surface, owing to the deposition of amorphous apatite. Newly formed worm-like HAp crystals can be observed after soaking for 24 h (Figure 3c). After 120 h (Figure 3d), HAp crystals are turned to be plate-like with thin edges oriented perpendicular to the surfaces of glass powders. The plate-like HAp crystals aggregate in clusters to exhibit a rose-like morphology. It is suggested that the plate-like morphology is promoted by the adsorption of ions such as carbonate,[13] which is in accordance with our FT-IR results. However, further work is necessary to ascertain the factors.

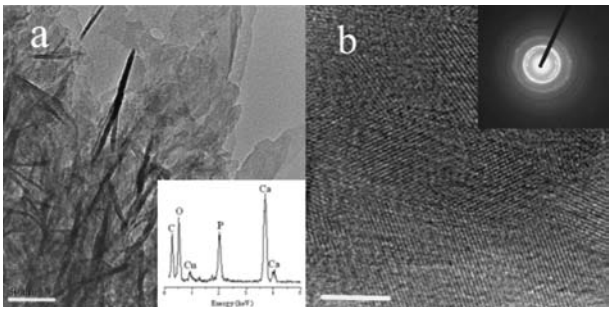

Figure 4. TEM images of MC15BS80 after soaking in SBF for 120 h at different magnifications (The scale bars are 50 and 5 nm for (a) and (b), respectively.).

Under TEM imaging (Figure 4a), the plate-like HAp crystals are also seen to be aggregated together, with both plate-like and rod-like morphology. The seeming nanorods are essentially due to the thin edges of the plate-like HAp crystals. The crystallinity of the newly formed HAp phase is further confirmed by high-resolution TEM investigations (Figure 4b). It is clear that HAp crystals have varying orientations, which in accordance with selected area electron diffraction (SAED) patterns (inset of Figure 4b), on which diffraction spots arranged in circular rings are present. Moreover, energy-dispersive X-ray (EDX) analyses (inset of Figure 4a) reveal that plate-like HAp crystals are calcium-deficient with an average Ca/P molar ratio of 1.50. Calcium-deficient HAp (d-HAp) can be represented by a general formula, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}\,(OH)_{2-x}$,[14] where x ranges from 0 to 2, giving a Ca/P molar ratio between 1.67 and 1.33, while $x = 0$ corresponds to the stoichiometric HAp (s-HAp). d-HAp is of greater biological interest than s-HAp because biological HAp is calcium-deficient and plays some important roles in processes such as bone remodeling and formation.[12,15]

In conclusion, wormhole-like mesoporous bioactive CaO–B_2O_3–SiO_2 glasses have been prepared by a combination of surfactant-templating, sol–gel method, and EISA processes. Plate-like, carbonated, and calcium-deficient HAp crystals may finally deposit over the entire surface of MCBS glasses after soaking in SBF for a short period of time. The combination of the diversified morphologies due to the sol–gel process and the superior in vitro bioactivity, together with the porous nature, properties that are useful for carrying drugs and immobilizing enzymes, make the brand-new MCBS glasses promising for applications in clinical orthopedics, controlled drug delivery, tissue engineering etc.

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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