Hierarchically Porous Bioactive Glass Scaffolds Synthesized with a PUF and P123 Cotemplated Approach

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Received March 28, 2007. Revised Manuscript Received June 19, 2007

Hierarchically porous bioactive glass ($CaO-SiO_2-P_2O_5$) scaffolds have been prepared by using nonionic block copolymer $EO_{20}PO_{70}EO_{20}$ (P123) and polyurethane (PUF) as cotemplates. It exhibits a hierarchical structure with interconnected macropores (about 200−400 or 500−700 μm), which provide the potential for tissue ingrowth and the neovascularization, and uniform mesopores (3.68 nm), which cause an increase in the specific surface area and enhanced bioactivity and release of ionic products. The ordered hierarchically porous scaffold has the advantages of both mesoporosity and macroporosity, which may find potential applications in tissue engineering and drug storage.

Introduction

In recent years, hierarchically porous materials of multiple length scales have attracted much attention because of their multilevel porous structure and high surface areas, which can provide many novel properties and have important prospects in practical industrial processes, such as catalysis, adsorption, separation, chemical sensing, transportation/storage of fluids and gases, and some biological applications.[1−5] Such materials show improved diffusion of the guest molecules through the inorganic network via pores and channels, as larger pores allow for better molecular accessibility, whereas the smaller pores provide high surface areas and large pore volumes.[2] Hierarchically porous silica has been prepared by the multiphase process,[3] wood cells,[4] and polyurethane foams.[5]

Bioactive glasses (BGs) have attracted much attention since the pioneering work by Hench et al. in 1971.[6] The inorganic part of the human bone is hydroxycarbonate apatite (HCA), and when BGs are implanted in the human body, a HCA layer capable of bonding with living bone is formed on the surface of the bioactive material. To repair large defects, we require 3D scaffolds, rather than usual powder or granules, in which form bioactive glasses are currently commercially available,[7,8] to provide a template and support tissue growth. Ideally, the scaffold must consist of an interconnected network with large pores (larger than 100 μm) to enable tissue ingrowth and nutrient delivery to the center of the regenerated tissue, and micropores (<2 nm) and/or mesopores (2 nm < pore size < 50 nm) to promote cell adhesion, adsorption of biologic metabolites, and resorbability at controlled rates to match the process of tissue repairing.[9−11] However, the conventional sol−gel-derived bioactive glass is heterogeneous in composition, disordered in pore channel arrangement and nonuniform in pore size distribution.[12−14]

The conventional sol−gel-derived bioactive glass scaffolds have been reported to show good bioactivity; however, the scaffolds, almost the same as the corresponding BGs, still suffer from the heterogeneity in composition, nonuniformity in the mesoporous structure and size. These scaffolds can further improve their bioactivity through increasing surface area by creating an ordered meso- or microporosity. Highly ordered mesoporous bioactive glasses (MBGs) with uniform and controllable pore sizes and relatively large pore volumes have therefore been synthesized by templating processes with block copolymers.[15,16] MBGs possess better bone-forming bioactivities in vitro compared to conventional sol−gel-

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10.1021/cm0708564 CCC: $37.00
Published on Web 07/26/2007

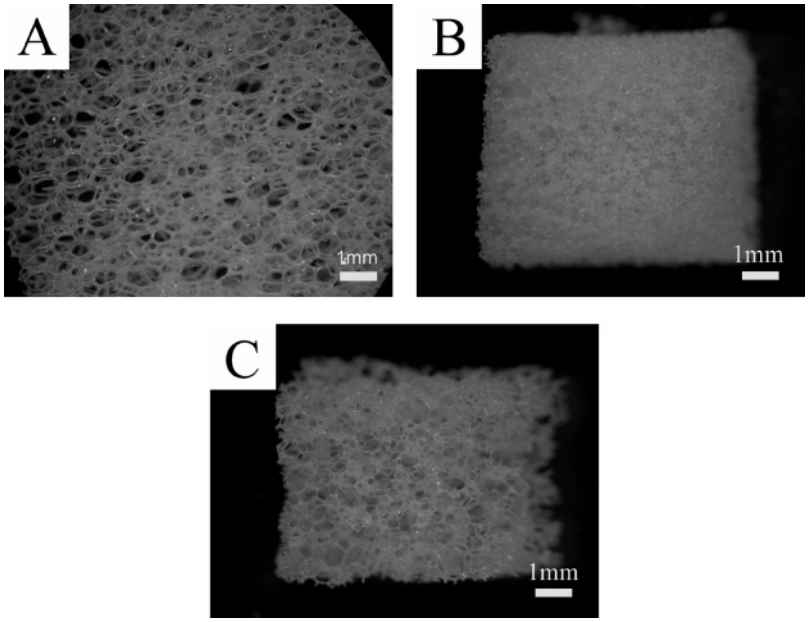

Figure 1. Optical micrographs of (A) the polyurethane foam used for preparing the scaffold-S, (B) hierarchically porous bioactive glass scaffold-S; and (C) scaffold-L.

derived BG. However, the currently reported MBGs have only a single kind of mesopore with a size of several nanometers, and their use has been restricted to powders. To the best of our knowledge, there has been no report in the literature on ordered hierarchically meso/macroporous bioactive glass scaffolds. Herein, we present the first synthesis of the hierarchically porous bioactive glass scaffold using polyurethane (PUF) for macroporosity and a block copolymer P123 as the templating surfactant for mesoporosity. The foams exhibit a hierarchical structure with interconnected macropores (about 200−400 or 500 − 700 μm), which provide the potential for tissue ingrowth, and mesopores (3.68 nm), which enhance bioactivity and release of ionic products.

Experimental Section

The hierarchical bioactive glass scaffolds were synthesized by using nonionic block copolymer $EO_{20}PO_{70}EO_{20}$ (P123) and PUF as the cotemplate. P123 was obtained from BASF. Polyurethane foam (Xinfeng Sponge Factory, China) is a kind of sponge with an open porous structure and strong liquid-absorbing properties.

In a typical synthesis, P123 (4.0 g), tetraethyl orthosilicate (TEOS, 6.7 g), $Ca(NO_3)_2{\cdot}4H_2O$ (1.4 g), triethyl phosphate (TEP, 0.73 g; 80:15:5 Si:Ca:P molar ratio), and 0.5 m HCl (1.0 g) were dissolved in ethanol (60 g) and stirred at room temperature for 1 day. Afterward, the polyurethane foam was completely immersed into the sol and compressed in order to force the sol to migrate into all pores; the excess sol was then squeezed out. The struts of the sponge body tissue were uniformly coated with the appropriate sol while the pore remained open. After 1day of drying, the same procedure was repeated. The raw porous body was dried for several days in air at room temperature and then dried at 100 °C for 24 h. After the samples were completely dry, they were heat-treated at a slow heating rate of 1 °C/min to 700 °C and then kept at that temperature for 5 h. This stage was aimed to decompose and burn out the polyurethane foam support without collapsing the scaffold. Two kinds of sponge with different porosities were used to prepare the hierarchical bioactive glass scaffolds. The two samples prepared using the PUF with the small and large pore sizes were named as scaffold-S and scaffold-L, respectively.

The bioactivity of the scaffold-S in vitro was tested by immersing the scaffold samples with a dimension of $5 \times 5 \times 5$ mm^3 in simulated body fluid (SBF) at a solid:liquid ratio of 1 mg/mL at 37 °C in the static state to monitor the formation of hydroxyapatite on the surface of the sample with time, and the SBF medium was not refreshed. Afterward, the scaffold was taken out, washed three times with acetone, and dried in air for further characterization.

X-ray diffraction patterns of the prepared samples were recorded on a Rigaku D/Max-2200PC X-ray diffractometer with Cu target (40 kV, 40 mA). Field-emission TEM analysis was conducted with a JEOL 2010 electron microscope operating at 200 kV. FE-SEM analysis was conducted with a JEOL JSM 6700F electron microscope. N_2 adsorption−desorption isotherms were carried out on Micromeritics Tristar 3000 analyzer at 77 K under a continuous adsorption condition. BET and BJH methods were used to determine the surface area, pore volume, and pore size distribution. The points used for the calculation of the BET specific surface area are at the pressure smaller than 0.2 P/P_o. The pore volumes were calculated according to BJH desorption cumulative pore volume of pores between 17 and 3000 Å diameter. FTIR spectra were obtained on a Nicolet Thermo NEXUS FTIR spectrometer. The concentrations of Ca, P, and Si in the SBF solutions before and after soaking were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Varian Co., USA).

Results and Discussion

Macroporous Structure. Optical micrographs of the scaffold-S and scaffold-L show that the two kinds of scaffolds have recorded a reticular structure (images B and C of Figure 1) of the original PUF sponge (Figure 1A). However, due to the shrinkage during the calcination process, the macropore diameter of the scaffold-S decreased to around 200−400 μm from the original about 700 μm of the PUF sponge. Scaffolds with different geometries can be prepared simply by cutting the polyurethane foam into the desired shapes. The representative field-emission scanning electron microscopy (FE-SEM) images of the scaffold with different

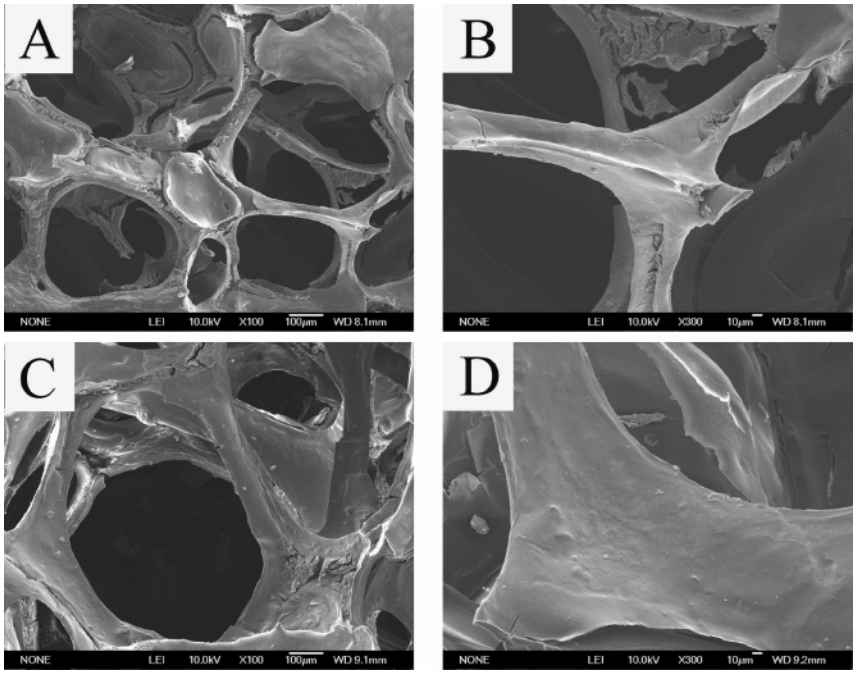

Figure 2. Representative FE-SEM images of the samples scaffold-S (A, B) and scaffold-L (C, D) after calcination at 700 °C.

macropore diameters are shown in Figure 2. It can be seen that the scaffold-S exhibits a macroporous structure with interconnected open pores of 200−400 μm in size and the thickness of the pore walls about 30 μm. When a PUF template with much larger pore size is used, the scaffold-L with interconnected open pores of about 500−700 μm and the thickness of pore walls of about 100 μm can be obtained.

Tissue regeneration techniques involve the implantation of scaffolds into defects to regenerate a tissue in situ. In tissue engineering applications, the scaffolds are seeded with cells in vitro to produce a base for tissue before implantation.[11] To obtain a high density of cells seeding into the scaffold and to promote neovascularization when being implanted in vivo, the scaffolds, in addition to biocompatibility and biodegradability, should have high porosity, large surface area, suitable pore size, and highly interconnected pore structure.[17] It is difficult to draw a clear conclusion about the optimum macropore size for the bone substitutes, which largely depends on the features of materials. However, it is generally thought that the macropore diameter and the macropore interconnections should be larger than 50−100 μm.[18] If the diameters of these interconnected macropores are not large enough, tissue ingrowth and vascularization will not occur efficiently in vivo and the damaged tissue cannot be fully regenerated.[10] Most studies considered that pores between 50 and 150 μm determine osteoid growth and pores larger than 150 μm facilitate proliferation of cells, vascular ingrowth, resorption, and internal mineralized bone formation.[10,17,18] In this study, highly hierarchically porous bioactive glass scaffolds were successfully fabricated, which showed a homogeneous distribution of open macropores with about 200−400 or 500−700 μm in pore diameter and were likely beneficial in facilitating cell infiltration and bone ingrowth.

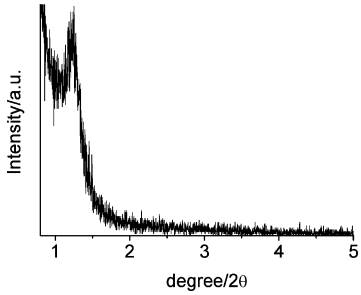

Figure 3. Small-angle XRD pattern of the scaffold-S.

Mesoporous Structure. The small-angle X-ray diffraction (SAXRD) pattern of the scaffold-S in Figure 3 indicates that such a prepared sample possesses an ordered mesoporous structure. The nitrogen adsorption−desorption isotherm of the scaffold-S shown in Figure 4A, which can be attributed to type IV behavior, is the characteristic of capillary condensation in mesopores. For the scaffold-S, a high Brunauer−Emmet−Teller (BET) specific surface area of 334 $m^2\ g^{-1}$ and a total pore volume of 0.47 $cm^3\ g^{-1}$ can be obtained. A narrow peak in the Barrett−Joyner−Halenda (BJH) pore-size distribution curve is centered at 3.68 nm (Figure 4B), which is indicative of the uniform mesopores in this material. Figure 5 shows a typical TEM image of the scaffold-S, illustrating uniform and homogeneously distributed mesopores, as also indicated by the SAXRD and nitrogen adsorption−desorption isotherm analyses.

The conventional sol−gel-derived glasses have been found to exhibit enhanced resorbability and bioactivity in vitro and improved bone bonding in vivo compared to melt-derived bioactive glasses.[19] This has been attributed to the mesopo-

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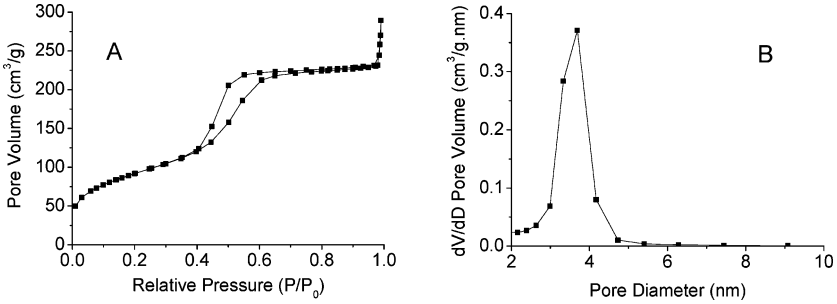

Figure 4. (A) Nitrogen adsorption−desorption isotherm and (B) corresponding pore-size distribution curve (obtained by the BJH method using the desorption branch of the isotherm) of the sample scaffold-S.

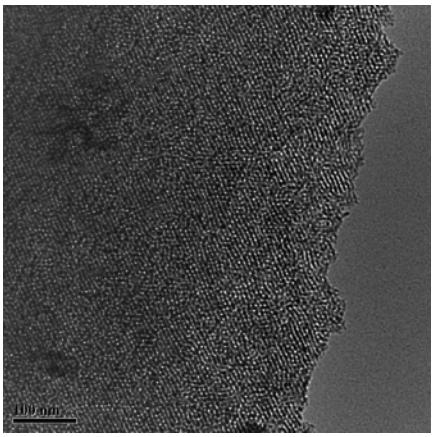

Figure 5. Typical TEM image of the sample scaffold-S.

rous texture (pores in the range of 2−50 nm in diameter) of the gel glasses, which causes an increase in the specific surface area and has been regarded as the advantage of the sol−gel process.[10] However, the conventional sol−gel-derived bioactive glass is heterogeneous in composition. In addition, the pores in conventional BG are disordered and the pore sizes are not uniformly distributed.[12−14] Comparatively, MBGs have more uniform mesopores, more homogeneous distribution in the network for all the components, higher specific surface area and pore volume, and all these features could greatly enhance bone-forming bioactivity in vitro relative to the conventional BG.[15,16] Therefore, it can be inferred that the present hierarchically porous bioactive glass scaffolds will lead to much improved bone-forming bioactivities in vitro compared to conventional sol−gel-derived bioactive glass scaffolds. The subsequent bioactivity results confirm this inferrence. In addition, the mesoporosity offers a means of adsorbing and desorbing a range of biologically active substances, such as proteins and growth factors, without the loss of conformation and biologic function, and thus promotes the bioresorption of the materials.[9]

Formation Mechanism of the Hierarchically Porous Bioactive Glass Scaffold. In this study, highly hierarchically porous bioactive glass scaffolds were successfully fabricated by using the PUF sponge replication method combined with P123 as mesoporous structure-directing agents. On the basis of previous reports,[5,20] the formation mechanism of the hierarchically porous bioactive glass scaffold can be described as follows. The primary sol is a homogeneous solution of soluble Ca, P, and Si species, P123, and HCl prepared in ethanol−water solvent. After the PUF sponge is immersed into the sol, the sol enters the interior of the struts of a reticulate PUF sponge because of the porous structure of the struts. During the solvent evaporation process, progressively increased block copolymer P123 concentration lets the surfactant concentration exceed the critical micelle concentration and promotes the self-assembly among Ca, P, Si species and P123 micelle and the formation of the mesophases on the surface and at the interior of the PUF struts. After the PUF sponge and block copolymer P123 are burned out, a positive replica of the PUF sponge with mesoporous structured bioactive glass struts is obtained.

Bioactivity of the Hierarchically Porous Bioactive Glass Scaffold. It has been reported that a significant characteristic of bioactive materials is their ability to bond with living bone through the formation of a HCA interface layer on their surface both in vitro and in vivo. The in vitro bioactivity of the scaffolds has been investigated by soaking them in simulated body fluid. SEM images of the scaffold before and after soaking in SBF for 8 h are shown in Figure 6. Before being soaked in SBF, the strut surface of the scaffold was smooth. After being soaked in SBF for 8 h, small deposits of about 150 nm on the surface of the scaffold struts were visible. FTIR spectroscopy measurements were also carried out to evaluate the changes of the scaffold as a function of soaking time. Figure 7 summarizes FTIR spectra of the scaffolds before and after soaking in SBF. Before soaking, the spectrum shows characteristic absorption bands of Si−O bonds at 1040, 800, and 470 cm^{-1}. After 4 h of soaking in SBF, an intense band at 603 cm^{-1} corresponding to an amorphous phosphate is observed. After 8 h of soaking, the band at 603 cm^{-1} split itself into a doublet at 562 and 603 cm^{-1}, corresponding to a crystalline phosphate. The absorption bands of the phosphate groups at 1040, 962, 603, and 562 cm^{-1}, together with the absorption bands of the carbonate groups at 1488, 1432, and 870 cm^{-1} and that of −OH at 630 cm^{-1}, are in accordance with the FTIR spectra of HCA. Figure 8 shows the Ca, Si, and P concentrations in SBF for different soaking times of the scaffold. The Ca level increases during the first 8 h and thereafter slowly decreases toward the end of the soaking period. The P concentration gradually decreases because of the formation of HCA by consumption of P from SBF solutions, though there are some P species released from the scaffold. The Si concentration gradually increases during soaking. Xynos et al. have reported that bioactive glass dissolution products cause rapid

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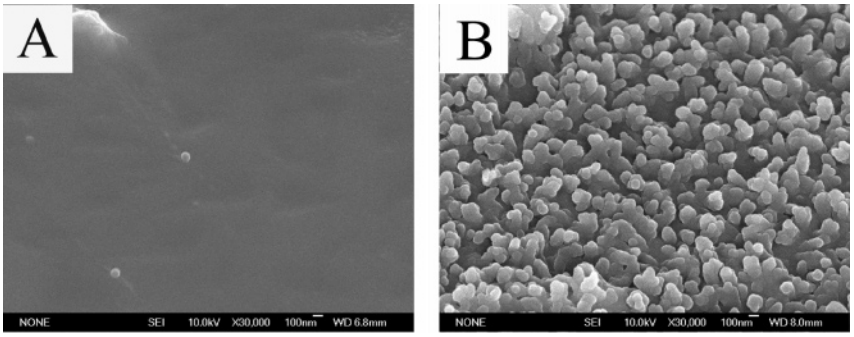

Figure 6. FE-SEM images on the struts of the sample scaffold-S (A) before and (B) after being immersed in SBF for 8 h.

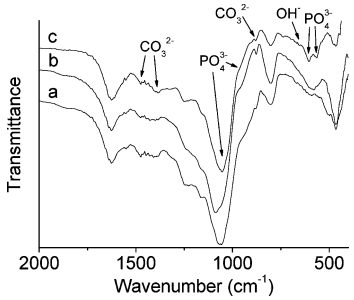

Figure 7. FTIR spectra of the sample scaffold-S (a) before and after being immersed in SBF for (b) 4 and (c) 8 h.

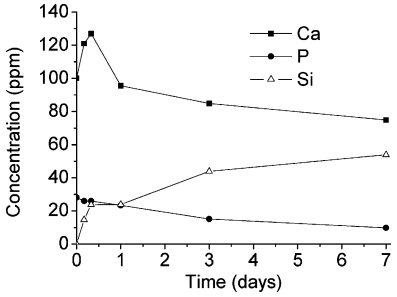

Figure 8. Concentration variations of the sample scaffold-S after being immersed in SBF for different time periods by ICP measurement.

expression of genes that regulate osteogenesis and the production of growth factors.[21] The present study shows that hierarchically porous bioactive glass scaffolds induced the precipitation of a HCA layer on their surface in SBF even for short soaking periods. It has been reported that this bonelike apatite layer could provide a suitable substrate for osteoblast-like cell proliferation and function, which allows strong bonding of the materials to the surrounding bone tissue.[17] The above results show that after being prepared as the scaffold, the hierarchically porous bioactive glass scaffolds have preserved the bioactivity of MBGs to form a HCA layer in SBF.[15] Further studies will be carried out to test the properties of the hierarchically porous bioactive glass scaffolds in cell cultures for future applications for tissue repair.

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Conclusions

We have demonstrated the synthesis of ordered hierarchically porous bioactive glass scaffold using P123 and PUF as cotemplates. P123 functions as a soft template for the formation of mesoporosity and PUF as a positive hard template for macroporous structures. The hierarchical materials exhibit fully interconnected macropores 200−400 or 500−700 μm in size and uniform mesopores of 3.68 nm; the materials have a high BET specific surface area of 334 $m^2\ g^{-1}$ and a total pore volume of 0.47 $cm^3\ g^{-1}$. The hierarchically porous bioactive glass scaffolds induced the precipitation of a HCA layer on their surface in SBF in 4 h and converted to crystalline HCA in 8 h. These ordered hierarchically porous materials have the advantages of both mesoporosity and macroporosity, which may find potential applications in tissue engineering and drug storage.

Acknowledgment. We gratefully acknowledge the support of this research by the National Science Foundation of China (Grant 20571081, 20633090) and Shanghai Nanoscience Program (Grant 0652nm014).

CM0708564