Synthesis of hierarchically porous bioactive glasses using natural plants as template for bone tissue regeneration

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Abstract A series of highly ordered hierarchically porous silica and bio-glasses materials with macropore size of 8–1,000 μm and mesopore size of 3.1–5.6 nm have been synthesized using six plant based materials as templates. However, the as-obtained porous structure was reported for the first time with interconnected 3D macropore up to 1,000 μm . The porous silica materials were used as the host for drug loading and release, which showed a good sustained delivery function. The as-synthesized bio-glasses materials indicated the highly bioactive capability in the bone regeneration. This method can be utilized to synthesize other multi-porous bioactive glasses using different plants as templates for bone tissue repairing.

Keywords Hierarchically pore silica · Bio-glasses · Plant template · Drug release · Osteogenic property

1 Introduction

Hierarchically porous materials have received enormous attention due to the multiple dimensions of their pore structures, high surface area and complex morphologies [1]. These materials combine the advantages of the two kinds of pores: macropores can improve diffusion and transport of large molecules, and the high surface area and large pore volumes of mesopores are beneficial for loading

large amounts of guest molecules. Thus, the materials will have promising prospects for industrial processes involving catalysis, adsorption, separation, chemical sensing, storage of fluids and gases in transportation [2–4], and enzyme immobilization [5]. Generally, hierarchical porous materials have been prepared by multiple template methods, including hard templates for macropores [6–13], and soft templates for preparation of meso-/micro-pores [14]. Constructing novel hierarchically porous materials with natural biological templates is an brand-new field. They have many advantages compared to artificial ones due to their abundant, renewable, and environmentally friendly properties. They also have various structures, splendid morphologies, and good biocompatibilities [1, 15–17]. At present, all kinds of biological materials have been used as hard template such as plants: wood [18], bamboo [19], diatoms [20]; animal tissue: cuttlebone [21], echinus bone [22–29]. However, the materials prepared by these natural templates contain only 1–5 μm wide macropores in general. So far, few highly ordered hierarchically porous materials have been synthesized with macropores bigger than 10 μm [18, 19] using natural plants as template.

Recently, these hierarchically porous materials have been used in tissue regeneration. Ideally, a scaffold for bone repaired should have three important characteristics: (1) an interconnected framework with large pores ($>10 \mu\text{m}$) to enable tissue growth and nutrient delivery to the center of the regenerated tissue; (2) a large specific BET surface area provided by a microporous or mesoporous phase to promote cell adhesion, drug storage and delivery, and adsorption of biologic metabolites [30–33]; (3) a favorable bio-compatibility (i.e. the formation capability of hydroxyapatite (HAP) for repairing of bones).

Bioactive glasses (BGs) have been an interesting topic since the pioneering work by Hench et al. [34]. To date

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macro-/meso-porous bioactive glasses (MMBGs) have been studied by several research groups for bone tissue regeneration [35–38]. These reports focus mainly on the synthesis of BG materials using granular polyethylene glycol, methyl cellulose and polyurethane sponges as macropore templates and nonionic block copolymers as mesopore templates. Osteogenic properties of multi-level pore materials were also studied. Till now, no BGs with macropore size larger than 10 μm have been synthesized using natural templates.

In this paper, we successfully synthesized a series of highly ordered hierarchically porous silica materials with macropore sizes ranging between 8 and 1,000 μm and mesopore sizes between 3.1 and 5.6 nm using six plants as templates for macropores and the block copolymer P123 as mesopore template. To the best of our knowledge this is the first report about hierarchically pore structures with interconnected 3D macropores up to 1,000 μm . In addition, ibuprofen (IBU) was employed as a model drug to study the drug loading/release profiles of these silica materials. Furthermore, we achieved the first synthesis of a hierarchical porous bioactive glass scaffold using plants and P123 as co-template by adding calcium and phosphate ions during synthesis of the silica materials. The BGs also exhibit a hierarchical structure with interconnected macropores (about 20–200 μm) and 3.1–4.1 nm wide mesopores, the bioactivity of the BGs for bone tissue regeneration was simultaneously investigated revealing superior in vitro bone-forming bioactivities of the prepared BGs.

2 Experimental section

2.1 Materials

All the chemicals were purchased from commercial sources and used without further purification: $\text{EO}_{20}\text{PO}_{70}\text{EO}_{20}$ (P123, Aldrich Chemical Co., USA), Tetraethoxysilane (TEOS, Tiantai Co., Tianjin China), hydrochloric acid and ethanol (EtOH, Harbin Chemical Co., Harbin, China), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, Tianjin Chemical Co., Tianjin, China), triethyl phosphate (TEP, Shenyang Chemical Co., Shenyang, China) and ibuprofen (IBU, Tianjin Chemical Co., Nanjing). Plants were obtained from Harbin, China.

2.2 Characterization

Samples were characterized by X-ray diffraction (XRD) using a SIEMENS D5005 diffractometer with $\text{Cu K}\alpha$ radiation at 40 kV and 30 mA. N_2 adsorption/desorption isotherms were measured at liquid nitrogen temperature using a Micromeritics ASAP 2010M system. The pore

sizes distributions were calculated from the adsorption branches of the N_2 adsorption isotherms using the Barrett–Joyner–Halenda (BJH) model. Scanning electron microscopy (SEM) was performed using a Hitachi S-4800 instrument operated at an accelerating voltage of 200 kV. An SEM–EDS accessory was used to observe the HAP growth on the surfaces of samples. Transmission electron microscopy (TEM) images were recorded on JEOL 2010 F and Philips CM200 FEG instruments with an acceleration voltage of 20 kV. UV–vis spectra were measured on a 752 Spectrophotometer made in Shanghai. The concentrations of Ca, P, and Si in simulated body fluid (SBF) solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES; Varian Co., USA) before and after soaking of plant.

2.3 Synthesis of hierarchical porous materials

In a typical procedure, a surfactant solution was prepared by adding 0.9 g P123 to a mixed solution containing 10.0 g of ethanol, 0.8 g of water and 0.1 g of 2 mol L^{-1} hydrochloric acid, and then 2.08 g TEOS was added. The mixture was stirred for 2 h, the dried plants were cut into $1 \times 1 \text{ cm}^3$ including calla peel, paulownia stem, poplar stem, abutilon stem, artichoke stem and calla stem, and then they were soaked in the solution for 3 days at 60 $^\circ\text{C}$ in a sealed polypropylene container. The plant based materials were then removed from the solution and re-soaked in a new solution for another 3 days at 60 $^\circ\text{C}$. Finally, the samples were taken out from the sol-like mixture, air-dried, and calcined at 550 $^\circ\text{C}$ for 5 h in air. The obtained hierarchical porous materials were named as HPM1 (calla peel), HPM2 (paulownia stem), HPM3 (poplar stem), HPM4 (abutilon stem), HPM5 (artichoke stem) and HPM6 (calla stem), respectively.

2.4 Drug load and release profiles

For the studies of IBU release, HPM4 and HPM5 were selected randomly as samples. A typical drug release experiment was performed as follows: 206 mg of porous composite material and 206 mg of IBU were dispersed in 10 ml of n-hexane solution by stirring for 2 h at room temperature. Then the loaded samples were separated from the solution by vacuum filtration and dried under ambient conditions. The filtrates were diluted by n-hexane solution and the amount of loaded IBU was measured with the UV–vis spectrophotometer.

The release profiles of the samples were obtained by soaking the drug-loaded powders in phosphate buffer solution (pH = 6.8). The release experiment was performed at 37 $^\circ\text{C}$. At predetermined time intervals, 3 ml of sample was withdrawn and another 3 ml of fresh phosphate

buffer solution was added immediately. The withdrawn samples were diluted to 25 ml and the drug concentration in the sampled fluid was measured with the UV–Vis spectrophotometer.

2.5 Preparation of the MMBG scaffolds

The mesopore/macropore bioactive glass scaffolds (MMBGs) were synthesized by using nonionic block copolymer P123 and plant peelings as co-templates. In a typical synthesis, P123 (4.0 g), TEOS (6.7 g), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (1.4 g), TEP (0.73 g), and 0.5 mol L^{-1} HCl (1.0 g) were dissolved in ethanol (60 g) and stirred at room temperature for 1 day. Afterwards, paulownia stem, artichoke stem, and abutilon stem were immersed into the solution for 3 days at 60°C in a sealed polypropylene container. After evaporating the solution for 24 h at room temperature, the samples were re-soaked in a new solution for another 3 days at 60°C . Finally, the samples were taken out from the sol-like mixture, air-dried, and heated at a slow rate of $2^\circ\text{C}/\text{min}$ to 550°C to obtain the final MMBG scaffolds named as MMBG1 (paulownia stem), MMBG2 (artichoke stem) and MMBG3 (abutilon stem).

2.6 In vitro bioactivity of the MMBG scaffolds in SBF

The assessment of the in vitro bioactivity of the MMBG scaffolds was carried out in SBF. The SBF solution had a composition and ionic concentrations similar to those of

human plasma [39]. MMBG1, MMBG2 and MMBG3 were used to investigate the bioactivity. Each type of MMBG was soaked in 100 ml SBF solution in a polyethylene bottle at 37°C . The ratio of MMBG powder weight to SBF solution volume was 1.5 mg/ml [40]. The samples were taken out from the SBF solutions after soaking for 1, 3, 5 or 7 days, then rinsed with acetone and air-dried at room temperature.

3 Results and discussion

3.1 Characterization of the porous composite materials

Figure 1 shows the morphologies of the macroporous scaffolds by SEM images. It can be seen that the six samples exhibited different pore structures. HPM1 and HPM2 have smaller pore size ($8\text{--}10 \mu\text{m}$), while HPM3 and HPM4 both have two sets of macropores whose sizes are $8\text{--}10$, $30\text{--}40 \mu\text{m}$ and $8\text{--}10$, $60\text{--}80 \mu\text{m}$, respectively. The pore size of HPM5 decreases gradually from the edge ($200 \mu\text{m}$) to the center ($80 \mu\text{m}$), and the pore size of HPM6 ranges between 500 and $1,000 \mu\text{m}$.

Figure 2a shows the small angle XRD patterns of several hierarchically pore silicon. HMP4 and HMP5 clearly show a [100] peak, which indicates that these samples possess a hexagonally ordered mesoporous structure. The three MMBGs show very weak diffraction peaks (Fig. 2b) compared to the pure silicon materials, indicating that the mesopore structure is different with the addition of Ca and

Fig. 1 SEM image hierarchically macro/meso scaffolds **a** HPM1, **b** HPM2, **c** HPM3, **d** HPM4, **e** HPM5, **f** HPM6

Fig. 2 XRD patterns of samples at low diffraction angles **a** HMP4, HMP5, **b** MMBG1, MMBG2 and MMBG3

P. TEM images confirm the highly ordered hexagonal pore arrangement (Fig. 3).

The nitrogen adsorption–desorption isotherms and the corresponding pore size distributions of all samples (Fig. 4) indicate a type IV isotherm. The surface areas, pore volumes and sizes are listed in Table 1. It can be seen that all samples possess large volumes from 0.23 to 0.4 cm³/g and the specific BET surface area reaches 300–420 m²/g. Narrow peaks in the BJH pore-size distributions are centered at 3.13–5.56 nm. It can be inferred

that the mesoporosity provides a way for loading larger amounts of guest molecules, by which could improve in vitro bioactivity.

Each plant consists of cellulose, hemicellulose and lignin, interconnected vessels, tracheids and sieve tubes are contained in each part of the plant. The primary as-synthesized sol is a homogeneous solution. After soaking of plant into the solution, the silicate species were mineralized in the inner of the vessels, tracheids and sieve tubes. After the calcination, the silica scaffold preserved the original

Fig. 3 TEM images of **a** HMP4, **b** HMP5 recorded along the [100] directions, **c** MMBG1, **d** MMBG2 recorded along the [001] directions

Fig. 4 Nitrogen adsorption/desorption isotherm and mesopore distribution of the samples **a** HPM4, HPM5, **b** MMBG1, MMBG2 and MMBG3

Table 1 BET surface area pore volume and average pores diameter of the samples

Sample	BET surface area (m²/g)	Pore volume (cm³/g)	Average pore diameter (nm)
HPM4	306.03	0.40	5.56
HPM5	347.56	0.23	3.75
MMBG1	420.85	0.35	3.52
MMBG2	409.49	0.30	3.13
MMBG3	299.25	0.29	4.13

morphologies and structure of the plants, and “tube” types have been replicated so that macroporous materials were obtained [41].

3.2 Drug release of hierarchically pore silica

Figure 5 shows the high angle XRD patterns of IBU, the mechanical mixture of IBU and HPM4, IBU stored in

Fig. 5 XRD patterns at high diffraction angles. *a* IBU, *b* IBU mixed with HPM4, *c* IBU stored in HPM4

HPM4. It can be seen that the IBU and the mechanical mixture exhibited obvious XRD spectrum peak of the drug, it indicated that the drug molecules exist still crystal form, and the assembly does not appear the diffraction peaks of the drug, it indicated that the drug molecules has loaded into the mesopores of the material [42].

Figure 6 shows the cumulative release profile of the samples in buffer solution of pH = 6.8. It can be observed that IBU showed a similar, two-step release behavior for both samples with an initially fast and a relatively slow subsequent release through the whole period. About 20 and 45 wt% of the IBU were released from HPM4 to HPM5 within 1 h, respectively, but the IBU release reached similar values of 58.3 and 59.1 wt% when the release rate approached zero after 48 h. Maybe this is because that the

Fig. 6 Drug release kinetics of hierarchically pore silica HPM4, HPM5

Fig. 7 SEM images of the MMBG1 before and after soaking in the SBF solution for 1, 3, 5 days. **a** Before soaking, **b** after soaking 1 day, **c** after soaking 3 days, **d** after soaking 5 days

Fig. 8 SEM images of the MMBG2 before and after soaking in the SBF solution for 1, 3, 7 days. **a** Before soaking, **b** after soaking 1 day, **c** after soaking 3 days, **d** after soaking 7 days

specific surface area is mainly determined by the mesoporous phase. The drug was mainly loaded in the mesoporous channels, and only a little amount was adsorbed on

the macropore surface. The release of the drug from the pore materials may involve: solvent diffusion into the mesopores with dissolution of the drug, followed by its

Fig. 9 SEM images of the MMBG3 before and after soaking in the SBF solution for 1, 3, 5 days. **a** before soaking **b** after soaking 1 day **c** after soaking 3 days **d** after soaking 5 days

release from the mesopores into the macropores, and eventually release of the drug from the macropores to the outside solution. Thus, the macropores shall play a buffer role in the drug release. When the drug concentrations in the macropores and outside medium reached a homeostatic equilibrium, then drug molecules were no longer released from the pores. Consequently the drug could not be released completely.

3.3 Bioactivity of the hierarchically porous bioactive glass scaffolds

The ability to bond with living bone through a HAP interface layer on their surface is a significant characteristic of MMBGs, which has been widely studied both in vitro and in vivo [43]. The deposition/growth of HAP of the scaffolds in vitro has been investigated here by soaking them in SBF at 37 °C. SEM images of the scaffolds before and after soaking for 1, 3, 5 or 7 days are shown in Figs. 7, 8 and 9. It can be seen that before soaking the three MMBGs show a smooth and homogeneous surface. After 1 day soaking, the different morphologies of HAP appeared inside the large pores and surface of the samples. Spherical HAP has grown inside the macropores of MMBG1 and MMBG2 while layered HAP formed inside the macropores of MMBG3. After 3 days the macropores and the outer surface of the bio-glasses had been almost

Fig. 10 EDS spectra of sample MMBG1, MMBG2 and MMBG3 immersed for 7 days

completely covered by HAP particles. At the same time, the macropore walls had become thicker gradually. After 5 days almost all macropores of MMBG1 and MMBG3 were blocked and HAP had completely covered the sample surface, whereas it needed 7 days for MMBG2 to reach that state. The reason may be that the pore size of MMBG2 is larger than those of MMBG1 and MMBG3. There are different morphologies and growth speed of HAP for the three bio-glasses, which may meet different demands of practical bone regeneration.

The EDS results of the glasses through 7 days immersion (Fig. 10) indicate that the precipitated layers are

Fig. 11 **a** Ca, P, Si concentration change with soaking time in SBF after MMBG1 being soaked by ICP measurement. **b** Variations of Ca, Si, P of the average quality ratio on the surface of MMBG1 by EDS measurement

composed of Ca and P with a Ca/P atomic ratio of 1.61 (MMBG1), 1.63 (MMBG2) and 1.72 (MMBG3), respectively. The atomic ratios are close to the theoretical value 1.67 Ca/P ratio of apatite [44]. Figure 11a shows that the concentrations of Ca, Si, and P in SBF for various immersion periods. The results indicate that silicon was released from the glasses, while calcium and phosphate were deposited on their surface, as reported by Li et al. [36]. The Si content increase with extension of soaking time, while the concentration of P decreased continually, because phosphorus diffused slowly from the samples in SBF. The Ca^{2+} concentration is controlled by both the release of Ca^{2+} from the sample and the formation of HAP. The Ca^{2+} concentration increased during the first 3 days for the rapid calcium dissolution; and then decreased slowly, the reason can be attributed to the rapid growth of the apatite nuclei formed on surface of the sample, which overcame the release rate of Ca^{2+} to the solution. It can be concluded that a HAP layer has formed from SBF to the samples, and the materials can induce the growth of HAP on their surface. On the other hand, EDS analysis of Ca, Si, and P on the sample surfaces during different times (Fig. 11b) roughly indicated that Ca and P increased continually while Si decreased. This also confirmed the growth of HAP on the surface of the samples. Results indicated that these novel bioactive glass scaffolds with good bioactivity can induce the formation of HAP layers in SBF, and thus may have potential application in tissue regeneration engineering.

4 Conclusions

In summary, for the first time we reported the synthesis of a series of highly ordered, porous silica and bio-glasses with large pore sizes of 8–1000 μm and mesopore sizes of 3–5 nm using six plants as templates. The novel porous silica materials exhibit sustained drug delivery profiles, and the porous bioactive glass scaffolds can induce the precipitation of HAP layers on their surface in SBF within

1 day, which are converted into crystalline HAP within 7 days. The morphologies and the growth speed of HAP differ for the three MMBGs, so it is difficult to come to a clear conclusion about the optimum macropore size for bone regeneration. The unique interconnected multimodal porosity distribution and excellent in vitro bioactivity of MMBGs make them a good candidate for bone regeneration and drug delivery.

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