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Three-Dimensional Mesoporous−Giantporous Inorganic/Organic Composite Scaffolds for Tissue Engineering

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Tissue engineering, which can be defined as the science of persuading the body to heal itself by its own intrinsic repair mechanisms, has opened up whole new areas of research.[1–4] In the field of tissue engineering, the conversion from cell suspension into a three-dimensional (3D) tissue structure is guided by 3D biodegradable and biocompatible scaffolds, because the cells in a cell culture normally do not self-assemble into a 3D tissuelike structure. The scaffolds used for this application should have three-dimensional and highly porous structures with an interconnected pore network to facilitate the cell growth and flow transport of nutrients and metabolic waste. A suitable surface chemistry for the attachment, proliferation, and differentiation of cells and suitable mechanical properties to match those of the tissues at the site of implantation are also required.[3a,4] Moreover, the scaffold should exhibit bioactivity and osteoconductivity. Recently, the use of mesoporous materials, which have pores ranging in size from 2 to 50 nm, has been 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.[5–12] Highly 2D hexagonal[7] and 3D[10] cubic ordered mesoporous bioactive glasses (MBGs) with superior bone-forming bioactivities in vitro compared with normal BGs have therefore been synthesized by templating with a triblock copolymer.

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

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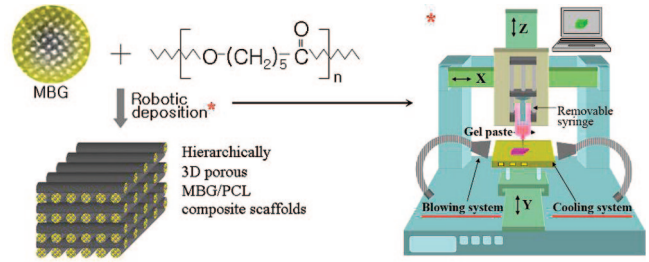
mesosized pores are too small to promote cell growth. Ideally, the scaffold must consist of a 3D interconnected pore network with a pore size of at least 100 μm (giant pores), in order for them to allow for both cell penetration and the proper vascularization of the ingrown tissue.[1] To overcome this pore size limitation, our group first proposed and successfully prepared hierarchically 3D porous BG ceramic scaffolds fabricated using a combination of the sol–gel, double-polymer templating, and rapid prototyping (RP) techniques, which can generate a physical model directly from computer-aided design data.[10a] Shi et al. also very recently reported the synthesis of the hierarchically porous BG scaffolds using polyurethane and a block copolymer template.[11] Although both of these new 3D scaffolds have favorable porous structures and sizes, porous ceramic scaffolds are too brittle to use as bone scaffolds. Therefore, composites of ceramics and polymers are being developed with the aim of increasing the mechanical scaffold stability of the scaffolds and improving their tissue interaction properties.[3] Shi et al. reported preparation of MBGs and poly ϵ-caprolactone (PCL) composite scaffold by using a solvent-casting−particulate-leaching method.[12] However, the particulate-leaching method still has some shortcomings in controlling the mechanical stability and in both the structure and interconnectivity of pores, which may limit their application in terms of cell penetration in tissue engineering.[13] To overcome this limitation, many researchers have proposed the RP technique.[13,14]

We report herein the first process that can be used for the efficient and reproducible preparation of hierarchically 3D porous MBG/polymer composites using a combination of the sol–gel, polymer templating, and RP techniques with a gantry robotic deposition apparatus. A biodegradable polymer, PCL, was adopted as the matrix of the MBG. The triblock copolymer, $EO_{20}PO_{70}EO_{20}$ (P123), $EO_{100}PO_{65}EO_{100}$ (F127), acts as a template, inducing the formation of mesopores, and the RP technique is used to produce the giant pores. We made use of a heat-controlled blowing system in the RP technique to maintain the 3D scaffold morphology followed by the rapid solvent evaporation of the solvent. The hierarchically mesoporous−giantporous BG/PCL scaffolds so obtained have good molding capabilities, enhanced mechanical properties, superior in vitro bone-forming bioactivities, and good biocompatibilities.

The synthesis strategy is illustrated in Scheme 1. We preliminarily prepared MBGs by our previously reported method and the MBGs were then ground and sieved.[10b] Granules with a size less than 25 μm were selected. The ground MBGs retained their 3D cubic mesostructure morphology as well as their large specific surface area of 505 $m^2\ g^{-1}$ (see the Supporting Information, Figure SI 1). PCL was dissolved in chloroform at 40 °C, and the predetermined amounts of MBG powders (from 20 to 80 wt % MBG with respect to PCL) were then mixed with this to produce a homogeneous MBG/PCL paste. The 3D scaffolds were fabricated by the direct exclusion of the paste onto a cooled

Scheme 1. Processing Routes of MBG/PCL Composite Scaffolds with Hierarchically 3D Pore Structure



substrate using a robotic deposition device with a heat-controlled blowing system. The experimental details are given in the Supporting Information, section SI 2. The rapid elimination of the solvent in the paste plays a key role in inducing the rapid solidification of the scaffold and subsequently in maintaining its 3D scaffold morphology, because the solvent remaining in the paste can cause the fusion of the extruded strut and subsequently induce the collapse of 3D architecture. The plotting liquid medium such as ethanol is generally used for this purpose.[15] However, the chloroform in the PCL paste is not easily eliminated with ethanol medium and the collapse of the 3D structure occurs as a consequence. A heat-controlled blowing system as well as the a chilled substrate were employed to solve this problem. The heat-controlled blowing at 40 °C allows the instantaneous evaporation of chloroform after extruding the MBG/PCL paste. The chilling of the substrate below 10 °C makes it possible to induce the fast solidification of the PCL. As a result, 3D porous PCL scaffolds were obtained. The feature comparison of the PCL scaffolds between those made using the ethanol plotting medium and those made using the blowing system is shown in the Supporting Information, Figure SI 3.

The 3D scaffold obtained by combining the bioactive ceramic and biodegradable polymer exhibited more favorable morphological, mechanical, and biological properties.[3] The addition of the MBGs to PCL makes it easier to maintain the extruded MBG/PCL strut morphology than in the case of pure PCL. The ease of formation of the 3D scaffold was improved by increasing the MBG content to 60 wt % (60MBG). However, further increasing the amount of MBG relative to PCL tended to cause the deformation of the 3D structure because of the low viscosity of the paste, as well reduce its mechanical strength. Figure 1A shows the optical images of the 3D PCL and 60MBG/PCL composite scaffolds. The 60MBG/PCL strut retained its cylindrical morphology well and enabled a 3D porous structure to be formed without difficulty, whereas the pure PCL strut partially collapsed and resulted in the production of a nonperiodic 3D porous structure. The size, thickness, and structure of the scaffold can be easily managed under computer control. A synergetic effect on the mechanical properties is expected to be obtained by adding MBG to PCL (Figure 1B). As mentioned above, the previously prepared hierarchically

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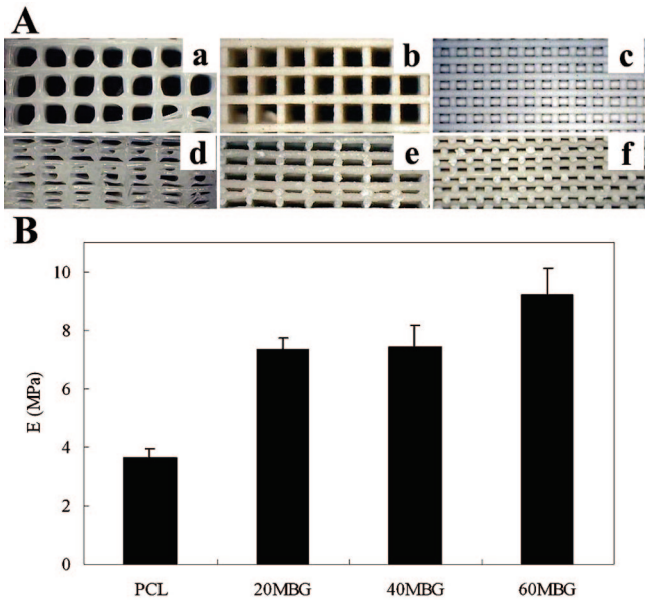

Figure 1. (A) Optical images of 3D periodic PCL (a, d) and 60MBG/PCL composite (b, c, e, f) scaffolds with simple tetragonal geometry (a, b, d, e) and with tetragonal geometry with offset layers (c, f). a−c are top views and d−f are side views. (B) Typical mechanical properties of PCL and MBG/PCL composite scaffolds with different MBG/PCL weight ratios ($n = 5$).

porous BG scaffolds could not overcome the typical handicap of the poor mechanical strength of ceramic scaffolds (compressive modulus <1 MPa). The mechanical properties are largely enhanced by adding MBG to PCL. The compressive modulus of the 60 MBG/PCL composite is 9.3 ± 0.9 MPa, whereas the compressive modulus of PCL is 3.9 ± 0.3 MPa.

BGs have been widely studied, because they have the ability to chemically bond with living bone tissue through the formation of a biologically active apatite layer at the implant−tissue interface.[2,16] The chemical compositions of the BGs and architectures of the pores are closely related to get capable bioactivity. The high porosity in the MBGs favors the rapid formation of an apatite-like layer, because it facilitates the rapid and massive release of Ca^{2+} ions.[2]b,[7] The dissolution of the MBG should result in nucleation and growth of an apatite layer on the surface of the polymer scaffold, which should further affect the polymer degradation behavior in addition to providing the required osteoconductivity. The bone-forming activity of the MBG/PCL scaffold (and that of the PCL scaffold for comparison) in vitro was tested in SBF in order to monitor the formation of apatite on the surface of the scaffold over time, as shown in Figure 2. The FE-SEM image reveals that the surface of the MBG/PCL scaffold undergoes important changes when it reacts with the SBF. The formation of apatite-like nanoparticles is observed on the surface of the MBG/PCL scaffold after soaking the sample for 4 h (images e and k in Figure 2), whereas no change is observed on the surface of the PCL scaffold after the same period of time (images b and h in Figure

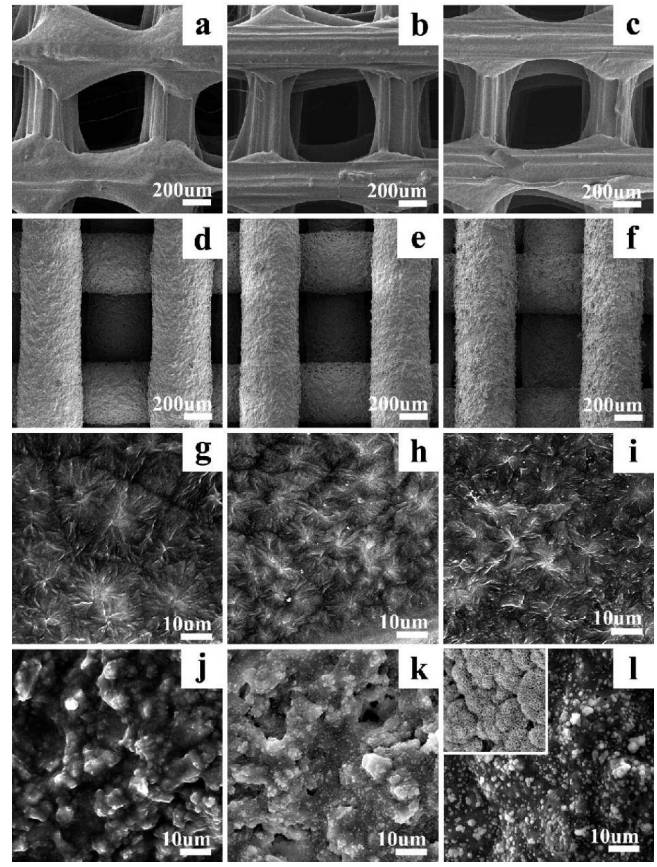

Figure 2. FE-SEM micrographs of PCL (a−c, g−i) and 60MBG/PCL composite (d−f, j−l) scaffolds after they were immersed in SBF for 0 h (a, d, g, j), 4 h (b, e, h, k), and 24 h (c, f, i, l). a−f are low-magnification images and g−l are high-magnification images. The inset of l is the 10-fold enlarged image of l.

2). It is interesting to note that the surface of the MBG/PCL scaffold was almost fully covered with newly formed apatite having a platelike morphology after being soaking for 24 h (Figure 2l), whereas there was only a partial apatite-like particle precipitation on the surface of the PCL scaffold (images c and i in Figure 2). The EDX results of each sample show the same result as the FE-SEM observation (see the Supporting Information, Figure SI 4). These results indicate that the addition of MBG to PCL improves the ability of the scaffolds to induce apatite formation, because the large surface area of the MBG allows for high bone-forming bioactivity. The apatite layer could provides a suitable substrate for the osteoblast-like cell proliferation and function, which allows for the strong bonding of the materials to the surrounding bone tissue.[17]

The biocompatibilities of the PCL, MBG/PCL scaffolds with different amounts of MBG, and BG (general sol–gel-derived BG)/PCL scaffolds were evaluated in vitro by studying the MG63 behavior for two passages, viz. after 1 day and 4 day, using the MTT test as shown in Figure 3. There were no significant differences in the cell number after 1 day of cell culture. It is surprising to note that the cell numbers seeded on the MBG/PCL scaffolds are significantly larger than those on the PCL scaffold, regardless of the amount of MBG. The released Ca^{2+} ions may stimulate the

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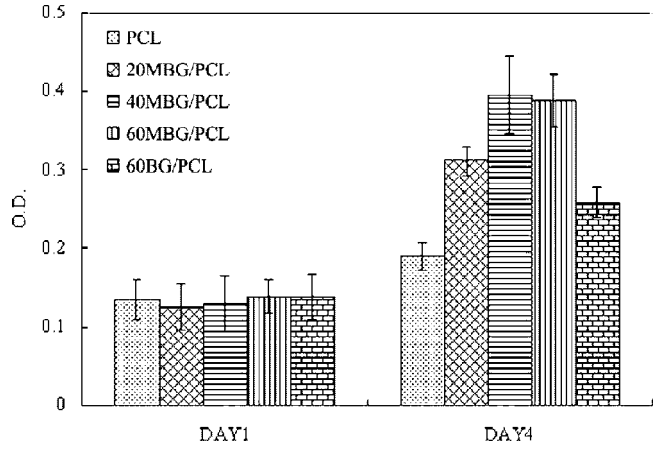

Figure 3. MTT assay for proliferation of MG-63 cultured at 1 and 4 days incubation onto 3D PCL, MBG/PCL composite scaffolds with different MBG/PCL weight ratio, and 60BG/PCL, which do not use polymer template. Error bars represent means ± SD for $n = 5$.

proliferation of the osteoblast cells, thus causing the rapid expression of genes that regulate osteogenesis.[18] The difference in the cell number between the 60MBG/PCL and BG/PCL scaffolds may come from the difference in their bioactivities, that is, MBG shows superior bioactivity to the general sol–gel-derived BG, as already reported by Zhao et al.[7] According to the results of this study, it can be inferred at this stage that the MBG/PCL scaffolds have no negative effect on the attachment and proliferation of cells and that they are in vitro biocompatible. Further studies of both their in vitro and in vivo properties are currently in progress.

In conclusion, 3D structured scaffolds were designed by a computer and well-produced from the MBG/PCL paste by using a RP technique with a heat-controlled blowing system. Each strut, which forms giant pores, contains 3D cubic structured mesopores. The combination of MBG and PCL brings about a significant enhancement of the molding capabilities, mechanical properties, and in vitro bone-forming bioactivities of the scaffold. These materials are excellent candidates as scaffolds for bone tissue regeneration. This simple and reproducible synthetic method can be used with various materials, such as other polymers and ceramic particles, for the preparation of various hierarchical porous materials and have the potential to be used in applications involving biomedical devices, drug delivery systems, filters, catalysis, and optics. A more detailed structural investigation and studies of their mechanical and biological properties are currently in progress and will be the subject of future publications.

Supporting Information Available: Optical images of the PCL scaffolds fabricated by different solvent elimination systems; detailed synthesis procedure and characterization methods; structural information of MBG; EDX spectra of PCL and 60MBG/PCL scaffold after they were immersed in SBF for different times (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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