Supporting Information

Three-dimensional Mesoporous-Giantporous Inorganic/organic Composite Scaffolds for Tissue Engineering

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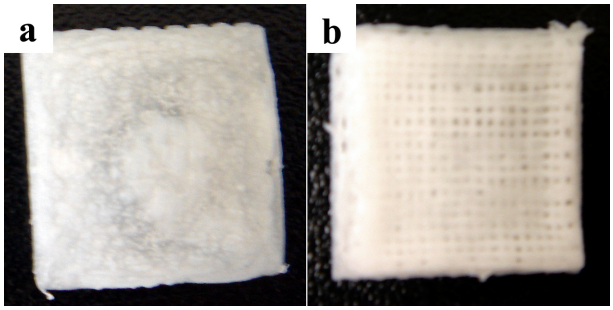


SI 1. PCL scaffolds fabricated by a robotic deposition with a plotting medium (a) and a heat-treated blowing system (b).

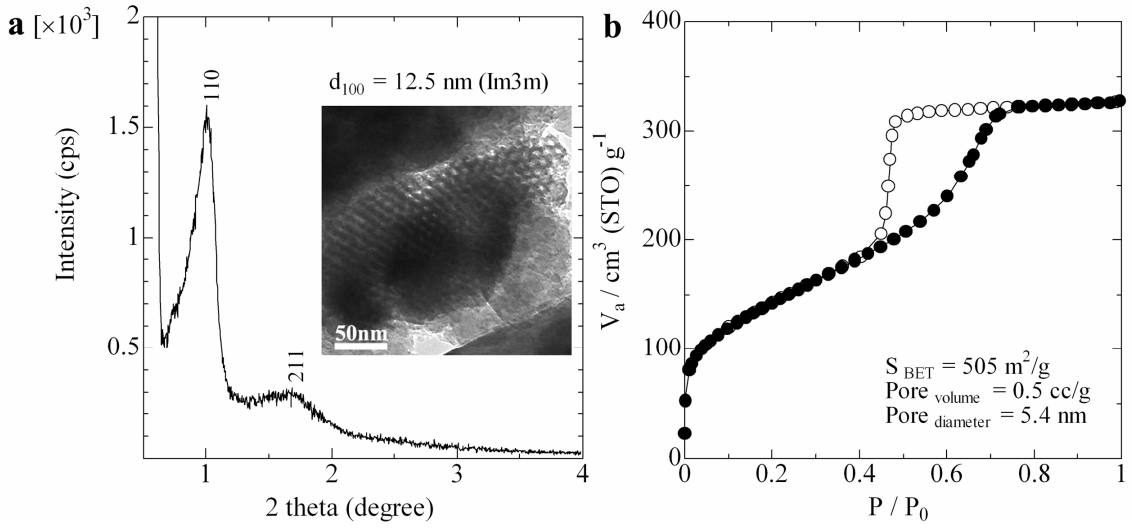
SI 2. Experimental details

Synthesis of gel paste: MBG with 3D cubic pore structure were prepared using tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), and calcium nitrate tetrahydrate (CaNT) as inorganic precursors, and a triblock copolymer, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (Pluronic F127, $EO_{100}PO_{65}EO_{100}$, M_{ave} = 12600), as the meso-structure-directing agent through an evaporation-induced self-assembly process. In a typical synthesis, 2.88 g of F127 is dissolved in 18.1 ml of ethanol (EtOH). Stock solutions, which were prepared by mixing 1.36 g of CaNT, 0.26 ml of TEP, 6 ml of TEOS, 0.95ml of HCl (1M), 7.62 ml of EtOH and 2.86 ml of H_2O, were added to this solution after stirring them separately for 1h, and were vigorously stirred together for another 4h at 40 °C. The molar composition was TEOS: CaNT: TEP: F127=1: 0.2: 0.05: 0.008 in this case. The reactant solution was transferred to a polystyrene vessel without a cap, and aged at 40 °C, 40 RH% for 48h without stirring. Gel films were obtained on evaporating the solvent. These gel films were easily separated from the vessel after aging and were calcined at 600 °C for 6h in air to remove the template. The calcined MBGs were then ground and sieved. Granules with size in below 25 μm were selected. PCL was dissolved in chloroform at 40 °C and the determined amounts of MBG powders (from 0 to 80 wt. % of MBG to PCL) were then mixed with this to produce homogeneous PCL/MBG paste.

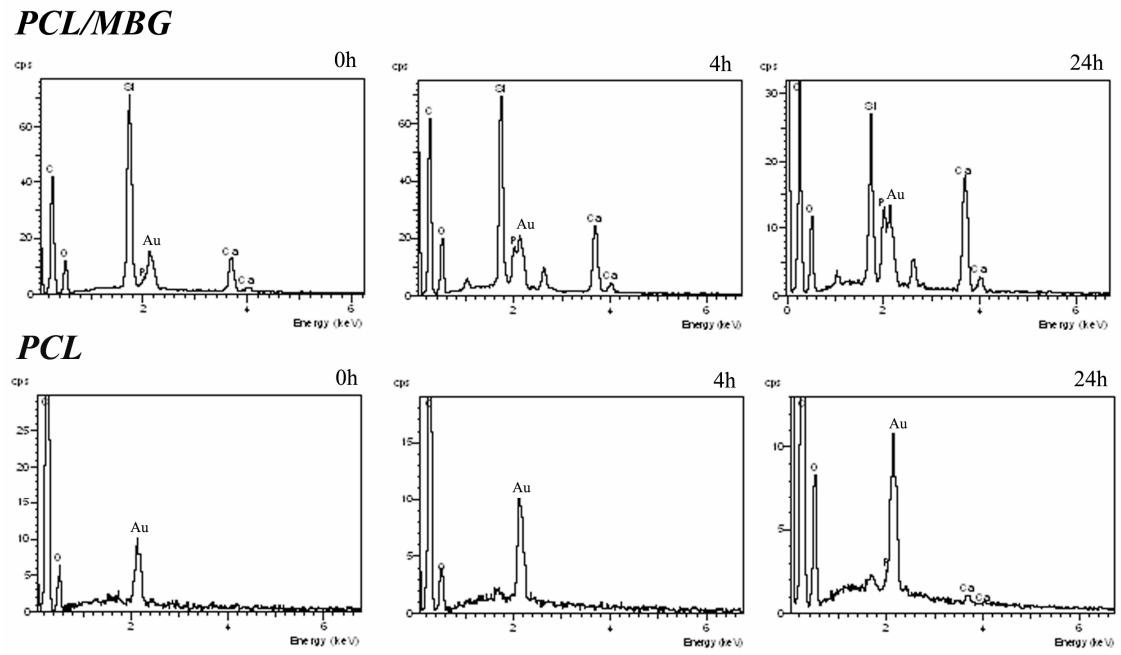
Robotic deposition of scaffolds: Scaffolds were fabricated by directly excluding the paste gel onto a chilled substrate using a robotic deposition device. A gantry robotic deposition apparatus was used with specially-altered systems such as an actuator to control the position of deposition nozzle for scaffold fabrication and a heat-controlled blowing system to maintain 3D scaffold morphology followed by the rapid solvent evaporation. Three axes of motion control (x, y, and z-axis) were provided by the gantry system, and a material delivery assembly composed of a syringe as a reservoir was affixed on the z-axis motion stage. The z-axis motion stage assembly was mounted on a moving x gantry to enable controlled motion of the mounted syringe in three dimensions. The gel paste housed in the syringe was deposited through a cylindrical nozzle (17~26 gauge (G), 24 G (≈ 500 μm) is generally used). A linear actuator served to depress the plunger of the syringe at a fixed speed such that the volumetric flow rate could be precisely controlled. The excluding strength and speed is controlled 200~250 μl/min and 5~10 mm/sec, respectively, depending on the viscosity of gel paste. The gel paste excluded onto chilled substrate (5~10 °C) to the fast condensation followed by the solidification of PCL. The shapes and sizes of scaffold can be designed at discretion and can be controlled by computer system.

In Vitro bioactivity: The assessment of the in vitro bioactivity of the hierarchically porous scaffolds was carried out in SBF at 37 °C. The scaffold was immersed in SBF after washing with EtOH and drying. SBF contained 142.0 mM Na^{+}, 5 mM K^{+}, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 147.8 mM HCl^{-}, 4.2 mM HCO^{3-}, 1.0 mM $HPO4^{2-}$, and 0.5 mM $SO4^{2-}$. Its chemical composition was similar to that of human plasma. The solution had a pH of 7.4 and was kept at 37 °C before use.

In vitro biocompatibility test: The fabricated 3D scaffolds were repeatedly washed with EtOH and freeze dried to remove extra remained chloroform before cell seeding. Human osteoblast-like cells, MG63, were seeded onto square-shaped specimens (height = 8 mm, width = 8 mm, depth = 3 mm) at a density of 5×10^5 cells/ml and cultured with Dulbecco's Modified Eagle Medium, 10 % fetal bovine serum (FBS), and 1 % Penicillin/Streptomycin. The cell culture was maintained in a gas-jacket incubator equilibrated with 5 % CO_2 gas at 37 °C. The cell viability and proliferation were determined using the MTT assay. The absorbance of the formazan produced by the cells was measured at 570 nm with a micro-plate reader (ELISA). 5 specimens were examined for each sample.



SI 3. XRD pattern, TEM image, and N_2 adsorption-desorption isotherms of MBG, templating by F127.



SI 4. EDX results of PCL and PCL/MBG composite scaffolds after immersing them in SBF (Au was coated to FE-SEM observation).