Supplementary Material (ESI) for Chemical Communications

Supporting Information

Bioinspired Fabrication of 3D Hierarchical Porous Nanomicrostructures of Calcium Carbonate for Bone Regeneration

Experimental Section

Fabrication of nanoporous $CaCO_3$ microcrystals: Typically, a glass Petri dish was filled up with 1 M calcium nitrate (99%, A.C.S. reagent, Sigma-Aldrich) solution and then placed in a closed desiccator containing a 10-mL vial of ammonium carbonate (A.C.S. reagent, Aldrich). After 3-day reaction at room temperature (~25 °C), the white products were removed from the Petri dish, washed with DI water for 3 times and dried at 65 °C for 3 h. As a result, different sized calcium carbonate microcrystals were obtained to have 5 and 50 μm in size by prolonging the reaction time from 6 hours to 3 days. For heat treatment, the temperature of a furnace (Nabertherm, model N 11/H with a program controller, Germany) was first increased to a predetermined temperature of 900 °C. After being stabilized for 0.5 h, the as-prepared calcium carbonate microcrystals in a crucible were put into the furnace and then heated for 1 h. The as-decomposed product was then taken out of the furnace at the same temperature and cooled to room temperature at ambient conditions. Shape and morphology of the as-prepared samples were examined by JEOL JSM-6700 field-emission scanning electron microscope (SEM).

Fabrication of hierarchical $CaCO_3$ scaffolds: A special mold has been designed for the construction of $CaCO_3$ scaffolds with hierarchical pore sizes. The nano-/micro-porous $CaCO_3$ microcrystals were loaded into the mold as building blocks and the macro-pores of the scaffolds were generated in the mold by using pins with a diameter of 500 μm. A

supersaturated solution of calcium nitrate was added to interconnect the nano-/micro-porous $CaCO_3$ microcrystals by newly formed $CaCO_3$ in the CO_2 environment (same as the conditions for crystal growth).

In vitro evaluation: MC3T3-E1 osteoblasts were maitained in α-MEM medium supplemented with 2.0 mM L-glutamine, 1.0 mM sodium pyruvate, 10% fetal bovine serum and 1% penicillin-streptomycin solution. The osteoblasts were seeded onto the $CaCO_3$ scaffolds in 24-wells plate after the scaffolds were soaked with the culture medium for 1 h. After 14 days of culture, the scaffolds were taken out and rinsed with PBS for 2 times. Cell adhesion and proliferation were observed under confocal laser scanning microscopy (Olympus FV500, Japan) with the cells fixed and then stained with propidium iodide (PI, Invitrogen).

In vivo evaluation: Surgery of male Sprague Dawley rats was performed under general anesthesia and aseptic conditions. After a longitudinal skin incision to expose femurs, two large holes with diameters of 3 mm were made on each femur using surgical drill: one hole was filled with porous $CaCO_3$ while the other was left empty as control. New bone formation within the defect regions was evaluated using micro-computed tomography (micro-CT, Skyscan 1076, Belgium) imaging. Samples were scanned through 180° with a rotation step of 1° at a spatial resolution of 35 mm. The scan files were reconstructed at a step size of 1 using a modified Feldkamp algorithm and the reconstructed data were loaded onto the 3D modeling software, VGstudio (Volume Graphics GmbH, Germany) to stack the 2D image into a 3D model for further analysis. A threshold value of 180 was applied to segment the bone tissue from the surrounding soft tissue.

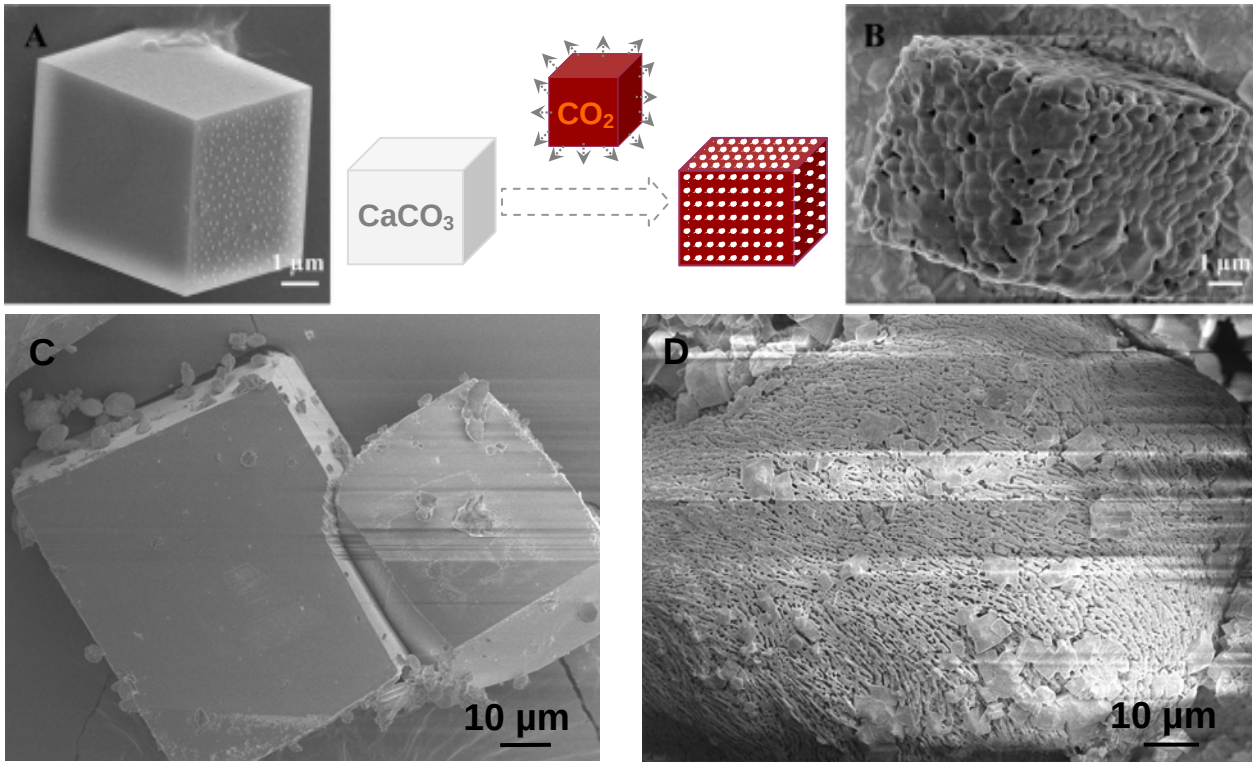

Fig. S1 SEM images of small $CaCO_3$ microcrystals of ~5 μm in size (A) before and (B) after 0.5-h heat treatment at 900 °C. SEM images of large $CaCO_3$ microcrystals of ~50 μm in size (C) before and (D) after 1-h heat treatment at 900 °C. Longer thermal treatment has been applied to larger microcrystals for complete formation of porous structure throughout the crystal. Schematic illustration of the formation process was shown in the middle.

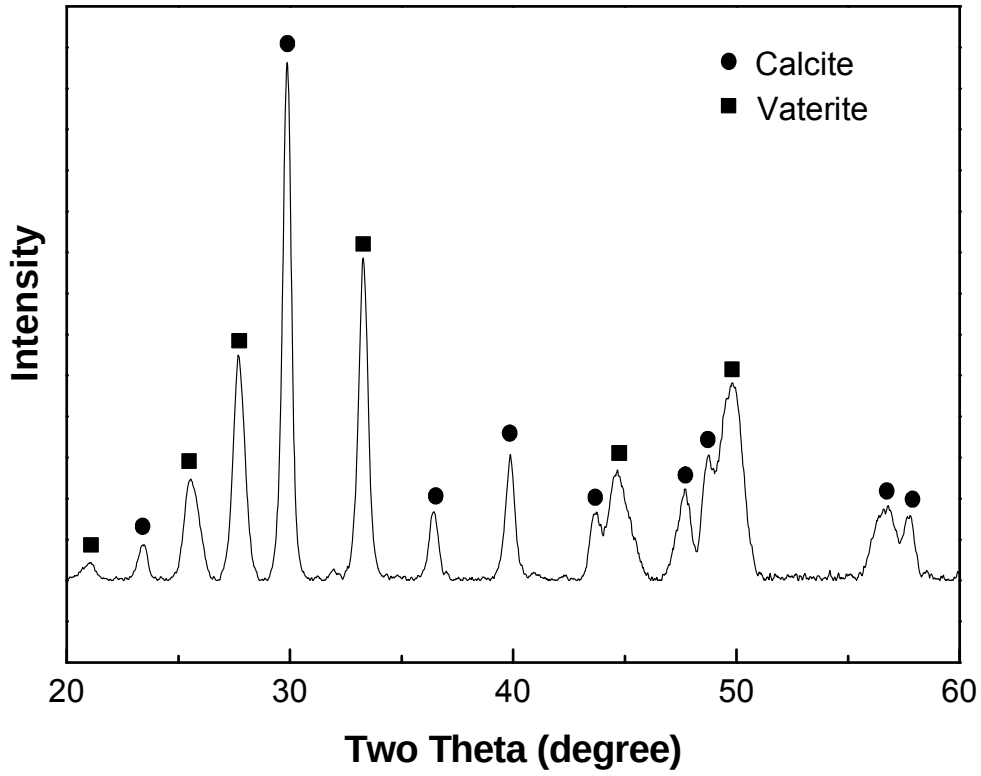

Fig. S2 XRD pattern of 50-μm $CaCO_3$ microcrystals that was treated in a furnace at 900 °C for 1 h before taking out at the same temperature.

Experimentally, the as-prepared $CaCO_3$ microcrystals were put into a furnace at 900 °C. After remaining at this temperature for 1 h, the thermally treated product was taken out and then cooled to room temperature naturally. The existence of two $CaCO_3$ polymorphs calcite and vaterite was revealed by XRD. The fast temperature increase from room temperature to 900 °C led to the formation of highly porous CaO structure, which reacted with H_2O and CO_2 to form $Ca(OH)_2$ and $CaCO_3$ and further converted into $CaCO_3$ quickly. This result suggested the porous structure throughout the crystals. XRD measurement was performed within 1 h after the thermal treatment.

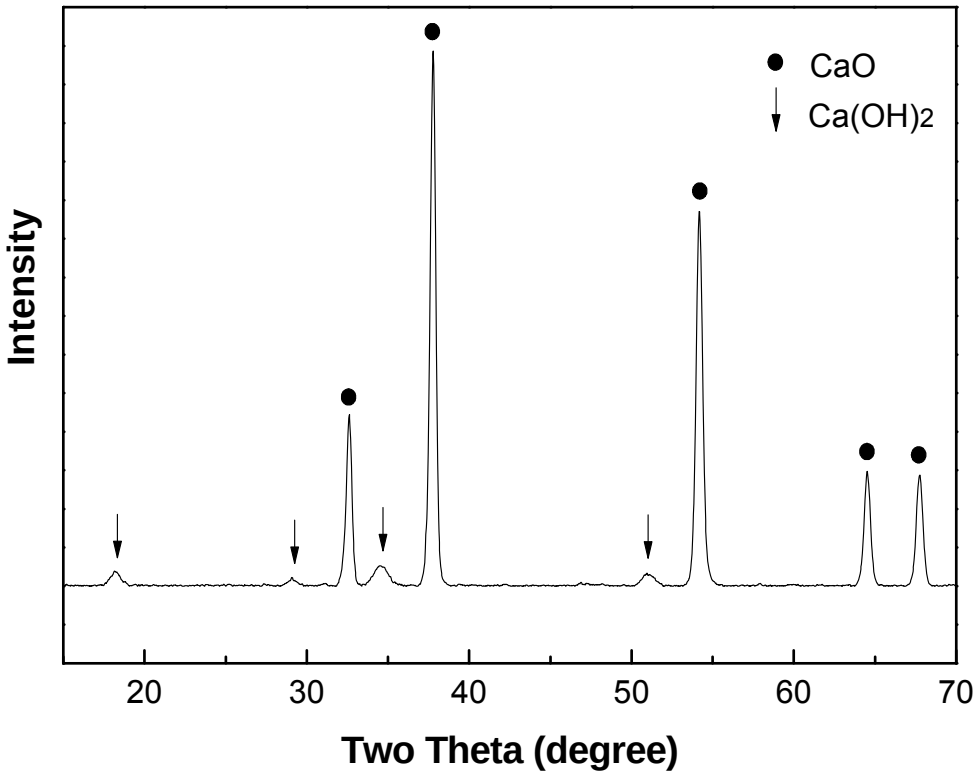

Fig. S3 XRD pattern of 50-μm $CaCO_3$ microcrystals that was first treated in a programmable furnace while temperature was going up to 900 °C followed by 1-h heat treatment at the same temperature before taking out.

Experimentally, the as-prepared $CaCO_3$ microcrystals were put into a programmable furnace which took 2 h to gradually increase to 900 °C. After remaining at this temperature for 1 h, the thermally treated product was taken out and then cooled to room temperature naturally. The co-existence of CaO and $Ca(OH)_2$ was revealed by XRD. The slow temperature increase from room temperature to 900 °C led to the formation of less porous CaO structure, which quickly reacted with H_2O to form dense surface coating of $Ca(OH)_2$ for protecting remained CaO internally while decreasing the temperature to room temperature. These remained CaO and $Ca(OH)_2$ slowly reacted with CO_2 and eventually converted into $CaCO_3$ after 5 days. XRD measurement was performed within 1 h after the thermal treatment.