Electronic Supplementary Information

Tailor-Made Poly(L-lactide)/Poly(lactide-co-glycolide) /Hydroxyapatite Composite Scaffolds Prepared *via* High-Pressure Compression Molding/Salt Leaching†

Jin Zhang,[a] Shu-Gui Yang,[a] Jian-Xun Ding,[b] Zhong-Ming Li*[a]

[a] College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, Sichuan, P. R. China; E-mail: zmli@scu.edu.cn (Z.-M.L.)

[b] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China; E-mail: jxding@ciac.ac.cn (J.-X.D.)

The self-made high-pressure compression molding apparatus is schematically shown in Fig. S1a. The diameter of the plunger is 20 mm, and the length of the cylindrical channel is 88 mm. Hydrostatic pressure supplied from a hydraulic jack can be up to 1000 MPa, while the magnitude of pressure inside the channel can be measured via the modulated pressure meter with an accuracy of ± 0.5 MPa, and the molding temperature is controlled via a thermocouple mounted 15 mm away from the channel, which ensures a temperature accuracy of 1 °C inside the cell. The sample inside the channel was heated by an electrical heating sheath, which was controlled by a temperature controller. Prior to the experiment, the temperature of the inside channel was carefully calibrated with the in-situ temperature measured by the thermocouple. Additionally, to avoid the thermal degradation of PLLA and PLGA, we used nitrogen to protect the sample during the high-pressure compression molding.

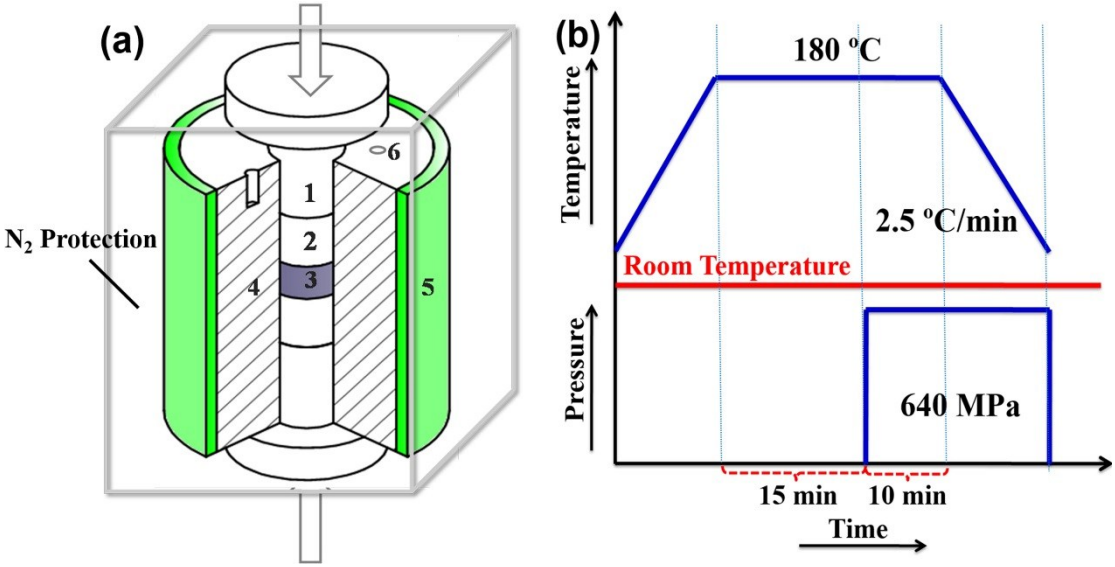

Fig. S1 Schematic of the high-pressure cell (1. Guide Pillar; 2. Mould Core; 3. Sample; 4. Mould; 5. Heater Circle; 6. Thermocouple) (a) and temperature and pressure protocol (b).

Sieved sodium chloride particulates 100 – 200 μm in diameter were added into the melted PLLA/PLGA/HA mixtures in an internal mixer at 180 °C and 50 rpm. The weight ratio of salt particulates to the composites was 9:1. Porous scaffolds were fabricated by a novel method named high-pressure compression molding/salt leaching techniques. The temperature and pressure protocol is shown in Fig. S1b. At first, a

given amount of PLLA/PLGA/HA/NaCl mixture was put inside the channel of the mould, heated to 180 °C and kept the temperature for 15 min. After that a predetermined pressure of 640 MPa was applied to the sample during 2 min. Both the temperature and pressure were kept for 10 min to achieve a steady state. Then the material was cooled to room temperature at a rate of 2.5 °C/min and released the pressure. The interconnected porous structure was acquired by leaching the molded parts with distilled water for long enough time until the weight remained constant after dried in a vacuum oven. Fig. S2 illustrates the detailed experimental procedures about the fabrication of porous PLLA/PLGA/HA composite scaffolds as described above.

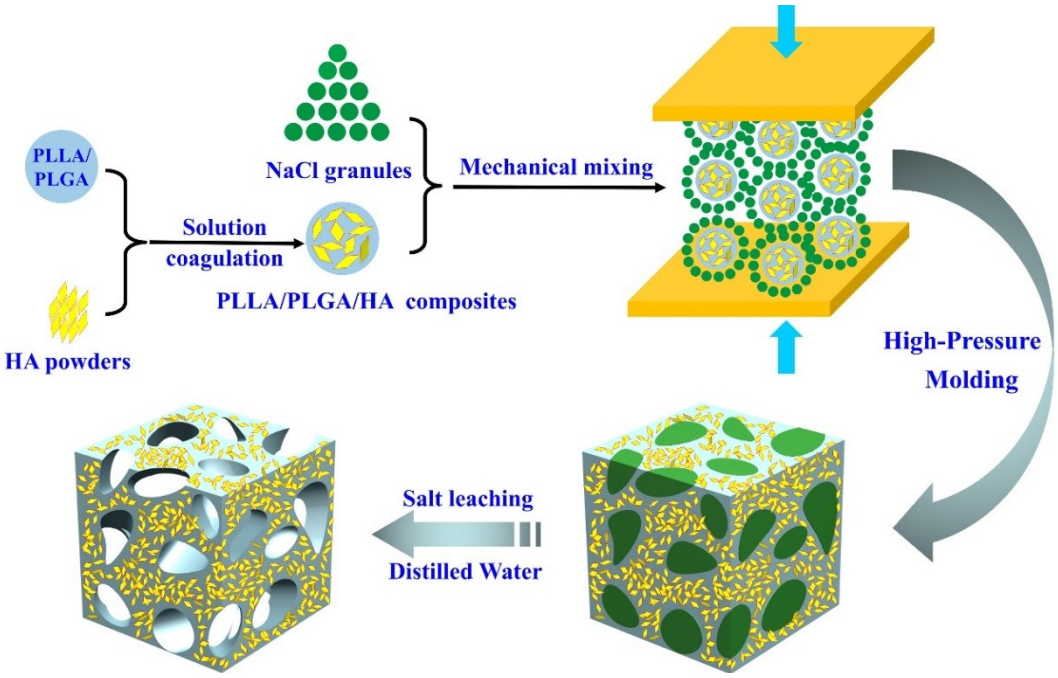

Fig. S2 Schematic of fabrication procedures of porous PLLA/PLGA/HA scaffolds.

Fourier transform infrared (FTIR) spectrometer was employed to further evaluate the existence of HA by confirming its functional groups. Fig. S3 illustrates FTIR spectra of the porous scaffolds with and without HA. In comparison with the pure scaffold (Fig. S3b), two strong peaks appear in the spectrum of such composite scaffold (Fig. S3a). The peaks at 563 cm^{-1} and 602 cm^{-1} represent the triply degenerated bending mode $v_{4c'}$ and $v_{4a'}$ of the O-P-O bonds of the phosphate group,

respectively,[1] indicative of the presence of HA in the porous scaffolds. Stable existence of HA particles can be attributed to two aspects. Firstly, concentration of the ethylic acid is extremely low (below 5 %) so that the acid-base reaction proceeds at a slow rate. Secondly, an ion-dipole bond might be formed between the oxygen in the ester group of PLA (C=O) and the Ca in HA,[2] guarantee that HA could be tightly-bonded to PLA substrate.

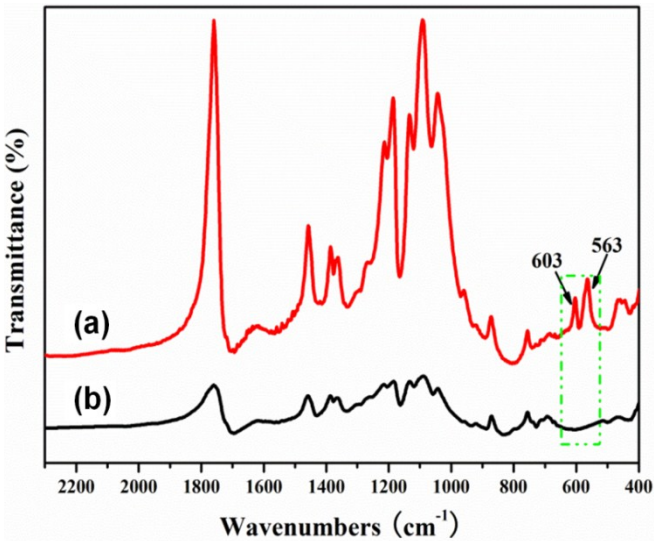

Fig. S3 FTIR spectra of porous scaffolds with (a) and without (b) HA particles.

The distribution of HA particles in PLA scaffolds was observed with a transmission electron microscope (TEM) at an accelerating voltage of 200 kV. The sample section for TEM observation was obtained using a Leica EMUC6/FC6 microtome, whose thickness was 80 nm. It can be seen from Fig. S4 that HA is integrated into the matrix along with a certain degree of agglomerations.

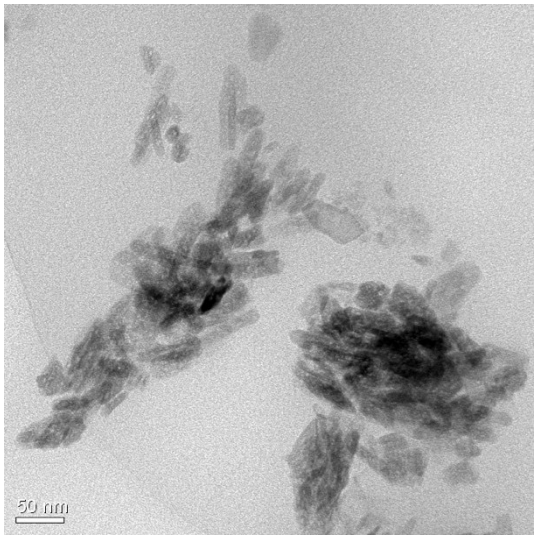

Fig. S4 TEM image of the porous composite scaffolds with HA particles.

The morphology and proliferation of cells on the high-pressure compression molded (HPCM) scaffolds are compared with those on the low-pressure compression molded (LPCM) scaffolds. It is worthy to be noticed that the LPCM scaffolds are fabricated under the compression pressure of 5 MPa, which indeed belongs to the traditional compression-molding/salt leaching samples.

In order to elucidate the effect of high-pressure compression molding on the attachment and growth of cells in scaffolds, SEM and Cell Counting Kit-8 (CCK-8) assays are employed to qualitatively and quantitatively evaluate the cellular viability, respectively. The relative cellular adhesion and SEM microimages of osteoblast-like MC3T3-E1 cells are illustrated in Fig. S5A. After incubation for 24 hours, MC3T3-E1 cells cultured in the HPCM and LPCM scaffolds are densely presented within the pores in a 3D fashion, thus most of the macropores become sealed off by a continuous layer of cells as well as a fibrous ECM. In line with the cellular morphology revealed by SEM micrographs, cellular adhesion rates of both scaffolds are comparable to that of Termanox^{TM} cover slip ($P > 0.05$), verifying the desirable adhesion of MC3T3-E1 cells on the surface of porous composite scaffolds.

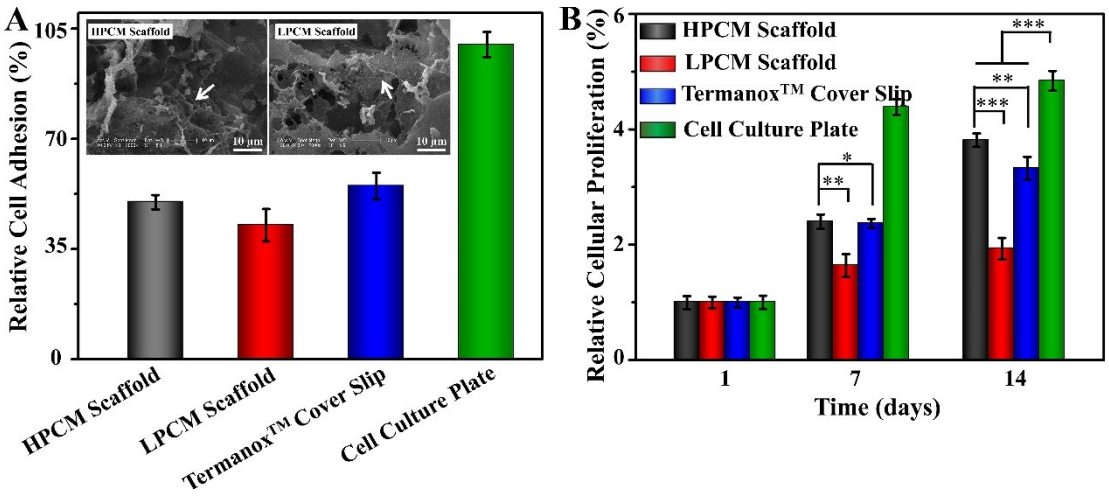

Fig. S5 Relative cellular adhesions (A) and proliferations (B) of MC3T3-E1 cells after culture on HPCM and LPCM scaffolds, Termanox™ cover slip, and cell culture plate at predetermined time points. Cellular number of cell culture plate is regarded as the criterion to calculate the relative adhesion rate. SEM micrographs of HPCM and LPCM scaffolds after co-culturing with MC3T3-E1 cells for 24 hours are inserted in Fig. S5A. Statistic data were presented as mean ± standard deviation ($n = 3$; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

The *in vitro* proliferations of MC3T3-E1 cells in the HPCM and LPCM scaffolds, Termanox™ cover slip, and cell culture plate are examined by CCK-8 tests, as shown in Fig. S5B. The cellular viabilities in both HPCM and LPCM scaffold groups remain high, implying low toxicities of our scaffolds towards MC3T3-E1 cells. The HPCM scaffold shows a noticeable cellular proliferation, about 4 times increase of cellular number in relative to that of the first day. This is distinctly higher than that for the LPCM scaffold, and even better than that for Termanox™ cover slip. The efficient cellular viability of the HPCM scaffold mainly benefits from the highly porous structure and sufficient mechanical properties ensuring the structural integrity.[3] Additionally, it is well-known that cells generally have a favorable growth state in the cell culture plate;[4] result comparable to the control sample fully verifies that the HPCM scaffold possesses high cellular compatibility and has great potential to effectively accelerate the cells' infiltration.

References

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