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Supplementary Materials for

3D printing of Haversian bone–mimicking scaffolds for multicellular delivery in bone regeneration

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/12/eaaz6725/DC1)

Movie S3 (.avi format). The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds with different diameters of Haversian canals for mechanical and porosity tests.
Movie S4 (.avi format). The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds with different numbers of Volkmann canals for mechanical and porosity tests.

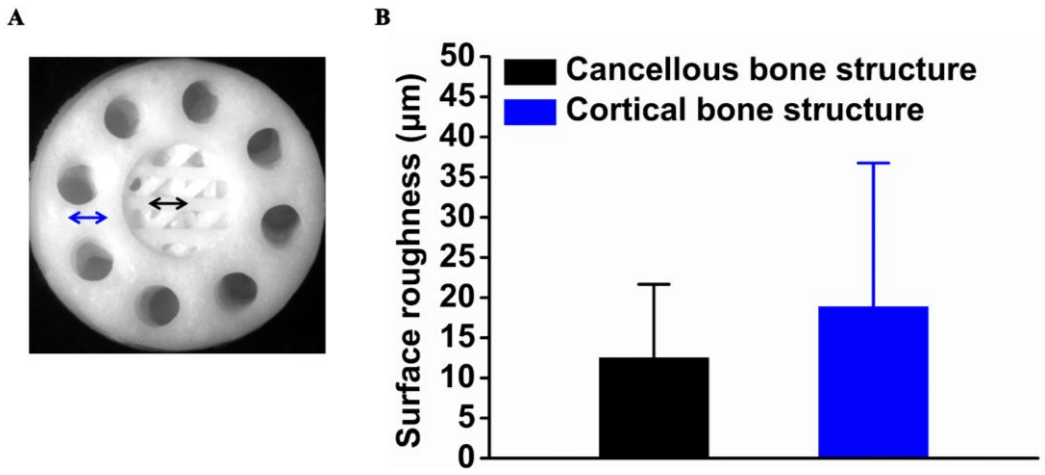

Fig. S1. Surface roughness of the Haversian bone–mimicking bioceramic scaffolds. (**A**) Schematic diagram of the scanning path of the cancellous bone structure (the double-headed black arrow) and the cortical bone structure (the double-headed blue arrow) of the scaffold. (**B**) Surface roughness of the cancellous bone structure and the cortical bone structure of the scaffold.

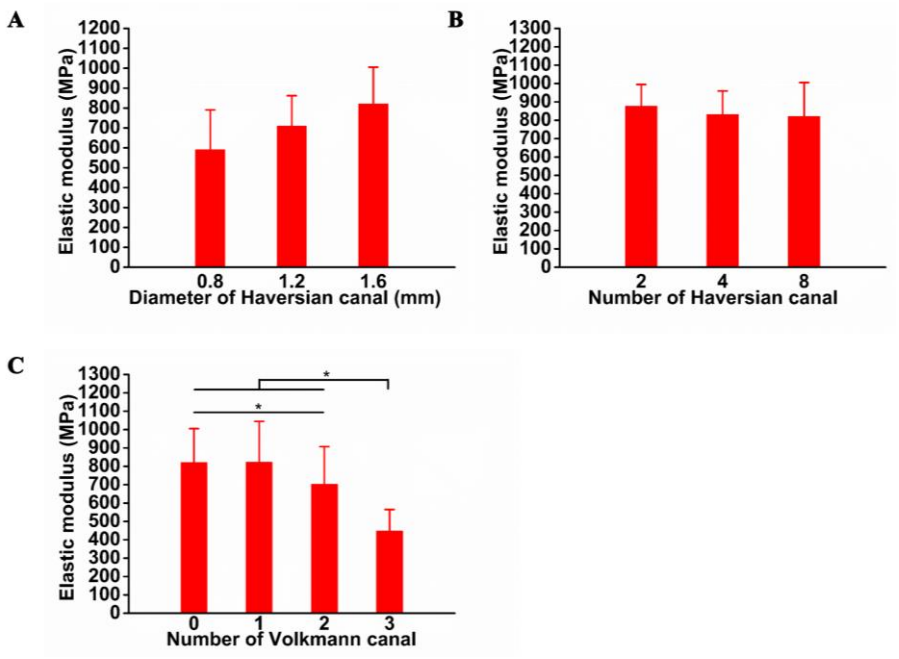

Fig. S2. Compressive modulus of the Haversian bone–mimicking bioceramic scaffolds. (**A** to **C**) Compressive modulus of scaffolds with (A) different diameters of Haversian canals, (B) different numbers of Haversian canals and (C) different numbers of Volkmann canals. n = 6 replicates. *$P < 0.05$.

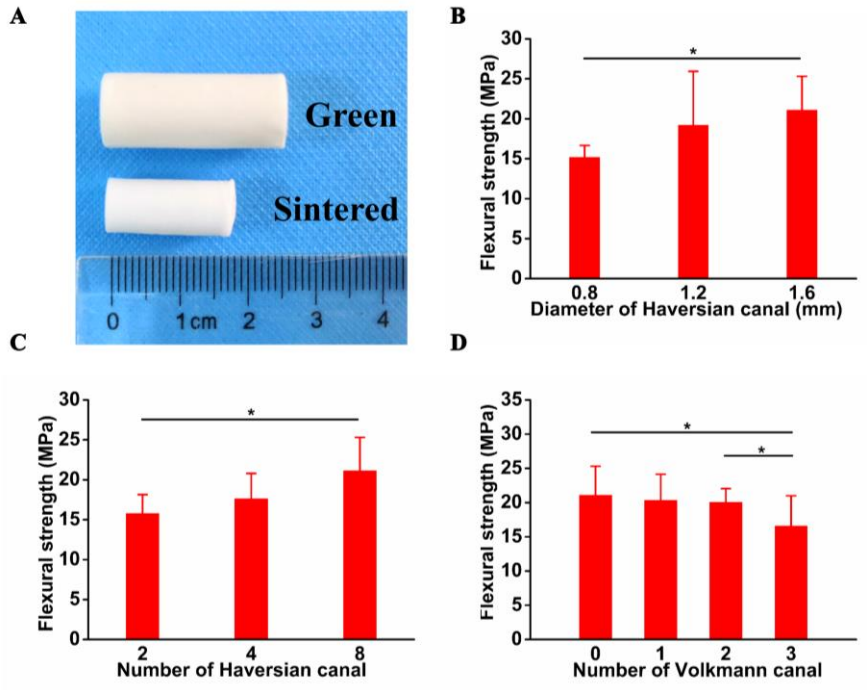

Fig. S3. Flexural strength of the Haversian bone–mimicking bioceramic scaffolds.

(**A**) Scaffolds before and after sintered for the flexural strength test. (**B** to **D**) Flexural strength of scaffolds with (B) different diameters of Haversian canals, (C) different numbers of Haversian canals and (D) different numbers of Volkmann canals. n = 6 replicates. *$P < 0.05$.

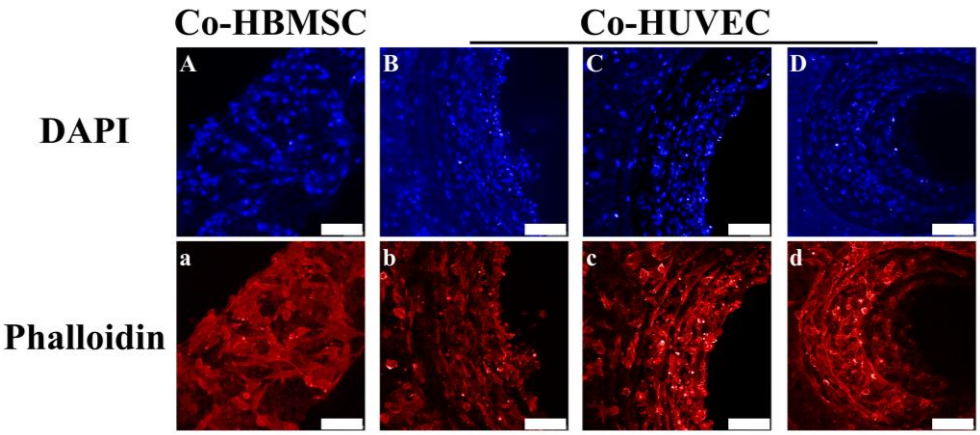

Fig. S4. CLSM images of cocultured HBMSCs and HUVECs stained with DAPI and phalloidin. Co-HBMSC (co-cultured HBMSCs) seeded on the cancellous bone structure (**A** and **a**) and Co-HUVEC (co-cultured HUVECs) seeded on the Haversian canal with different diameters, (**B** and **b**) D = 1.6 mm, (**C** and **c**) D = 1.2 mm and (**D** and **d**) D = 0.8 mm. Scale bars, 100 μm.

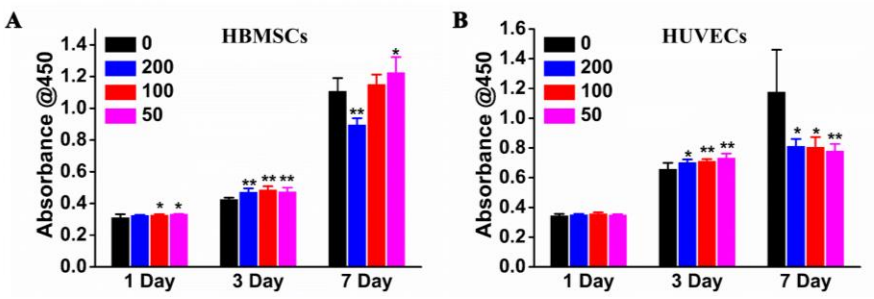

Fig. S5. Cell proliferation of HBMSCs and HUVECs cultured with the extracts from the Haversian bone–mimicking bioceramic scaffolds on days 1, 3, and 7. (**A** and **B**) Cell proliferation of (A) HBMSCs and (B) HUVECs cultured with different concentrations (scaffold mass to solution volume: 0 mg/mL, 200 mg/mL, 100mg/mL and 50mg/mL) of extracts from the scaffolds. n = 6 replicates. *$P < 0.05$, **$P < 0.01$.

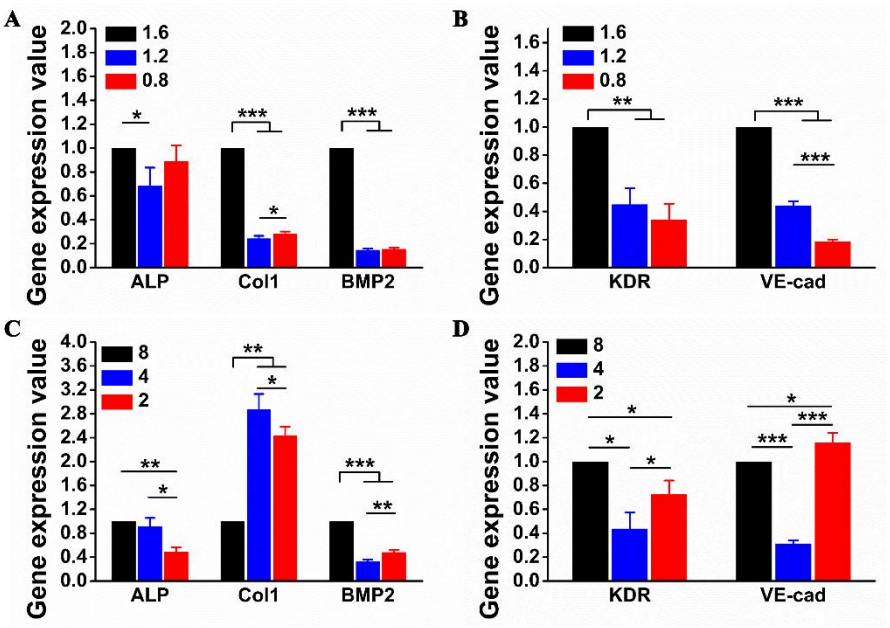

Fig. S6. The osteogenic and angiogenic gene expression of cocultured HBMSC-HUVEC seeded on scaffolds with different diameters and numbers of Haversian canals for 3 days. (**A** and **B**) The (A) osteogenic and (B) angiogenic genes expression of co-cultured HBMSC-HUVEC seeded on scaffolds with different diameters of Haversian canals. (**C** and **D**) The (C) osteogenic and (D) angiogenic genes expression of co-cultured HBMSC-HUVEC seeded on scaffolds with different numbers of Haversian canals. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

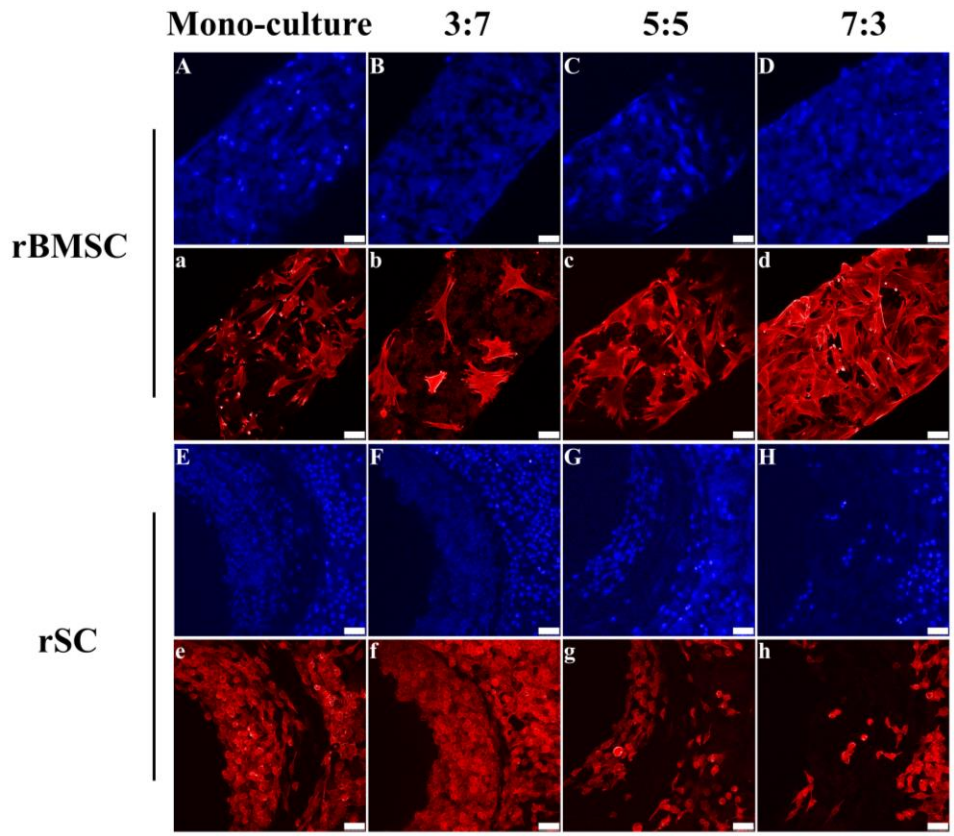

Fig. S7. The CLSM images of rBMSCs and rSCs in monoculture group and rBMSC-rSC coculture group with the ratio of rBMSCs to rSCs being 3:7, 5:5, and 7:3 stained with DAPI (blue) and phalloidin (red). (**A** to **d**) The CLSM images of rBMSC in (A and a) rBMSC mono-culture group and rBMSC-rSC co-culture group with the ratio of rBMSC to rSC being (B and b) 3:7, (C and c) 5:5 and (D and d) 7:3 seeded on the cancellous bone of scaffolds. (**E** to **h**) The CLSM images of rSCs in (E and e) rSC mono-culture group and rBMSC-rSC co-culture group with the ratio of rBMSCs to rSCs being (F and f) 3:7, (G and g) 5:5 and (H and h) 7:3 seeded on the Haversian canal of scaffolds. Scale bars, 50 μm.

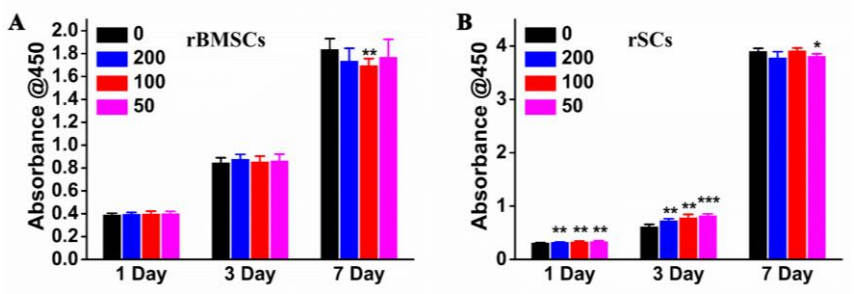

Fig. S8. Cell proliferation of rBMSCs and rSCs cultured with the extracts from the Haversian bone–mimicking bioceramic scaffolds on days 1, 3, and 7. (**A** and **B**) Cell proliferation of (A) rBMSCs and (B) rSCs cultured with different concentrations (0 mg/mL, 200 mg/mL, 100mg/mL and 50mg/mL) of extracts from the scaffolds. n = 6 replicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

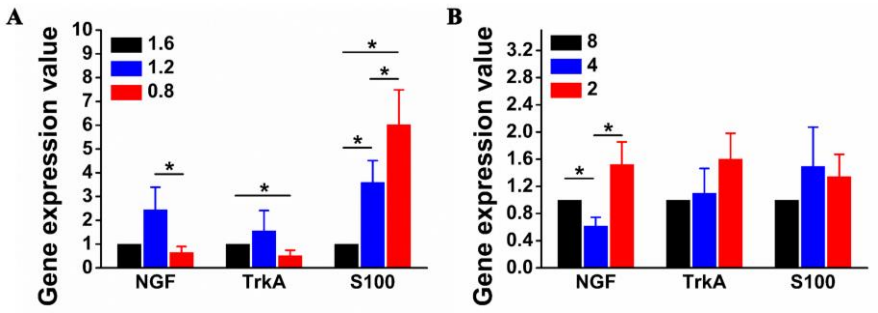

Fig. S9. The neurogenic gene expression of cocultured rBMSC-rSC seeded on scaffolds with different diameters and numbers of Haversian canals for 3 days. (**A** and **B**) The neurogenic genes expression of co-cultured **rBMSC-rSC** seeded on scaffolds with different (A) diameters and (B) numbers of Haversian canals. *$P <$ 0.05.

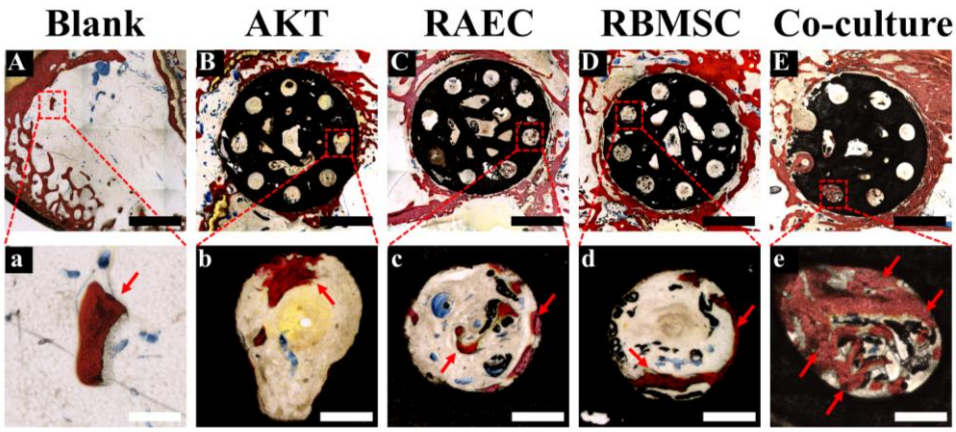

Fig. S10. New bone formation evaluated by histological analysis. The Haversian bone-mimicking bioceramic scaffolds-based rabbit bone marrow mesenchymal stem cell-rabbit aortic endothelial cell (RBMSC-RAEC) co-culture system enhanced the formation of new bone (red, indicated by red arrows) in rabbit femoral defects compared with those in RAEC mono-culture, RBMSC mono-culture, AKT and Blank group. (**A** to **E**) The sections from Microfil-perfused samples stained with picric acid-acid fuchsin. Scale bars, 2 mm. (**a** to **e**) Magnified images of the marked area in (A to E). The red arrows indicated new bone. Scale bars, 500 μm.

Movie S1. The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds for multicellular delivery. Five scaffolds with different numbers and diameters of Haversian canals were designed. The number of Haversian canals in the 5 scaffolds shown from left to right was: 8, 8, 8, 4, 2. The diameter (mm) of Haversian canals in the 5 scaffolds shown from left to right was: 0.8, 1.2, 1.6, 1.6, 1.6.

Movie S2. The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds with different numbers of Haversian canals for mechanical and porosity tests. Three scaffolds with different numbers of Haversian canals were designed. The number of Haversian canals in the 3 scaffolds shown from left to right was: 8, 4, 2.

Movie S3. The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds with different diameters of Haversian canals for mechanical and porosity tests. Three scaffolds with different diameters of Haversian canals were designed. The diameter (mm) of Haversian canals in the 3 scaffolds shown from left to right was: 1.6, 1.2, 0.8.

Movie S4. The bottom-up printing process to fabricate Haversian bone–mimicking scaffolds with different numbers of Volkmann canals for mechanical and porosity tests. Four scaffolds with different numbers of Volkmann canals were designed. The number of Volkmann canals in the 4 scaffolds shown from left to right was: 0, 1, 2, 3.