Supplementary file

S1. Attaching density of HUVECs on the inner surface of nutrient channels

The number of GFP-HUVECs $N_{TSM-HUVECs}$ in a TSM with a diameter of d_{TSM} (volume is V_{TSM}) can be calculated according to the GFP-HUVECs density of in the gelatin electrospraying ink $\rho_{TSM-HUVECs}$:

$$\begin{aligned} N_{TSM-HUVECs} &= \rho_{TSM-HUVECs} V_{TSM} \\ &= \frac{\pi}{6} \rho_{TSM-HUVECs} \times {d_{TSM}}^3 \end{aligned} \tag{S1-1}$$

Assuming that in the culture process, GFP-HUVECs can evenly attach to the lower half surface of the spherical nutrient channel formed by the TSM, the area of which is S_{semi}, and the diameter of the formed spherical nutrient channel is the same as the TSM, the attaching density of the GFP-HUVECs on the lower half surface of the spherical nutrient channel $\rho_{pore-HUVECs}$ can be obtained as follows:

$$\begin{aligned} \rho_{pore-HUVECs} &= \frac{N_{TSM-HUVEC}}{S_{semi}} \\ &= \frac{\frac{\pi}{6} \rho_{TSM-HUVEC} \times {d_{TSM}}^3}{\frac{\pi {d_{TSM}}^2}{2}} \\ &= \frac{\rho_{TSM-HUVEC} \times d_{TSM}}{3} \end{aligned} \tag{S1-2}$$

Thus, according to the correspondence between the diameter of the TSM and the applied voltage obtained in the paper, the centimeter-scale tissue with angiogenesis with different diameters of the on-demand nutrient channels can be constructed, and the attaching density of GFP-HUVECs can be predicted.

S2. Bioprinting of centimeter-scale bone tissue with TSM-B

3D bioprinting can play a significant role in organ transplantations. Among them, bone repairing has become a crucial application in clinical research. Thus, at the initial exploration of the biomedical application, we introduced TSM-B into the bioprinting of bone tissue by loading MC3T3-E1s (concentration of gelatin electrospraying ink was 5% w/v). Two kinds of culture models, namely cubic structures with controlled pores and without pores, were utilized as comparison. The structures (side length: 1cm) were respectively printed by 3D bioprinter with the TSM-B mixed with MC3T3-E1 cells.

The cultured structures were observed after 1, 4, 7 days of culturing. In terms of confocal fluorescence microscope observation, the sample was cut from the middle section since this part was the most difficult for materials exchanging. As shown in Figure S1, the cells in porous samples survived for 7 days of culturingwhile most of the cells died in non-porous structures. The results illustrate that the proposed TSM-B could provide sufficient nutrient supply for the encapsulated cells for their survival.

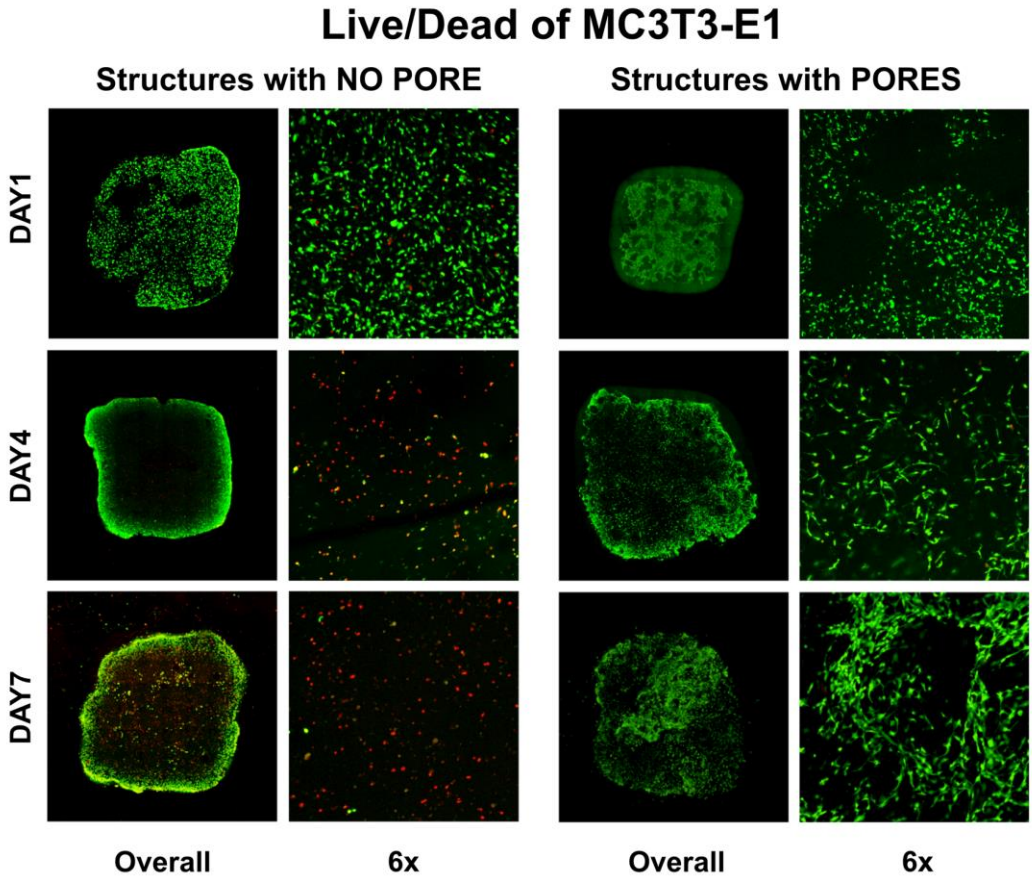

Figure S1. Live/Dead testing of MC3T3-E1s in the centimeter-scale structures

Moreover, the images stained by phalloidin and DAPI (Figure S2) showed that MC3T3-E1 cells in the porous structures could spread to a great extent in the 3D microenvironment while the cells in non-porous structures could only stay spheroid shape due to lack of enough nutrient and space.

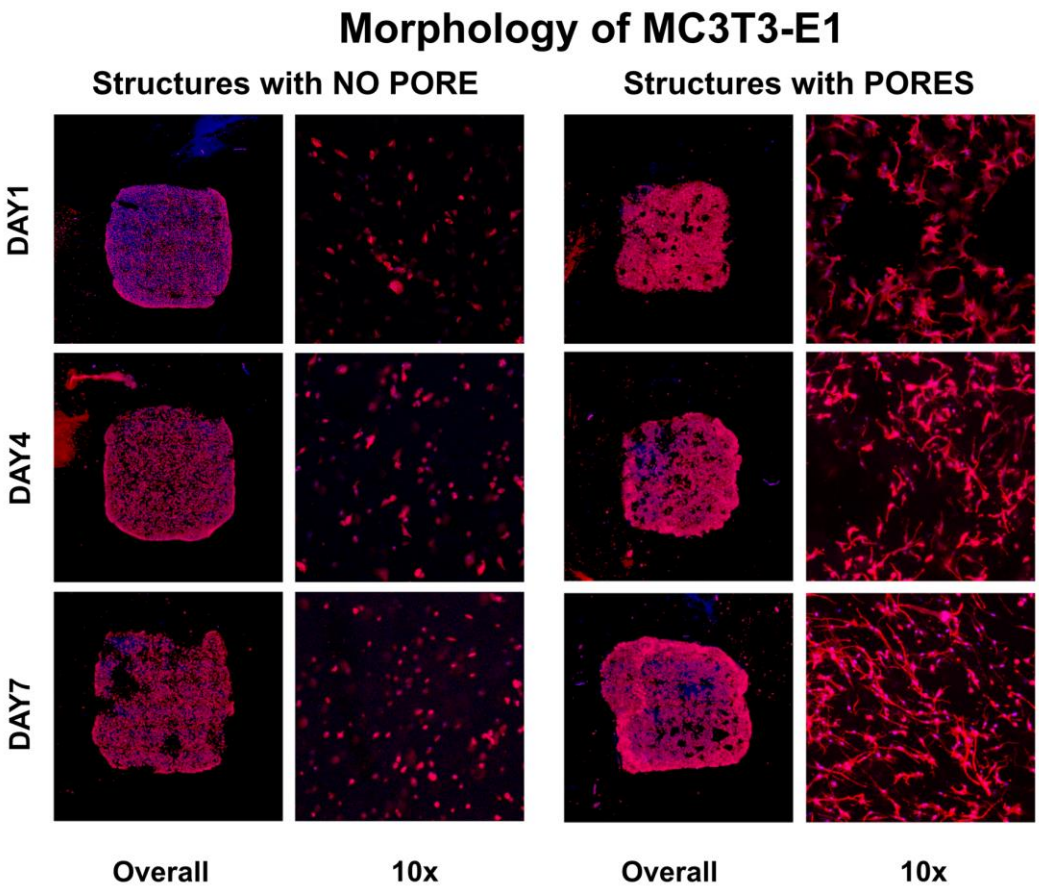

Figure S2. F-actin/Nucleus morphology of MC3T3-E1s in the centimeter-scale structures

Importantly, we analyzed the expression levels of alkaline phosphatase (ALP) of the two kinds of structures inducted by osteogenesis induction medium. High levels of ALP expression resulted in more purple color in the samples. To show the difference between two kinds of structure more obviously, the intact photos of the samples are displayed in Figure S3. As we can observe, on the first day, cells in two kinds of structures can synthesize ALP. However, on the 4th and 7th days, only cells inside the porous structures could realize ALP expression. This justified the importance of the proposed porous structures and the potential applications in the field of organ transplantation.

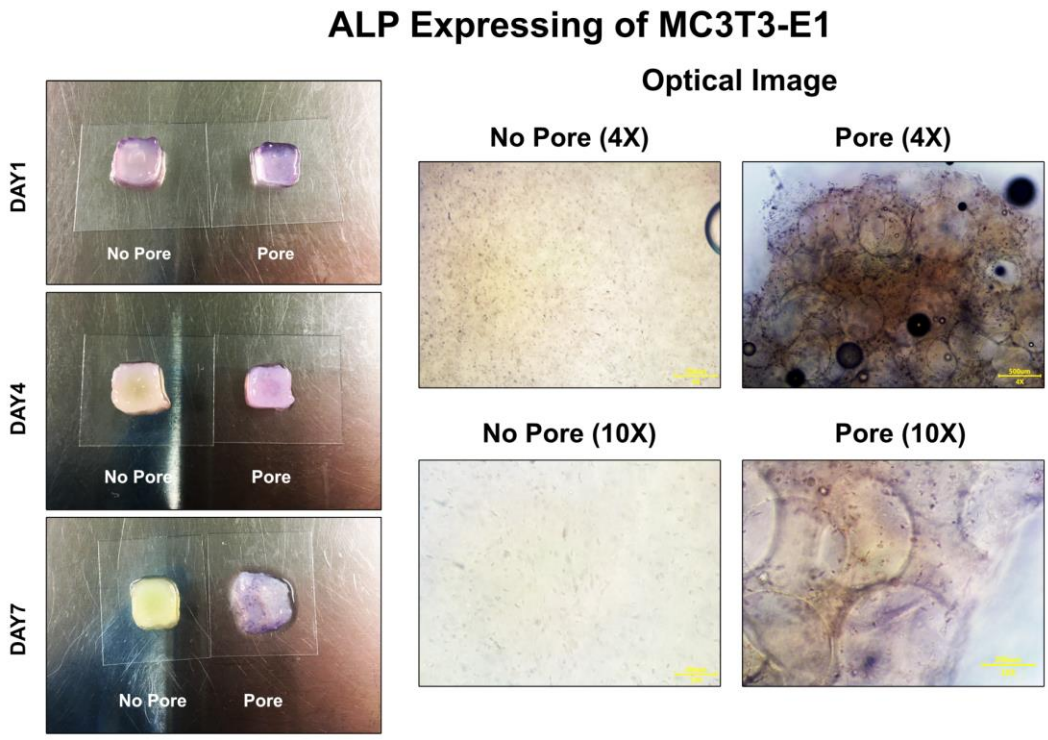

Figure S3. ALP expression of MC3T3-E1s in the centimeter-scale structures

S3. Degradation of hydrogel formed by the prepared GelMA precursor solution

GelMA hydrogel is the resulting product of gelatin and methacrylic anhydride (MA), which is one of the main materials in 3D bioprinting because of its good biocompatibility and light curing property. With the help of photoinitiator and light irradiation, GelMA can be rapidly crosslinked and form structures with elasticity. Inside the structures, dense pores are generated spontaneously and constitute the extra cellular matrix (ECM), which build the material exchanging channels for cell growth. What's more, on GelMA molecule chains, the arginine-glycine-aspartic acid (RGD) sequences and the target motifs of matrix metalloproteinase (MMP) deriving from gelatin were retained. Therefore, the cellular attachment and degradation profile can be achieved. Here, the degradation profile of crosslinked GelMA was examined by collagenase II (2 U/mL) and the results showed that crosslinked GelMA can be totally degraded in 24 hours (Figure S4).

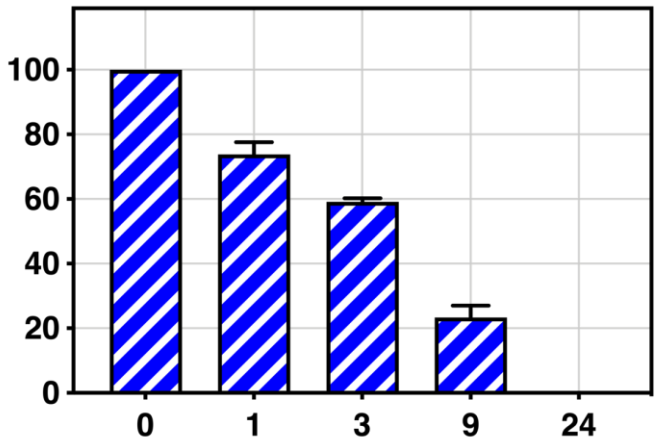

Figure S4. Degradation of hydrogel formed by the prepared GelMA precursor solution

S4. Continuity of the extruded TSM-B filament

A basic extrusion printability indicator is the continuity of the extruded filament. The images of the extruded TSM-B filaments with different nozzle sizes and nozzle moving speeds are shown in Figure S5.

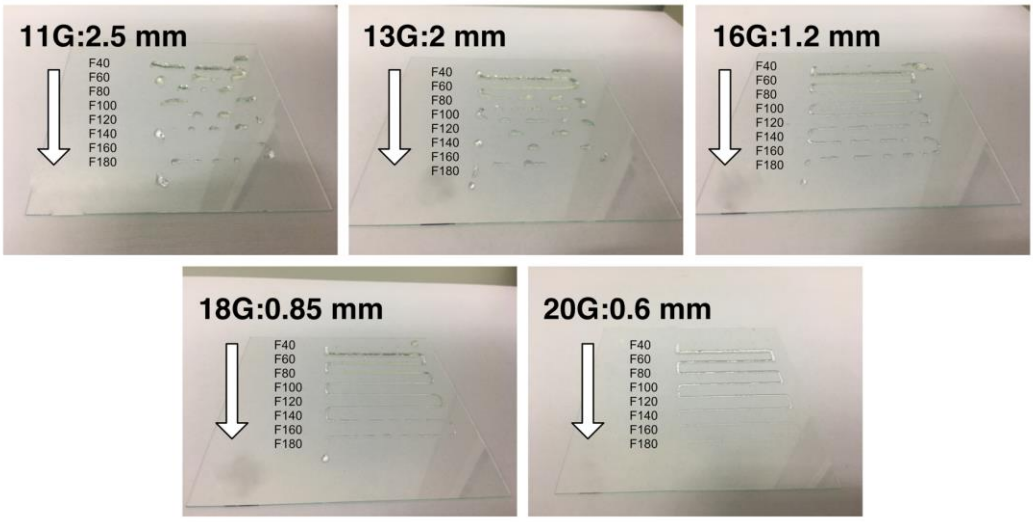

Figure S5. Continuity of the extruded TSM-B filament