SUPPLEMENTAL INFORMATION

1. Supplemental experimental procedures

1.1. Cell culture

Human placenta-derived stem cells (hPSCs, passages 4-7) were obtained from the Regenerative Medicine Clinical Center (RMCC) at the Wake Forest Institute for Regenerative Medicine (WFIRM). Human PSCs were isolated from the chorionic tissue of the placenta via enzyme digestion and cultured in α-Minimum Essential Medium (α-MEM) supplemented with 15% fetal bovine serum (FBS, HyClone, Logan, UT, USA), 17% AmnioMAX™ C-100 Basal Medium, 2% AmnioMAX™ C-100 Supplement, 1% GlutaMAX™ Supplement, and 2.5 μg/ml gentamicin.[72] The medium was changed every 3 days. All reagents for cell culture were purchased from Life Technologies (Carlsbad, CA, USA) unless stated otherwise.

1.2. Osteogenic differentiation of hPSCs

Human PSCs were seeded in six-well plates at a density of 3×10^5 cells per well in the growth medium for 3 days and replaced with the osteogenesis medium, consisting low glucose Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 nM dexamethasone, 10 mM β-glycerophosphate, 0.05 mM ascorbic acid 2-phosphate, 100 U/ml penicillin, and 100 U/ml streptomycin. The medium was replaced every 3 days, and the osteogenic differentiation of hPSCs was induced for up to 40 days in culture.

At the designated time points, Alizarin Red S staining, alkaline phosphatase (ALP) staining assay, and von Kossa staining were carried out to confirm the osteogenic differentiation of hPSCs. For Alizarin Red S staining, samples were treated with dye solution (pH 4.2) at room temperature for 45 min and then rinsed with DI water. For quantification, the dye were extracted using 10% cetylpyridinium chloride (CPC) in 10 mM sodium phosphate (pH 7.0). After 15 min, the supernatant was diluted 10-fold in 10% CPC solution, and Alizarin Red S concentration was determined by absorbance at 560 nm on a multi-plate reader (SpectraMax 190, Molecular Devices, San Jose, USA). ALP staining assay was performed using ALP kit (Millipore Sigma) by the manufacturer's instruction. For quantification, 100 μL 1% CPC was added to each well, and the plates were incubated with agitation for 30 min with shaking at 100 rpm. The plates were then read at 560 nm on a multi-plate reader. For von Kossa staining, pretreated samples were incubated with 2% (w/v) silver nitrate solution for 10 min in the dark, washed thoroughly with DI water, and then exposed to bright light for 15 min. The plates were rinsed in DI water after sodium thiosulfate (5%) was added for 3 min. Acid fuchsin counterstain was added for 5 min, and the plates were rinsed in DI water.

1.3. GelMA synthesis

Gelatin methacrylate (GelMA) was synthesized as a bioink. Briefly, 2 g of type A porcine skin gelatin (~300 g Bloom) was dissolved in 20 ml of PBS at 60°C. Methacrylic anhydride (MAA) was added dropwise (0.5 ml/h) to the gelatin solution under stirring at 50°C and allowed to react for 1 h. The resultant GelMA solution was added to 80 ml of PBS and dialyzed against DW using 12–14 kDa cutoff dialysis tubing (SpectraPor) at 40°C to remove salts and unreacted MAA. The

water was changed twice daily for 1 week. Finally, the GelMA solution was lyophilized and stored at -80°C until the use. The degree of methacrylate was calculated by NMR.

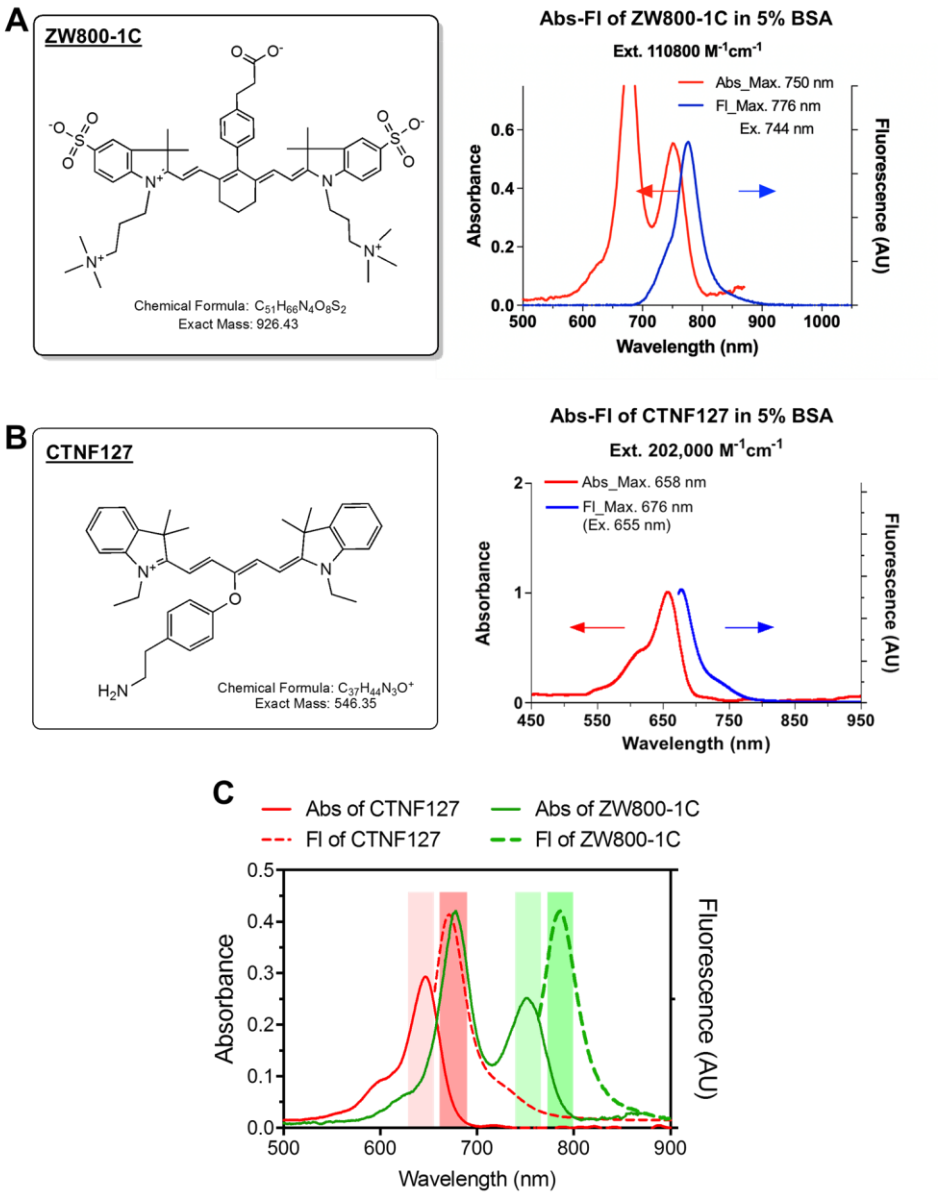

Fig. S1. NIR fluorophores of (A) ZW800-1C and (B) CTNF127. (C) Absorbance and fluorescence emission of CTNF127 and ZW800-1C in serum.

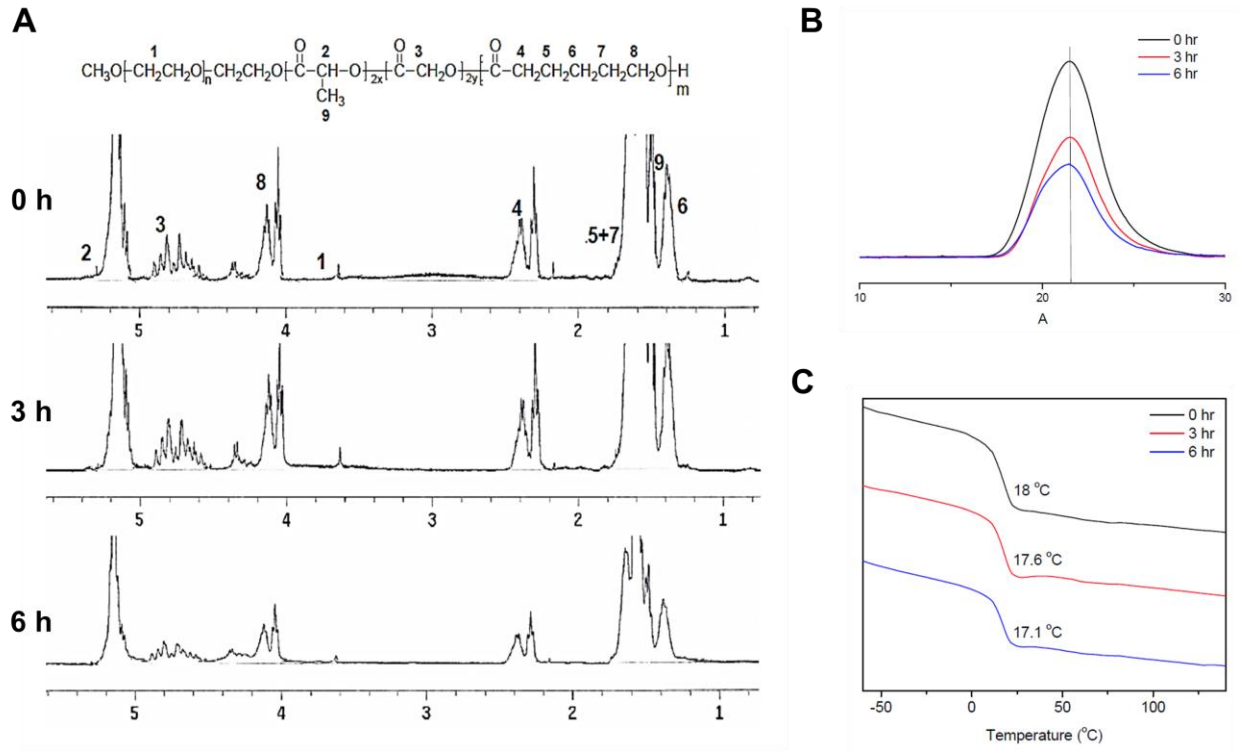

Fig. S2. Thermal stability of PCLG-2 copolymer at 100°C for up to 6 h. (A) ^{1}H-NMR, (B) GPC, and (C) DSC.

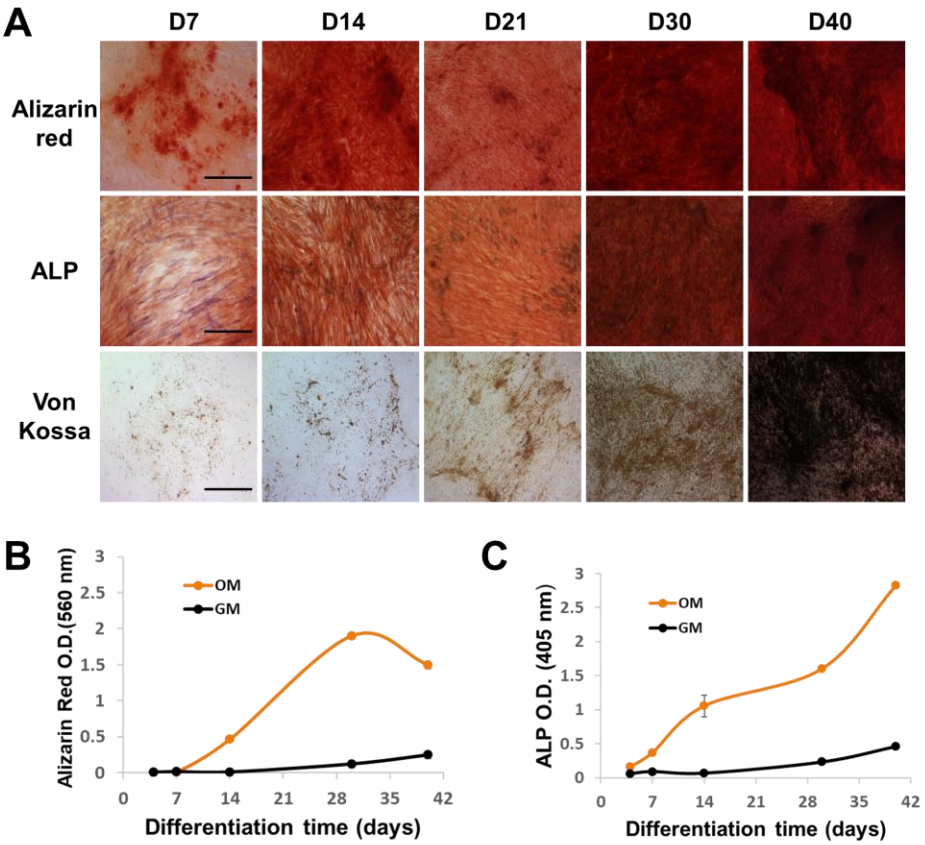

Fig. S3. Osteogenic differentiation of hPSCs. (A) Osteogenic differentiation of hPSCs was confirmed by Alizarin Red S, ALP, and von Kossa staining. (B) Calcium content measured by Alizarin Red S staining assay. (C) ALP assay.

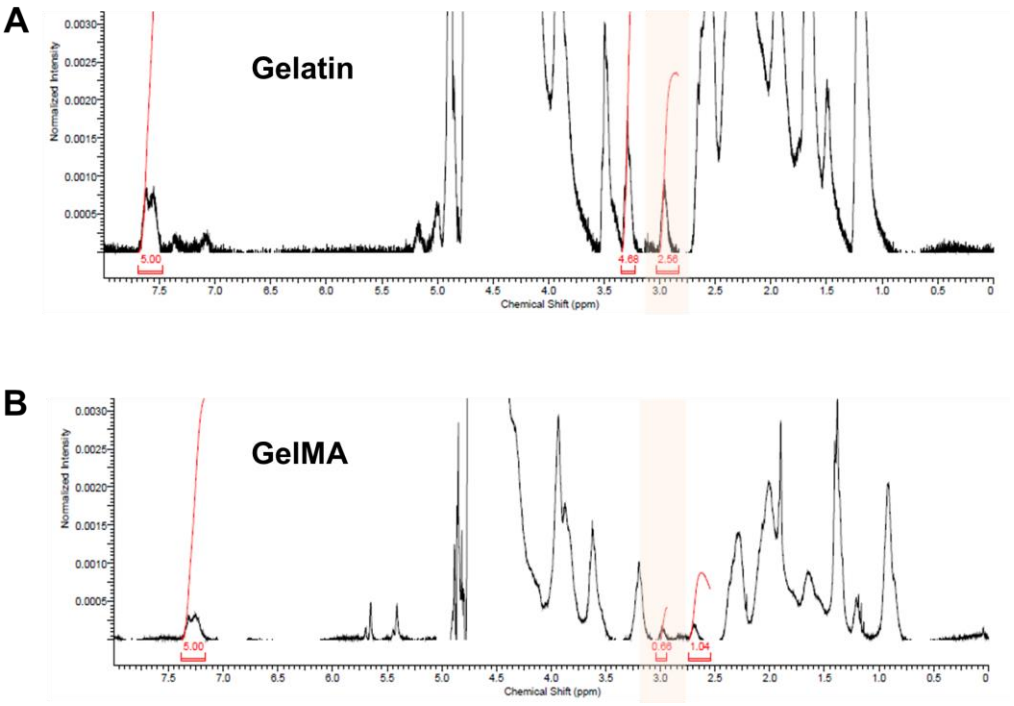

Fig. S4. Characterization of GelMA by ^{1}H-NMR. The MA modification of lysine residues with methacrylic anhydride addition can be confirmed by the decrease in the lysine signal at d = 2.9 ppm, and the increases in the methacrylate vinyl group signal at d = 5.4 ppm and d = 5.7 ppm, and the methyl group signal at d = 1.8 ppm.

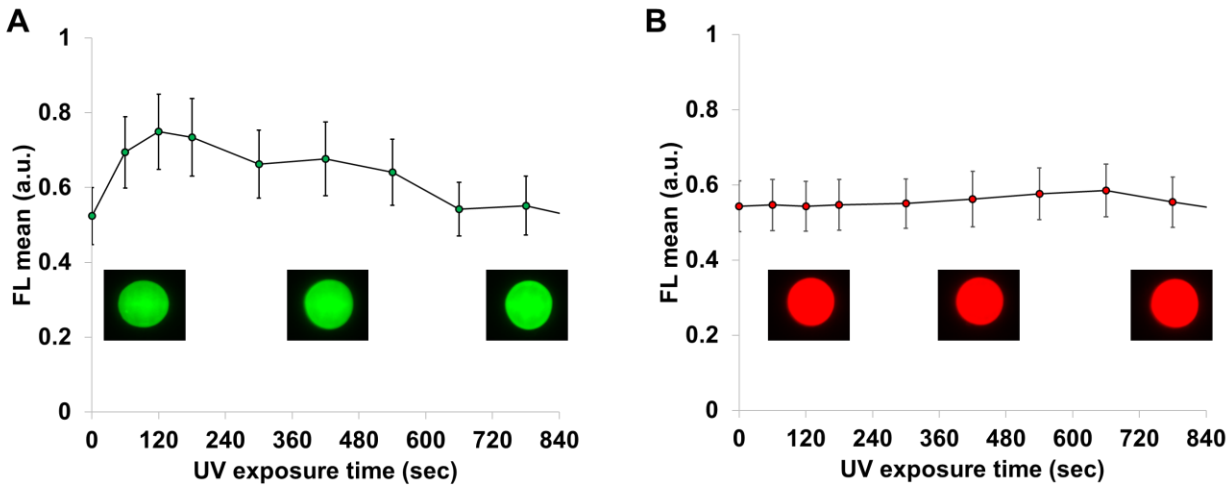

Fig. S5. UV stability of NIR fluorophores. (A) ZW800-1C, and (B) CTNF127.

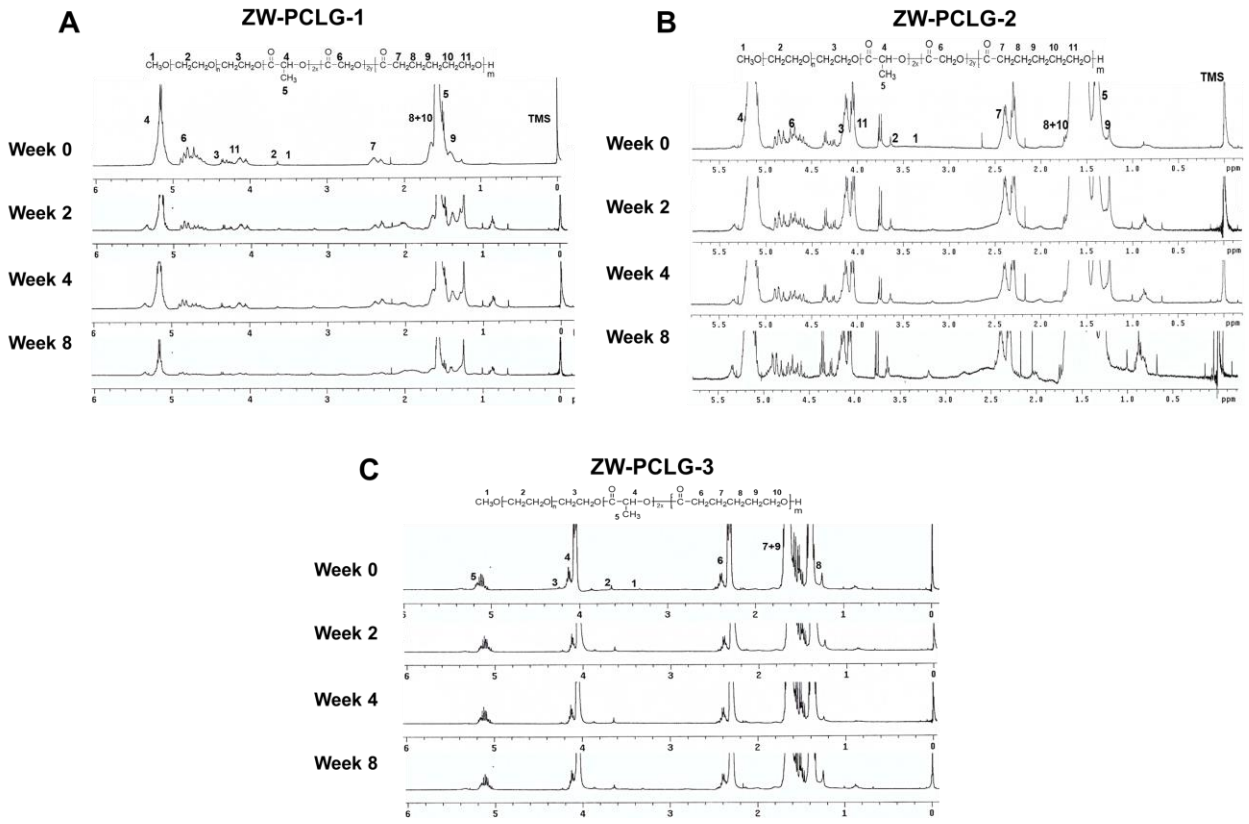

Fig. S6. Molecular weight changes of the printed ZW-PCLG scaffolds after implantation. ^{1}H-NMR spectra of (A) ZW-PCLG-1, (B) ZW-PCLG-2, and (C) ZW-PCLG-3.