Supporting Information

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SI Materials and Methods

All chemicals were purchased from Sigma Aldrich or Fisher Scientific unless otherwise stated. All animal and primary human cell experiments were reviewed and approved by an internal review board of Massachusetts General Hospital and the Norwegian Animal Research Authority.

Fabrication of Inverted Colloidal Crystal Hydrogel Scaffolds. Three drops of glass beads with four different diameter (D) ranges (75–106 μm, 150–180 μm, 250–300 μm, and 425–500 μm) dispersed in ethylene glycol were loaded into a glass vial [D = 6.5 mm, height (H) = 8 mm] until the thickness was roughly 1.5 mm. A vacuum was applied for 3–5 h to remove entrapped air. Then the glass vials were partially immersed in an ultrasonic water bath and were sonicated for 30 min to make a tightly packed and ordered structure of microspheres. Ethylene glycol was evaporated at 160 °C overnight, and the remaining glass beads were annealed for 4 h at 650–700 °C, depending on their diameters. During the annealing process, the slightly melted glass beads stuck together and formed a freestanding colloidal crystal. Next, colloidal crystal templates were transferred to other glass vials (D = 7 mm, H = 8 mm) and were infiltrated with 500 μL of hydrogel precursor solution composed of 30% (wt/wt) acrylamide monomer, 5% (wt/wt) bis-acrylamide cross-linker and 0.1% (vol/vol) N,N,N,*N*-tetramethylethylenediamine accelerator in N_2 gas-purged deionized water by centrifugation at 2000 × *g* for 10 min. The hydrogel precursor solution was radically polymerized by the addition of 50 μL of 1% (wt/wt) potassium persulfate. The hydrogel-integrated colloidal crystals then were taken from the glass vials, and bulk hydrogel was removed thoroughly using a razor blade. Subsequently, the glass beads were dissolved in 10% (vol/vol) hydrofluoric acid solution for 2–3 d. The remaining hydrogel matrices having an inverted structure of the colloidal crystal template were washed serially with 1 N·HCl solution and deionized water. Finally, the hydrogel inverted colloidal crystal (ICC) scaffolds were freeze-dried and kept in a dehydrated state until used.

Surface Immobilization of Rat-Tail Type I Collagen on Hydrogel Scaffolds. The day before cell seeding, the hydrogel scaffolds were sterilized by soaking in a 95% (vol/vol) ethanol solution for 20 min under UV light and were coated with type I collagen using an amine reactive and photoreactive heterobifunctional cross-linker (Sulfo-SANPAH; Pierce). Briefly, sterilized scaffolds were washed three times with conjugating buffer (0.15 M NaCl, 0.1 M Na_2HPO_4) and then were immersed in 1 mg/mL Sulfo-SANPAH solution for 4 h at 4 °C. After unbound reagent was washed away, each side of the hydrogel scaffold was exposed to UV light for 7 min, and the scaffold was immersed in 5 mg/mL of rat-tail–derived type I collagen solution at 4 °C overnight. On the next day the collagen-coated hydrogel scaffolds were washed three to five times with a PBS solution and then were stored at 4 °C until used.

The concentration of surface immobilized rat-tail type I collagen was quantified using an ELISA-like method. A collagen-coated hydrogel scaffold was chopped into four identical pieces (i.e., quarter-circle shapes) and immersed in 1% (wt/vol) BSA (R&D System) in PBS for 2 h to block nonspecific binding. The chopped scaffolds then were incubated serially in a 1:200 dilution of rabbit anti-rat collagen type I antibody (Millipore) and a 1:500 dilution of biotinylated goat anti-rabbit IgG antibody (Millipore) for 1 h each at room temperature. Subsequently the scaffolds were soaked in a 1:500 dilution of streptavidin-HRP solution (R&D System) for 30 min and then were exposed to 3,3′,5,5′-tetramethylbenzidine substrate solution (R&D System). Once strong color developed, the reaction was stopped by adding 2 M H_2SO_4 solution, and absorbance was measured at 450 nm with reference at 570 nm. Wells coated with a known concentration of rat-tail type I collagen were used as a reference.

Mechanical Property of Hydrogel Scaffolds. Storage moduli of hydrogel scaffolds and demineralized bone matrix with strains of 1–20% were measured at 1 Hz frequency and 26 °C setting via the Q800 dynamic mechanical analyzer (TA Instruments).

Preparation of Bioactive 2D Hydrogel Discs. Cleaned glass coverslips were covered with 700 μL of 0.1 M NaOH solution and were placed on a 70 °C hotplate. Once the solution evaporated, the coverslips were transferred to a fume hood and were covered with 350 μL of 3-aminopropyltruethhoxy-silane for 5 min. The coverslips then were rinsed three times with deionized water for a total of 10 min, were immersed in 0.5% glutaldehyde solution for 30 min, and were dried completely under N_2 gas. Next, 25–50 μL of complete hydrogel precursor solution was quickly sandwiched between a glass coverslip and another glass slide treated with dichlorodimethylsiloxane. After 20–30 min incubation, glass slides covered with hydrogel thin film were separated and coated with type I collagen following the protocol described above. Collagen-coated 2D hydrogel discs were placed in a six-well plate and were sterilized under UV light for 1 h before use.

Human Bone Marrow Stromal Cell Isolation and Expansion. Fresh human bone marrow aspirates were purchased from Lonza. Mononuclear cells were separated by Ficoll density gradient centrifugation (GE Healthcare) and were plated on a T-175 flask (1×10^6 cells per flask). Mononuclear cells were cultured at 37 °C with 10% CO_2 and 100% humidity. Bone marrow stromal cell (BMSC) expansion medium was composed of 15% FBS, 2% penicillin and streptomycin, 0.2% gentamicin, 1 ng/L fibroblast growth factor, and 3 g/L sodium bicarbonate in alpha-MEM with ribonucleotides and deoxyribonucleotides (Invitrogen). Medium was changed 1 wk later, and unbound cells were washed away. The following week, colony-forming adherent cells were replated in a new flask for expansion. Medium was changed every 3–4 d. BMSCs were subcultured when they reached 70–80% confluence. Only passage 2–5 BMSCs were used for experiments.

Isolation of Human Bone Marrow $CD34^+$ Cells. Mononuclear cells were separated from human bone marrow via Ficoll density gradient (GE Healthcare). Subsequently, $CD34^+$ cells were positively separated from mononuclear cells using a magnetically activated cell-sorting kit following the vendor's protocol (MACS system; Miltenyi Biotech). Typically 2–5 × 10^6 $CD34^+$ cells were isolated from 25 mL of fresh human bone marrow with more than 90% purity. Isolated $CD34^+$ cells were transplanted into the mice within 3 h.

Human Leukemia Cell Culture. The human bone marrow leukemia cell line TF-1a was a gift from Daniel Irimia (BioMEMS Resource Center, Harvard Medical School, Boston, MA). Phenotypically, TF-1a is $CD34^+/CD38^-$ (1). The culture was maintained in RPMI-1640 medium supplemented with 10% (vol/vol) FBS and 1% (vol/vol) penicillin and streptomycin. The medium was changed every 2–3 d.

Human BMSC Culture in 3D Hydrogel ICC Scaffolds and 2D Hydrogel Discs. A dense suspension of BMSCs ($1–10 \times 10^5$ cells in 20–30 μL) was dropped on top of the moderately dehydrated hydrogel

scaffold in a 24-well plate. Rapid rehydration process facilitates effective and homogenous BMSC seeding into the scaffolds, and 1.5 mL of culture medium was added in each well. After 4–6 h BMSCs were stably adhered to the hydrogel scaffold pore surface. The cell-laden scaffolds then were transferred to a new 24-well plate, and culture was maintained in a culture volume of 1.5 mL. Cell-seeding efficiency was calculated by counting the cells remaining in the plate. For 2D culture, type I collagen-coated 2D hydrogel discs were placed in a six-well plate, and 1–2.5 × 10^5 BMSCs were seeded. The culture volume was maintained at 1.5 mL, and half of the medium volume was changed every 2 d.

In Vitro Analysis of Human BMSC Secretory Function. Human BMSCs were cultured on different substrates: (*i*) conventional 2D tissue culture plate, (*ii*) 2D hydrogel discs, and (*iii*) 3D hydrogel scaffolds. After 3 d, the cultures were washed briefly with PBS, and BMSC expansion medium was replaced with conditioning medium, DMEM supplemented with 5% (vol/vol) penicillin and streptomycin and 0.05% (wt/vol) BSA. After 72 h of incubation, supernatants were collected, and human VEGF, IL-6, IL-8, and stem cell-derived factor-1 (SDF-1) levels were measured by an ELISA using VEGF, IL-8, and SDF-1 kits from R&D Systems and the IL-6 kit from BD Biosciences. Total cell numbers within the scaffolds were estimated using the PicoGreen dsDNA quantification kit (Invitrogen) or MTT assay (ATCC) and were applied to normalize ELISA data.

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient from whole human blood. PBMCs (1 × 10^5) were plated in each well of a 96-well plate with 50 μL of RPMI 1640 medium supplemented with 10% (vol/vol) FBS and 5% (vol/vol) penicillin and streptomycin. Subsequently 50 μL of the supernatants from BMSC cultures were loaded in each well. After 16 h of incubation, 50 μL of 30 μg/mL lipopolysaccharide solution in the RPMI medium was added to each well. The supernatants were collected after 5 and 24 h for human IL-10 and IFN-γ ELISA (OptEIA; BD), respectively.

Mice. Athymic Nu/Nu mice (male, 6–8 wk old) were bred at the Steele laboratory at Massachusetts General Hospital and were used for initial marrow-emulating interscaffold tissue development and short-term human hematopoietic cell homing experiments. NOD-*scid* IL2rγ^{null} (NSG) mice (6-8 wk old, originally a generous gift from Leonard D. Shultz, Jackson Laboratories, Bar Harbor, ME) were bred at the Vivarium, University of Bergen. NSG mice were used for the long-term engraftment study of human hematopoietic progenitor cell transplantation.

Subcutaneous Implantation of Scaffolds in Immunocompromised Mice. Before implantation, unseeded scaffolds and BMSC-seeded scaffolds cultured for 1–3 d were washed three times with PBS. Athymic Nu/Nu mice were anesthetized using i.p. injections of ketamine (50 mg/kg) and xylazine (10 mg/kg). An approximately 4-mm incision was made on the back to create an s.c. pocket, and the scaffolds were inserted gently using forceps. Up to four scaffolds were implanted per mouse, and the incisions were closed with surgical staples (Tyco Healthcare Inc.). NSG mice were shaved and depilated to remove all hair, and then scaffolds were implanted as described above.

Vascular Corrosion Casting and Decellularization. Athymic Nu/Nu mice bearing BMSC-laden and unseeded scaffolds were anesthetized and then were placed on an operating board using pins. The rib cage was opened surgically to access the heart. An 18-gauge catheter needle was inserted directly into the left ventricle, and then the right atrium was cut to make a drain. Then 30 mL of heparinized PBS solution, 40 mL of 10% (vol/vol) buffered formalin solution, and 30 mL of MV-120 Microfil corrosion casting reagent (FlowTech, Inc.) were perfused serially into the mouse at a steady-state flow rate of 10 mL/min using a syringe pump (Harvard Instrument). The resin was polymerized after overnight incubation at 4 °C. Tissues were removed corrosively from explanted scaffolds by immersion in 10% (wt/vol) KOH solution that left only the casted material and the hydrogel scaffold. To confirm the localization of interpore vasculature, some explanted scaffolds were fixed and histologically analyzed after H&E staining.

Mouse Irradiation. Bone marrow failure was induced by sublethal irradiation in mice. Briefly, athymic Nu/Nu mice were transferred to an irradiation pie cage (Braintree Scientific) and were irradiated at 450 rad. NSG mice were irradiated at 1.5 Gy.

Transplantation of Human Bone Marrow CD34$^+$ Cells or Leukemic Cells. Mice were sublethally irradiated 1 d before cell transplantation. For i.v. transplantation, mice first were warmed for a few minutes under a heat lamp and were placed in a rotating tail injector. Depending on the experiment, mice were injected via the tail vein with 200 μL of human leukemic cells (TF-1a) (2×10^6) or human bone marrow CD34$^+$ cells (1×10^5). For direct scaffold or skin injection, mice were anesthetized with isoflurane, and hair was shaved from NSG mice. Injection volume was maintained at 50 μL per scaffold, but cell numbers were varied to match the total number of transplanted cells in i.v. injected mice. For instance, if there was one scaffold, a 50-μL volume included all CD34$^+$ hematopoietic stem cells (1×10^5). If there were four scaffolds, a 50-μL volume contained 2.5×10^4 hematopoietic stem cells, retaining the same number of transplanted cells as in an i.v. injection. For the long-term reconstitution study, we purchased human bone marrow-derived CD34$^+$ cells from Stem Cell Technologies.

Flow cytometric analysis. Cell isolation. Mice were killed 4–5 wk after scaffold implantation, and cells were isolated from bone marrow, spleen, and peripheral blood. For peripheral blood collection, mice were deeply anesthetized. After the rib cage was opened, blood samples were drained from the inferior vena cava or from the heart by cardiac puncture or were acquired by submandibular bleed. Red Blood Cells (RBC) were lysed for 3 min in RBC lysis buffer (BD), and the remaining cells were collected by centrifugation. This step typically was repeated two or three times to remove RBCs completely from blood samples. To isolate bone marrow cells, tibias and femurs were dissected, and the marrow space was flushed with BMSC medium using a 23-gauge needle. Collected marrow cells were passed through 70-μm cell strainers to remove bony spicules and debris and then were treated with RBC lysis buffer for 1–2 min. For spleen cell isolation, a small incision (~1 cm) was made on the exposed peritoneal wall, and the spleen was extracted using a surgical forceps. The spleens were transferred to 70-μm strainers and were gently mashed with a plunger rod from a 3-mL syringe; cells were rinsed off the strainers with RPMI medium. Harvested spleen cells were treated with RBC lysis buffer for 3 min.

For interscaffold cell retrieval, explanted scaffolds were transferred to 70-μm cell strainers placed in a six-well plate. The scaffolds were minced into small pieces (1–2 mm) and incubated in 2 mg/mL of collagenase solution for 30 min. The chopped scaffolds then were thoroughly mashed with the plunger rod until the reddish scaffolds turned semitransparent. Extracted cell suspensions were passed through 40-μm cell strainers to exclude hydrogel debris. Then cells were centrifuged for 10 min at 1,500 rpm and were resuspended in 1–2 mL of FACS buffer (2% (vol/vol) FBS, 1% (vol/vol) penicillin and streptomycin in PBS). Typically 1–3 × 10^6 cells were retrieved from a single scaffold. Cell suspensions were passed through 40-μm strainers one more time immediately before flow cytometry to exclude completely large pieces of hydrogel debris.

Mouse Lin$^-$Sca-1$^+$C-kit$^+$ and cell lineage analysis. Athymic nude mice were killed 4 wk after implantation of BMSC-seeded and unseeded scaffolds, and cells were harvested from the bone marrow and scaffolds following the above protocols. Retrieved cells first

were stained with a biotinylated lineage-specific antibody mixture including CD3, CD5, CD19, CD11b, Gr-1, and Ter-119. Then the cells were stained with APC-Cy7–conjugated anti-biotin (1:50), APC-conjugated anti-c-kit (1:50), and PE-cy7–conjugated anti–scar-1 (1:100) antibodies (BD Bioscience). The cells were stained further with Hoechst nucleus staining dye immediately before flow cytometry to distinguish viable cells. For cell lineage analysis, harvested cells were stained with FITC–anti-mCD45, APC–anti-mCD19, PE–anti-mCD3, and PE-Cy7–anti-mCD11b antibodies (BD Bioscience) and with Hoechst nucleus dye to distinguish viable cells. Peripheral blood counts were obtained using a veterinary blood cell analyzer (HEMAVET; Drew Scientific).

Human cell engraftment analysis. For the short-term (3-d) engraftment study, human leukemia cells were stained with 10 μM of 5-chloromethylfluorescein diacetate (CMFDA) (Invitrogen) before i.v. transplantation. Percentages of fluorescent cells from extracted cells were analyzed by flow cytometry (BD). For the long-term $CD34^+$ cell-engraftment study, extracted cells were stained with PE-Cy7 human CD45 antibody (BD Bioscience), and percentages of fluorescently labeled cells were analyzed by flow cytometry.

Histological analysis. H&E and Von Kossa staining. Implanted scaffolds were harvested and fixed with 10% (vol/vol) buffered formalin solution at 4 °C overnight and were serially dehydrated in a tissue processor (Tissue-Tek Inc.). After being embedded in paraffin, tissue scaffolds were sliced to a 6-μm thickness (Jung Histocut) and were stained with H&E. Calcium deposition was characterized using a Von Kossa staining kit (American MasterTech) following the vendor's protocol.

Immunohistochemistry. For immunohistostaining of mouse CD31, implanted scaffolds were harvested and embedded in optimal cutting temperature (O.C.T.) compound using isopentane chilled with dry ice or liquid N_2. The scaffolds with embedded tissue were cut to a 5-μm thickness, and the tissue sections were stored at −80 °C until immunohistochemistry staining was performed. The sections were fixed briefly with 4% (vol/vol) paraformaldehyde in PBS and were blocked with 4% (vol/vol) normal rabbit serum diluted in PBS. The tissue sections were incubated with purified rat anti-mouse CD31 antibody (PECAM-1, 1:5 dilution; BD Bioscience), followed by biotinylated anti-rat IgG (Vector Laboratories Inc.) to identify endothelia of vessels. To distinguish human cells from mouse cells, anti-nuclei antibody (235-1, 1:100 dilution; Millipore) was used with the M.O.M kit (Vector Laboratories, Inc.) following the vendor's protocol, and the reaction was visualized with a 3-amino-9-ethylcarbazole (AEC) substrate (DakoCytomation).

For immunohistostaining of mouse VEGF receptor 3 (VEGFR3) and human CD45, implanted scaffolds were harvested and embedded in paraffin. The paraffin-embedded scaffolds were deparaffinized and rehydrated before immunohistochemical staining. Antigen retrieval was performed using retrieval solution (pH 6.0) (BD Biosciences) according to the manufacturer's protocol. For mouse VEGFR3, the sections were incubated in 1% (vol/vol) hydrogen peroxide solution and stained with rat anti-mouse VEGFR-3 (clone: FLT4; BioLegend) overnight at 4 °C. After washing with PBS, the sections were incubated with a biotinylated anti-rat IgG antibody (Vector Laboratories, Inc.) for 30 min at room temperature. A Vectastain ABC kit (Vector Laboratories, Inc.) and AEC substrate (Dako) were used for color development. For human CD45, the sections were stained with mouse anti-human CD45 (clone: 2B11 + PD7/26; Dako) for 30 min at room temperature. After washing with PBS, the sections were peroxidase blocked for 5 min, washed with PBS, and incubated with a biotinylated anti-mouse IgG antibody (Vector Laboratories, Inc.) for 30 min at room temperature. For color development, 3,3'-diaminobenzidine substrate (Dako) was used. All sections were counterstained with Harris hematoxylin solution. The images of slides were captured under an optical microscope or were digitized automatically at a magnification of 40× (NanoZoomer 2.0RS; Hamamatsu).

Microscopic analysis. Intravital confocal imaging. A dorsal skinfold chamber was installed on top of the implanted scaffold in athymic Nu/Nu mice following a previously described method (2). Then the anesthetized mouse was placed on an imaging stage and was positioned for data collection from the area of interest. Anesthesia was maintained and a heating pad was used throughout imaging. Intravital fluorescent imaging of the scaffold and tissue was performed using confocal microscopy (Olympus IV100; Olympus America). The target region was found by visualizing the colocalization of autofluorescent hematopoietic cells (emission: 670 nm) with the local vasculature containing a circulating fluorescent dye consisting of 100 μL of 10 mg/mL FITC-dextran (excitation: 488 nm/emission: 520 nm) injected via a tail-vein catheter. Human leukemia cells ($2–3 \times 10^6$) prestained with a DM-Dil CellTracker dye (Invitrogen) (excitation: 553/emission: 570 nm) also were introduced via the catheter. Real-time confocal imaging of the target region was performed for 20–30 min every 1–2 h for the first 6 h and was revisited 24 h later.

In vivo noninvasive whole-body fluorescent imaging. Nude mice were maintained on a low-manganese diet (Harlan) for 3–5 d before imaging to reduce the autofluorescence seen in the digestive tract of mice on a mouse chow diet. Human leukemia or $CD34^+$ cells (5×10^5) stained with 30 μg/mL of a near-infrared fluorescent dye (VT680; Perkin-Elmer) were injected directly into the scaffold or into corresponding s.c. sites of isoflurane-anesthetized mice. Mice underwent whole-body fluorescence molecular tomography (FMT) imaging on days 0, 1, 3, and 7 after cell injections using a commercial imaging system (FMT2500; Perkin-Elmer). Mice were anesthetized and placed in the imaging chamber. Data were collected in the 680-nm channel, and reconstructed 3D images were obtained using the FMT software. Volumes of interest were selected by drawing regions of interest that included cell-injected scaffolds or control skin regions. Total florescent signal was calculated by multiplying a mean fluorescent value by the volume of interest.

Scanning electron microscopic imaging. Explanted tissue scaffolds were fixed in 2.5% glutaraldehyde at 4 °C overnight and were serially dehydrated with 50, 70, 90, 95, and 100% (vol/vol) ethanol. Completely dried samples were deposited with a thin gold film using a sputter-coating machine (208HR; Cressington) and were observed using an Ultra55 field emission-scanning electron microscope (Zeiss).

Laser-scanning confocal microscopic imaging. Human BMSCs were stained with a CMFDA cell tracker dye (Invitrogen), and 5×10^5 cells were seeded into a hydrogel ICC scaffold. After 2–3 d culture, BMSCs residing in hydrogel scaffolds were imaged under a laser-scanning confocal microscope (FV300; Olympus) with a 488-nm laser.

Imaging process and analysis. Open source image analysis software, ImageJ, was used to process z-stack and time-lapse serial confocal images and to quantify nucleated portions of H&E-stained histology images.

Statistical analysis. Statistical comparisons of data were performed using SPSS version 17 software. Nonparametric tests, i.e., Kruskal–Wallis and Mann–Whitney tests, were applied for ELISA and LSK cell analysis, respectively. Comparisons of human cell engraftment in long-term engraftment studies were performed using an unpaired Student *t* test on GraphPad PRISM version 5.

1. Hu X, et al. (1998) Characterization of a unique factor-independent variant derived from human factor-dependent TF-1 cells: A transformed event. *Leuk Res* 22(9): 817–826.

2. Brown EB, et al. (2001) In vivo measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy. *Nat Med* 7(7):864–868.

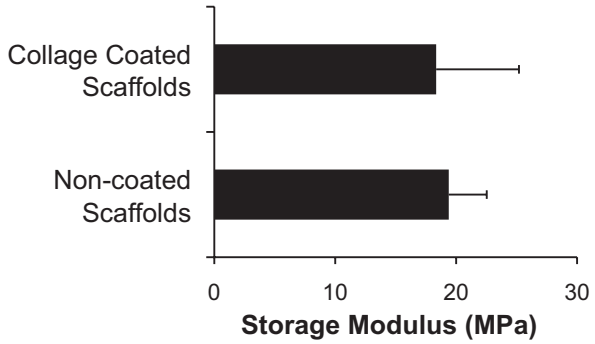


Fig. S1. Dynamic mechanical storage modulus measurement of hydrogel scaffolds at 5% strain before and after type I collagen coating.

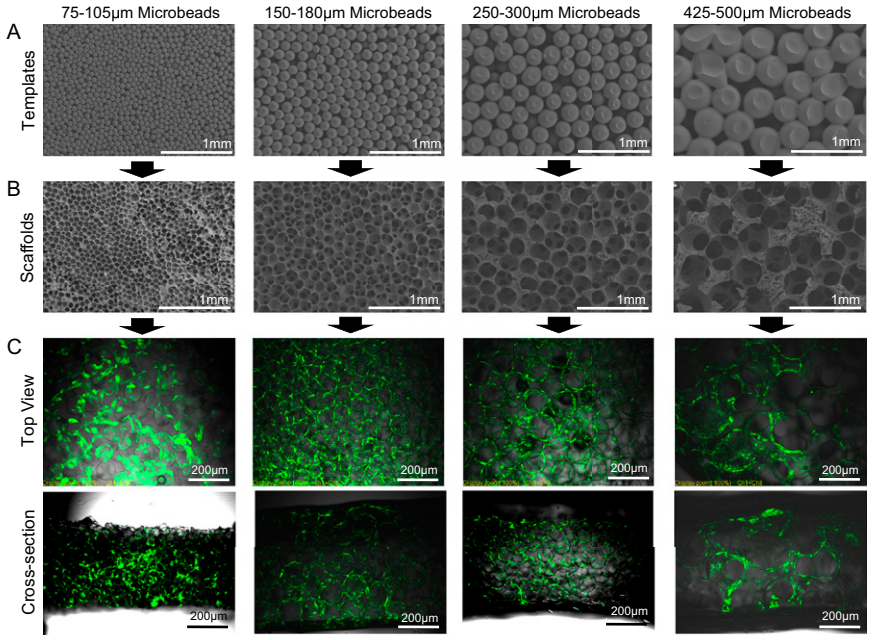


Fig. S2. Manufacture of hydrogel scaffolds with four different cavity ranges and BMSC seeding. (*A* and *B*) Scanning electron microscopic images of (*A*) colloidal crystal templates and (*B*) corresponding hydrogel scaffolds. (*C*) Confocal images of BMSCs 24 h after seeding.

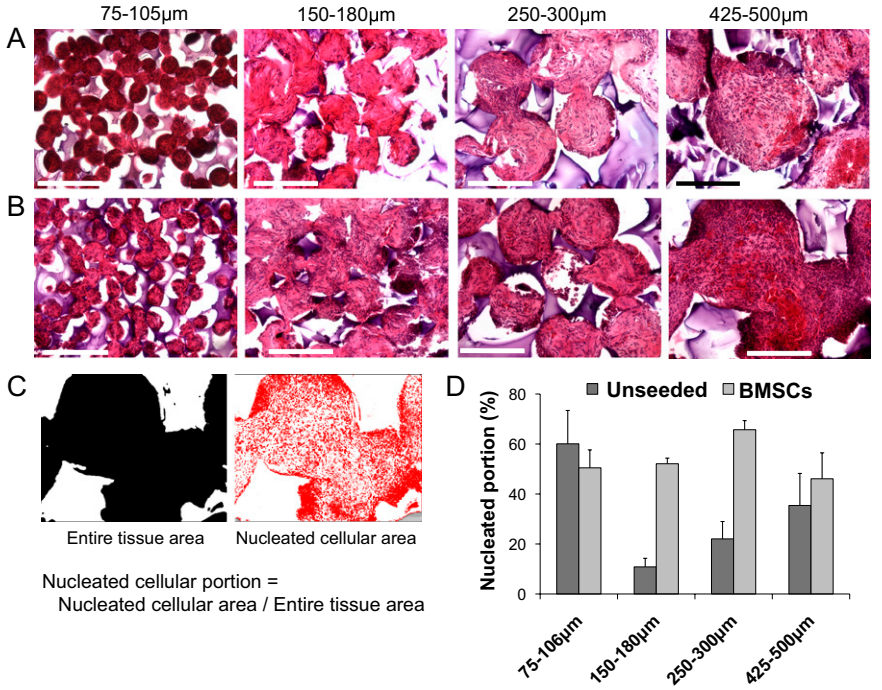


Fig. S3. Characterization of interscaffold tissue development in human BMSC-seeded and unseeded scaffolds having four different cavity ranges 4 wk after implantation. (*A* and *B*) H&E-stained unseeded (*A*) and BMSC-seeded (*B*) hydrogel scaffolds. (Scale bars, 250 μm.) (*C*) Example of the image analysis procedure for calculating the nucleated cellular portion from the entire tissue area. (*D*) Summary of image-processed nucleated cellular portion. (n = 5).

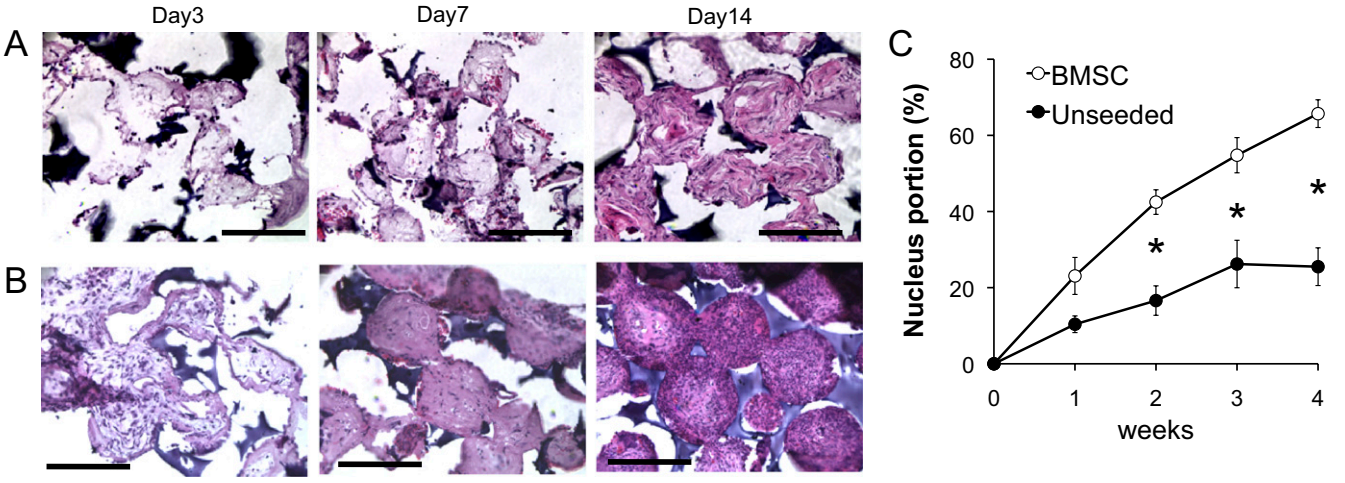


Fig. S4. Comparison of dynamic interscaffold tissue development in BMSC-seeded and unseeded scaffolds. (*A* and *B*) H&E-stained images of unseeded (*A*) and BMSC-seeded (*B*) scaffolds. (Scale bars, 250 μm.) (*C*) Comparison of nucleated cell recruitment kinetics in BMSC-seeded and unseeded scaffolds. (*$P < 0.05$).

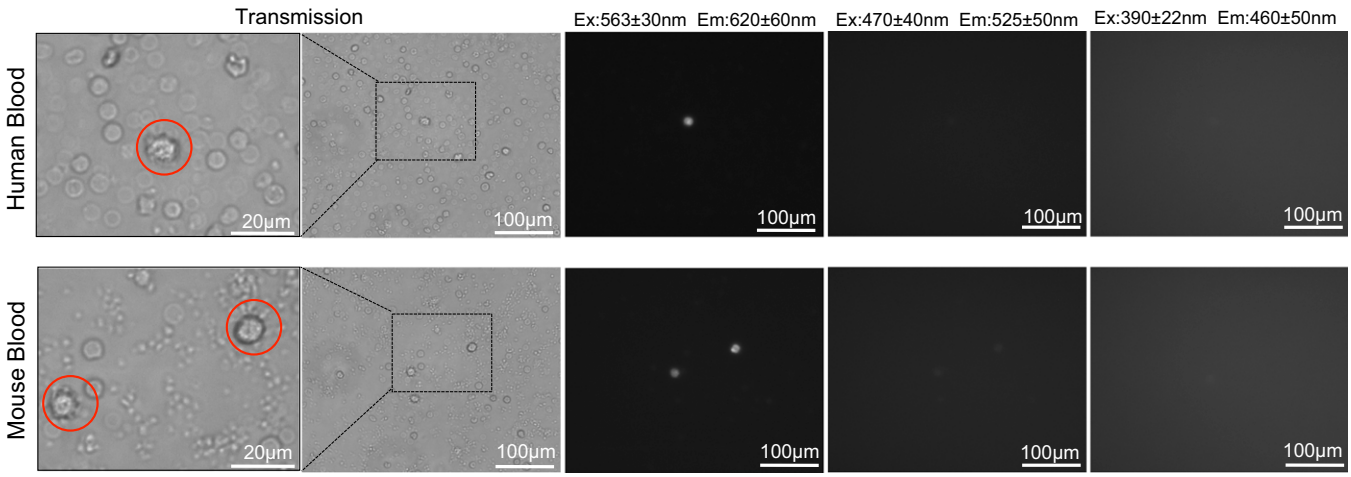


Fig. S5. Autofluorescence of human and mouse hematopoietic cells. Em, emission; Ex, excitation.

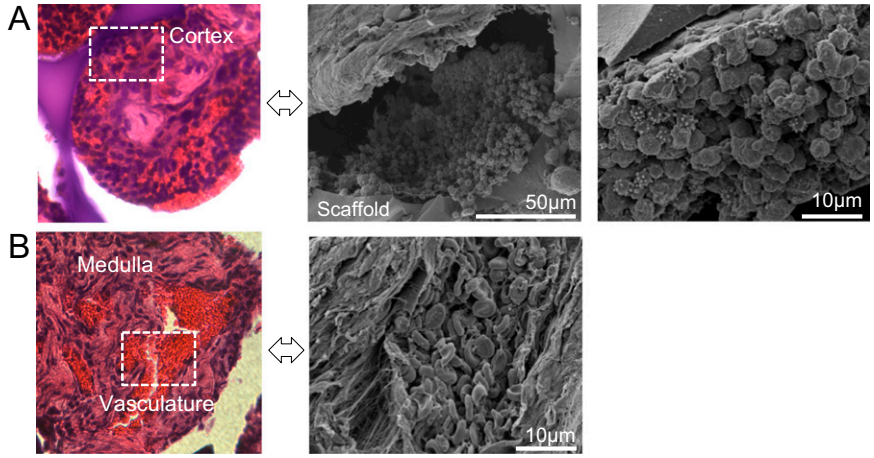
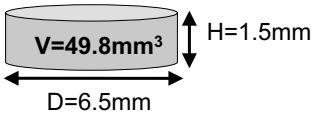


Fig. S6. H&E-stained histological and corresponding scanning electron microscopy images of intercavity tissue development of BMSC-seeded scaffolds 4 wk after implantation. (*A*) Cortex densely accommodating heterogeneous hematopoietic cells. (*B*) Vasculature retaining RBC at the pore center.

A Assumptions for estimating inter-scaffold cortex volume
(1) Standardized tissue-scaffold volume
(2) Complete inter-scaffold tissue development
(3) Homogenous inter-scaffold tissue development

B



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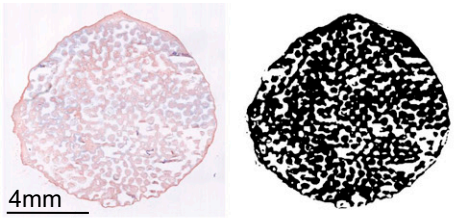
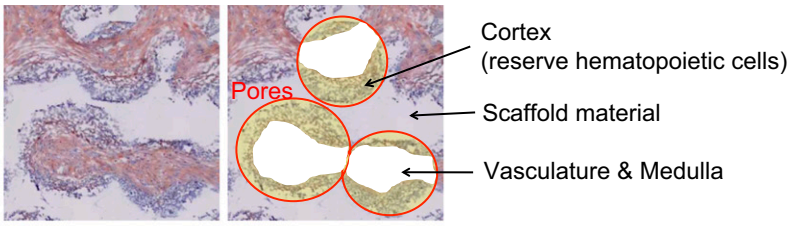


Tissue fraction in tissue-scaffold: 59.3 ± 7.7%

D



Volume fraction of inter-cavity cortex in pores: 39.6 ± 11.1%

E **Inter-scaffold cortex volume (reserve hematopoietic cells)**
Tissue-scaffold volume (49.8mm^3) x Tissue fraction (59.3 ± 7.7) x Inter-cavity cortex fraction (39.6 ± 11.1)
≈ 11.0 ± 3.5 mm^3 per scaffold

F

Species	BM Volume (ml) of Body weight (g)	Ref.
Dogs	2.1 ± 0.3%	1
Cats	1.9 ± 0.3%	2
Rabbits	2.3 ± 0.3%	3,4
Guinea-Pigs	1.6 ± 0.5%	5
Rats	3.0 ± 0.2%	6

Assumption: Total mouse BM volume will be 2% of body weight

G

Mice	Body Weight (g)	Estimated BM volume	Inter-scaffold cortex volume created by 4 scaffolds
Nu/Nu	30 ± 1.2	614 ± 24	7.2% of endogenous BM

Fig. S7. Assumptions and calculations used to estimate interscaffold cortex space and for comparison with endogenous mouse bone marrow (1–6). (*A*) Key assumptions. (*B*) Scaffold volume calculation. (*C*) Calculation of tissue fraction in tissue-scaffolds (n=3). (*D*) Calculation of volume fraction of intercavity cortex in pores (n=15-20 for each scaffold). (*E*) Estimation of interscaffold cortex volume. (*F*) Bone marrow volume of body weight reference (*G*) Estimated endogenous and ectopic marrow volume.

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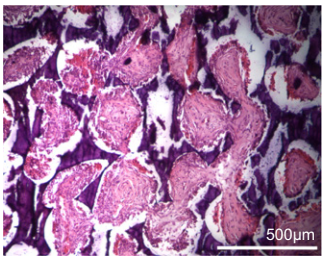
A



B

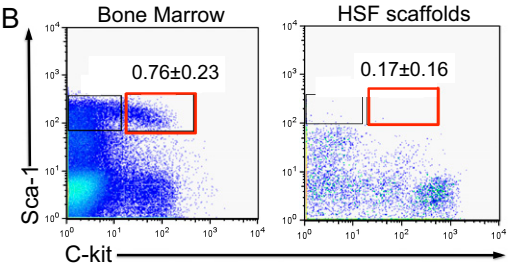


Fig. S8. Characterization of human skin fibroblast (HSF)-seeded hydrogel scaffolds 4 wk after implantation. (*A*) H&E staining and (*B*) Cytofluorimetry of LSK cells from explanted HSF scaffolds and endogenous bone marrow (n = 3).

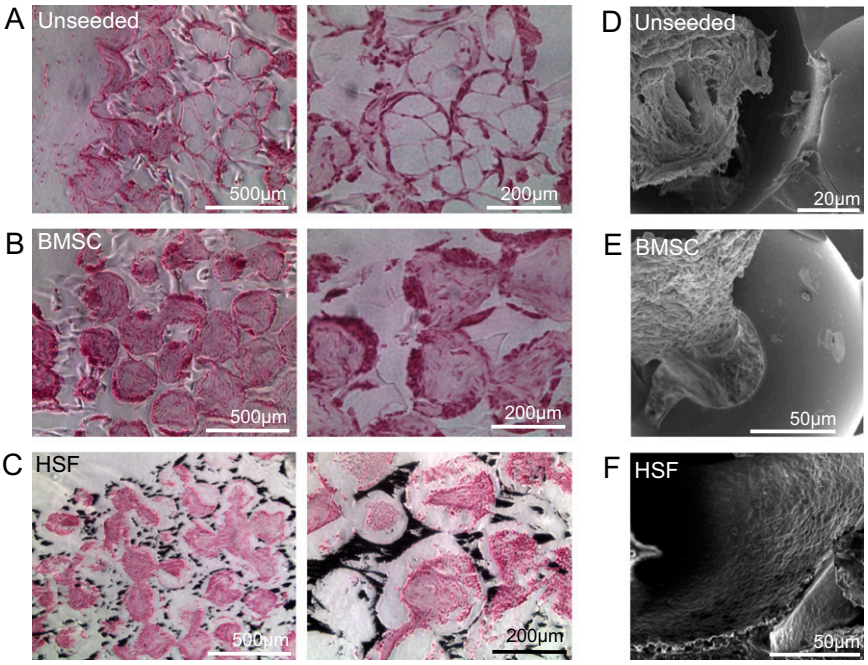


Fig. S9. (*A*–*C*) Von Kossa staining of 4-wk implanted (*A*) unseeded, (*B*) BMSC-seeded, and (*C*) HSF scaffolds. (*D*–*F*) Scanning electron microscopy images of the cavity surface of (*D*) unseeded, (*E*) BMSC-seeded, and (*F*) HSF scaffolds.

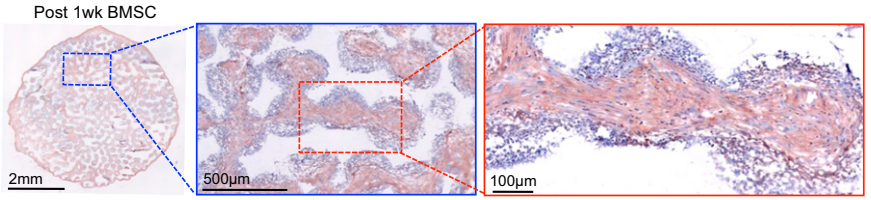
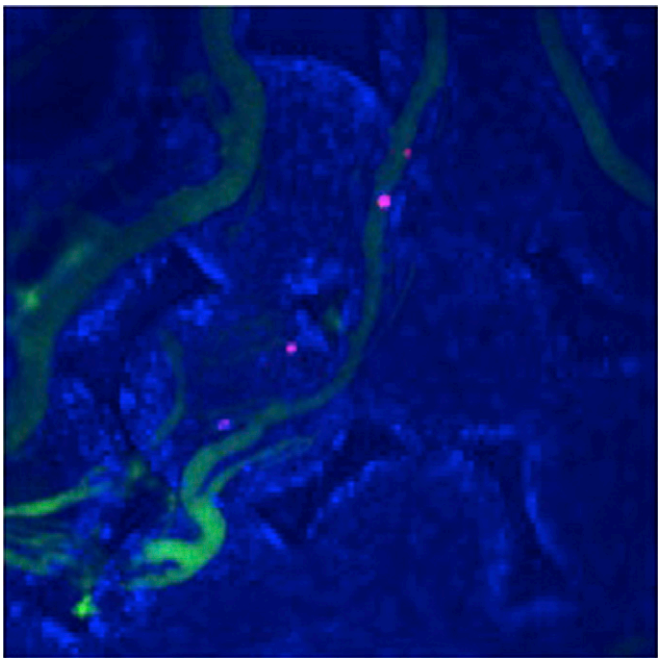
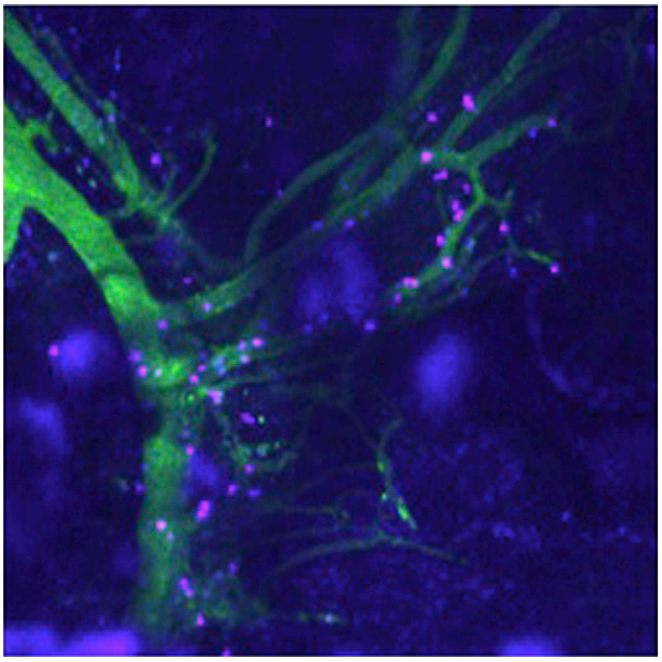


Fig. S10. Immunostaining of human nuclei 7 d after direct transplantation of human $CD34^+$ cells into BMSC-seeded scaffolds.



Movie S1. Intravital confocal movie of i.v. transplanted human TF-1a leukemia cells after 5 h in unseeded tissue scaffold vasculature (play speed, 15×).

Movie S1



Movie S2. Intravital confocal movie of i.v. transplanted human TF-1a leukemia cells after 5 h in BMSC-laden tissue scaffold vasculature (play speed, 15×).

Movie S2