Supporting information for:

Controlling the structural organization of regenerated bone by tailoring tissue engineering scaffold architecture

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Experimental procedure

Fabrication of collagen-apatite scaffolds with varied microstructures

Collagen/apatite scaffolds with varied microstructures were prepared by modifying the biomimetic co-precipitation method described previously [23]. Type I collagen was extracted from rat tail tendon, as described elsewhere [24]. A collagen-containing modified simulated body fluid (m-SBF) was prepared using the following procedures. Briefly, a diluted collagen solution was made by mixing 67 mL above prepared collagen solution (2 mg/mL) and 33 mL de-ionized water. The following chemicals at reagent grade were added to the collagen solution at the following order: 6.0 mM NaCl, 3.0 mM $K_2HPO_4.3H_2O$, 3.0 mM $MgCl_2.6H_2O$, 50 mM HEPES, 8.0 mM $CaCl_2$, 18 mM $NaHCO_3$. The pH of the collagen/m-SBF solution was adjusted to 7.0 by slowly adding 5 M NaOH solution with gently stirring. The collagen/m-SBF solution was then maintained in a water bath at 42°C for 24 h to facilitate collagen fibrogenesis and apatite crystal precipitation. After incubation, the resulted gel was centrifuged at 1.0×10^4 rpm for 15 min and washed with DI water for three times after removing the supernatant. The obtained pellet was then placed into a 35 mm Petri dish and further compressed into a disc shape.

Scaffold characterization

The apatite content in the scaffolds was determined by thermogravimetric analysis (TGA, TGA 2950, TA instruments, USA). The porosity, ε, of the scaffolds was calculated based on equation: $\varepsilon=1-D_A/D_R$ [25], where D_A is the actual density of collagen/apatite scaffold which was calculated by $D_A=(D_{col}R_{apa}+D_{apa}R_{col})/D_{col}D_{apa}$. Here $D_{col}=1.35$ g/cm^3 which is the density of collagen, $D_{apa}=3.16$ g/cm^3 which is the density of apatite, R_{col} and R_{apa} are the weight percentage of collagen and apatite in the scaffolds. D_R was calculated by $D_R=4M/\pi d^2h$. Here M is the mass of the scaffold, d is the diameter of the cylindrical sample, and h is the height of the sample.

Unidirectional, unconfined compression tests were performed on dry scaffold using DMA 2980 Dynamic mechanical Analyzer (TA instrument Inc., New Castle, DE). For each group of scaffold, four cylindrical specimens with a diameter of 8 mm and thickness of 4 mm were cut using biopsy punches (Acuderm Inc., Lauderdale, FL) at different locations of the scaffold sheet. All compression tests were performed perpendicular to the plane of scaffold sheet at a uniform stress rate of 0.5 N/min up to a maximum stress of 18 N. The compression modulus was calculated for each compression test from the slope of the linear elastic regime.

Donor cells isolation and expansion *in vitro*

OPCs were derived from pOBCol3.6GFPcyan transgenic mice. Neonatal calvarial cells were isolated from 4-6 day-old mice using a modified sequential digestion method described by Wang *et al* [27]. Briefly, after removal of sutures and adherent mesenchymal tissues, transgenic mouse calvariae was subjected to four sequential 15 min enzyme digestion at 37C in a solution containing 0.05% trypsin -EDTA and

0.1% collagenase P (Roche Diagnostics, USA). Cells released from the second to the fourth digestions were collected, centrifuged, re-suspended and plated at a density of 1.0×10^6 cells per 100-mm cell culture dish (Falcon, Fisher Scientific) in DMEM medium containing 10% FCS for 5-6 days. The cells were harvested from the dish with 0.25% trypsin/EDTA after they reached subconfluence and re-suspended in DMEM containing 10% FBS at a concentration of 1.0×10^6 cells/mL.

BMSCs were also derived from pOBCol3.6GFPcyan transgenic mice. Six to eight-week-old mice were sacrificed using CO_2 asphyxiation. Femurs and tibias were dissected from surrounding tissues. Bone marrow was harvested by flushing bones with α-MEM medium containing 10% FBS using a 25-G needle. The cells were filtered through a 70-μm cell strainer and plated at a density of 1×10^8 cells in 100-mm culture dish. Half of the culture medium was replaced by fresh medium to remove the non-adherent cells at day 4. The cells were allowed to grow for 7-8 days in α-MEM containing 10% FBS. Cells at a pre-confluent stage were harvested in 0.25% trypsin/EDTA and re-suspended in α-MEM containing 10% FBS at a concentration of 1.0×10^6 cells/mL.

Von Kossa staining procedure

Von Kossa staining was conducted at day 21 to assess the mineralization of both cell types. Briefly, cells were pretreated with saturated lithium carbonate solution after fixation in 2% paraformaldehyde. The plate was then incubated with 5% silver nitrate solution for 30 min under a bright light. After that, the plate was washed with

water and treated with 5% sodium thiosulphate for two min followed by more water washing and air-drying.