Bone Tissue Engineering Using Collagen Gel, Fibrin Sealant, and Stem Cells Derived From Postnatal Skeletal Muscle

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Statement of Purpose: Due to their biocompatibility, capacity to be remodeled and enhanced cell adhesiveness, naturally derived polymers, including fibrin and collagen, have been widely used for the delivery of osteogenic molecules or cells to promote bone regeneration (1). At specific concentrations, some of these materials are thermoset hydrogels: they exist as liquids at 4ºC and gel as the temperature approaches 37ºC. Previously we have shown that genetically engineered muscle-derived stem cells (MDSCs) seeded onto an absorbable gelatin sponge can heal a critical-sized calvarial defect (2). However, this delivery system generates excessive amounts of bone and thus may not be applicable for craniofacial reconstruction. We hypothesized that we could use fibrin sealant and collagen gel to deliver multipotent MDSCs and induce bone formation suitable for cranial applications.

Methods: MDSCs were isolated as described previously (3) and were retrovirally transduced to express either LacZ (MDSC-Lac) or LacZ and BMP4 (MDSC-B4). The level of functional BMP4 secreted by the transduced cells was determined (4). Two delivery systems were tested: bovine type I collagen gel (Neucoll, Inc., Campbell, CA) and Tisseel® fibrin sealant (Baxter, Mississauga, Ontario). Absorbable gelatin sponge Gelfoam® (Pharmacia & Upjohn, Kalamazoo, MI) was used as a control scaffold. 5-mm-diameter critical-sized calvarial defects were created in normal (C57BL/6J) 12-week-old male mice (ARCC protocol #28/04) under general inhalation anesthesia. Five hundred thousand MDSC-B4 or MDSC-Lac cells were mixed with the fibrin or collagen matrices just before implantation into the bone defect area. Gelfoam® was loaded with the same amount of cells 12 hours before implantation and incubated at 37ºC in proliferation medium. Mice in the nontreated defect group received no cells or scaffold. Animals were euthanized 6 weeks after surgery, and the skulls were processed for further radiographic, micro-CT, and histologic analysis. Northern Eclipse software was used to measure the remaining defect area from the radiographic scans, and the average area of regenerated bone was calculated. Student's *t* test was used for the statistical analysis.

Results/Discussion: All nontreated defects (n=10) remained open throughout the entire 6-week study period and developed a small area of regenerated bone (2.35 ± 1.78 mm^2, Figure 1). Defects in the MDSC-Lac treatment group exhibited nonsignificant improvements in bone healing upon comparison with the nontreated group. The area of new bone formed in the defects containing collagen gel, fibrin sealant, or Gelfoam® was 5.7 ± 1.69 mm^2 (n=10), 5.31 ± 0.91 mm^2 (n=8), and 5.72 ± 1.74 mm^2 (n=10), respectively. Implantation of any of the MDSC-B4-seeded matrices resulted in better bone defect healing than observed in the MDSC-Lac-seeded matrices. The use of fibrin sealant resulted in nearly complete healing in 8 of 10 mice (new bone area = 16.03 ± 4.03 mm^2), but the defects in 2 animals remained wide open. Four of the 8 mice treated with collagen gel exhibited substantial defect closure (new bone area = 11.7 ± 7.04 mm^2), but the other 4 mice showed minimal healing. Fully closed defects and the largest area of new bone (19.58 ± 0.11 mm^2, Figure 1) were detected in all mice treated with Gelfoam® (n=7). Even though the healing was significantly better in the Gelfoam® group than in the other scaffold groups, histologic and micro-CT analyses revealed hypertrophic protuberance of bony regenerate in that group. In contrast, the new bone regenerated after implantation of collagen gel or fibrin sealant better conformed to the cranium and displayed regular shape and compact structure (Figure 2).

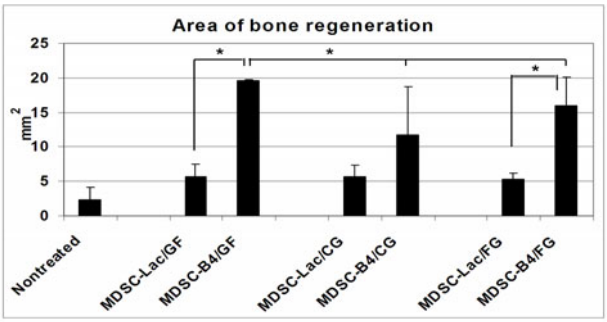

Figure 1: The area of regenerated bone 6 weeks after surgical implantation of MDSC-seeded Gelfoam® (GF), collagen gel (CG), or fibrin sealant (FG) matrices. Values are expressed as mean ± SD, * $P<0.05$.

Figure 2: Three-dimensional reconstructions of bone regenerated by MDSC-B4 cells delivered in Gelfoam® (A), collagen gel (B), or fibrin sealant (C).

Conclusions: Use of the appropriate delivery system is one of the critical aspects in bone-tissue engineering. Our study demonstrates that both collagen gel and fibrin sealant matrices can carry osteopotent MDSCs and promote skull defect healing in mice. The morphology of regenerated bone that formed after implantation of these scaffolds closely resembled the normal calvarium, which suggests that gel matrices may be more suitable for certain applications requiring limited bone growth to repair delicate anatomic structures.

References: 1. Leach K & Mooney D. 2004 *Expert Opin. Biol. Ther.* 2. Lee JY et al. 2000 *J. Cell Biol.* 3. Qu Z et al. 2002 *J Cell Biol.* 4. Peng H et al. 2001 *Mol. Ther.*