Development Of a BMP-2/SDF-1 Alpha Functionalized Mineralized Collagen Matrix Scaffold For Bone Regeneration Applications

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Disclosures:

Introduction: Localized bone loss associated with trauma, tumor, infection, periprosthetic osteolysis, or congenital musculoskeletal disorders represents a major worldwide socioeconomic problem frequently requiring surgical intervention. Previous studies showed that the bone regenerative effect of low doses of Bone-Morphogenetic Protein 2 (BMP-2) can be enhanced by the cytokine Stromal Derived Factor 1 alpha (SDF-1).(1) The aim of this study was to develop a BMP-2/SDF-1 functionalized biomimetic mineralized collagen type I matrix (MCM)-scaffold, evaluate its growth factor release kinetics in vitro, and test its bone regenerative potential in a murine segmental critical size defect model.

Methods: For the in vitro studies, 8 groups were investigated in triplicate. MCM-scaffolds were produced as described before (2) and functionalized with 75 mg heparin per 1 g collagen.(3) MCM-cylinders (2 mm diameter x 3 mm height) were incubated in 15 µL phosphate buffered solution (PBS) containing 0, 0.5, 2.5, 5, or 15 µg BMP-2 for 24 h. Additionally, 0.5, 2.5 and 5 µg BMP-2 groups were loaded with 10 µg SDF-1. After incubation with BMP-2 and SDF-1 scaffolds were rinsed with PBS and incubated for 6 weeks in Minimum Essential Medium Eagle Alpha Modification containing 10% fetal bovine serum and 1% penicillin/streptomycin. The medium was changed every 3rd day and supernatants were frozen. Collected media were analyzed for BMP-2 and SDF-1 by ELISA.

Sixteen 12-week-old nu/nu nude mice were randomized to 2 groups. All experiments were performed in adherence to the National Institutes of Health Guidelines for the Use of Experimental Animals and were approved by the Local Animal Care Committee (protocol no. 24-9168.11-1/2010-29). Critical size bone defects of 3 mm length were created at the right femur of each mouse and stabilized by an external fixator as described before.(4) Control and treatment groups received plain MCM-scaffolds and MCM scaffolds loaded with 2.5 µg BMP-2. After 6 weeks all animals were euthanized and µCT-scans of 20 µm voxel size were done on each femur to analyze regenerated bone volume. For histomorphological investigations, hematoxylin and eosin staining was performed. The degree of healing of the defect was evaluated by three independent observers on three representative sections per animal according to Huo et al. (5) using a scale ranging from fibrous tissue on grade 1 to bone (grade 10). Descriptive statistics included means and standard deviations. Unpaired t-tests or Fisher’s exact test were used for statistical analysis between the two groups of the in vivo experiment. Differences were considered significant when p<0.05.

Results: Long-term (6 weeks) BMP-2 (Figure 1) and SDF-1 (Figure 2) release was observed from the MCM-scaffolds. All animals survived the operations and the observation time. No intergroup differences regarding bone volume in and around the defect area were observed between the treatment group (4.8±1.3 mm³) and the control group (4.7±1.6 mm³; p=0.92). However, three-dimensional reconstructions (Figure 3) showed 6 out of 8 bony unions in the treatment group versus none in the control group (p=0.007). Histological analysis confirmed a higher degree of defect healing in the treatment group (7.9±1.8) compared with the control group (5.8±1.6, p<0.0001).

Discussion: After an initial burst of BMP-2 and SDF-1 from the scaffolds, there is long-term release of BMP-2 and SDF-1 of up to 6 weeks from the heparin-functionalized MCM-scaffolds. These results are promising for future in vivo studies, however in vitro conditions are not exactly comparable with in vivo situation and bioactivity of the growth factor and the cytokine after 6 weeks need further analysis. BMP-2 loaded scaffolds provided better conditions to form bridging callus whereas the control group resulted more in capping the bony ends. Combined functionalization by BMP-2 and SDF-1 may further enhance bone regeneration.

Significance: We demonstrated long-term release of BMP-2 and SDF-1 from heparinized MCM-scaffolds for up to 6 weeks. The use of BMP-2 functionalized MCM-scaffolds improved bone regeneration in vivo. Combining BMP-2 and SDF-1 release from MCM-scaffolds is a promising strategy to enhance bone regeneration.

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Figure 1: In vitro long term BMP-2 release from heparinized MCM-scaffolds. Released BMP-2 was analyzed from the culture supernatant via ELISA. Means and standard deviations are presented every three days up to 6 weeks.
Figure 2: In vitro long term SDF-1α release from heparinized MCM-scaffolds loaded with various BMP-2 concentrations and additional 10 μg SDF-1α. Released SDF-1α was analyzed from the culture supernatant via ELISA. Means and standard deviations are presented every three days up to 6 weeks.
Figure 3: Representative images of the 3D-reconstruction of the X-ray scans of the femoral defect areas. Defect areas of the group treated with mineralized collagen matrix (MCMA-scaffolds loaded with 25 µg BMP-2) are shown in the upper row. Six out of 8 mice showed unions of the defect. Two resulted in nonunions. The lower row shows images of the control group, treated with MCMA-scaffolds without BMP-2. All defects resulted in nonunions.

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