Fibrin Gel-Based Delivery of Growth Factors for Bone Healing in the Critical Size Rat Calvarial Defect

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Introduction: In the context of cartilage or bone repair, the use of an appropriate matrix to deliver cells and growth factors as well as to localize and enhance tissue healing is desirable. Fibrin Sealants have been used for cell delivery and shown to be a suitable delivery vehicle for growth factors (1,2), including Transforming Growth Factor-β1 (TGF-β1), released in a controlled manner (3). These fibrin gels consist of two human plasma-derived components: (a) a highly concentrated Fibrinogen Complex (FC) composed primarily of fibrinogen and fibronectin along with catalytic amounts of Factor XIII and plasminogen, and (b) a thrombin component. Members of the TGF-β family of growth factors have been shown to exert osteoanabolic and osteocatabolic effects, suggesting an influence on bone formation and also on bone remodeling (4). TGF-β1 has been shown to be involved in cartilage and bone development, most likely by inducing the differentiation of mesenchymal stem cells into the chondrogenic or osteogenic lineage. Bone Morphogenetic Protein-2 (BMP-2) is known to play a critical role in bone healing through its ability to induce differentiation of mesenchymal cells. The purpose of the present study was to analyze and compare bone healing in critical size defects of rat calvaria filled with fibrin gels supplemented with TGF-β1 or BMP-2 to evaluate the use of such matrices for bone repair in clinical applications.

Methods: Fibrin gels with or without the addition of a growth factor in the FC component (TGF-β1 or BMP-2, R&D Systems), were prepared on the day of the surgery in custom-made round silicon molds. Gels were prepared by diluting the components of a Fibrin Sealant product (Tisseel®, Baxter) to final concentrations of 25 mg/ml FC and 2 IU/ml thrombin in the gels. Critical size bone defects of 8 mm in diameter were drilled in rat cranium and plugged with the pre-formed gel constructs. Bone healing was analyzed at 3 and 6 weeks. At least four animals were included in each of the following groups for both time points: empty defect, fibrin gel alone, fibrin gel+TGF-β1 at 1, 5 or 25 μg/gel, and fibrin gel+BMP-2 at 5 μg/gel (60 animals total). At each time point, samples were harvested and fixed with 10% buffered formalin. Contact radiographs were taken to grossly assess the healing tissue. Bone replacement volumes were calculated as the ratio of new bone formation in the original defect volume using micro computed tomography (microCT). Finally, samples were embedded in plastic resin to be processed for hard tissue histology using an H&E stain.

Results: Contact radiographs revealed more new mineralized tissue in the BMP-2 group compared to all other groups. Defects not initially filled with a construct (negative controls) remained mostly empty. MicroCT results showed more new bone in the defect at 6 weeks compared to 3 weeks in all cases (Figure 1), although this was only significant for fibrin+5 μg BMP-2 ($P<0.01$) and fibrin+25 μg TGF-β1 ($P<0.05$) groups. New bone volume was significantly higher in fibrin+5 μg BMP-2 group than in all other groups at both 3 and 6 week time points ($P<0.001$). Fibrin+15 μg or 25 μg TGF-β1 gave more bone formation than fibrin+5 μg and fibrin alone at 6 weeks, but the difference was not statistically significant. Histology results showed new bone formation consistent with the microCT results.

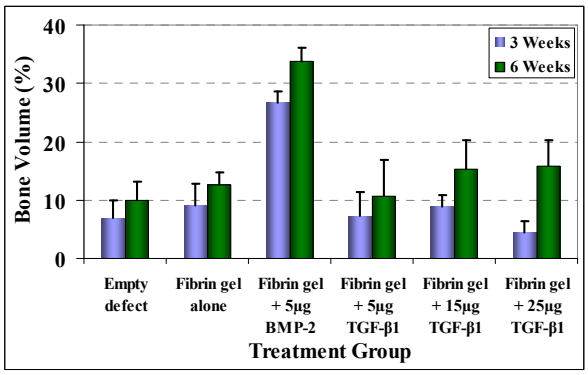

Figure 1. MicroCT results

Discussion and conclusions: The need to control and prolong the presence of growth factors at bone regeneration sites remains a challenge in tissue engineering. Fibrin gel has been shown to be a suitable delivery matrix for this purpose (3). The current study investigated the dose and time response effect of fibrin gels loaded with TGF-β1 or BMP-2 on new bone formation in rat calvarial critical size defects.

Radiographs, microCT quantification of bone volume, and histological evaluation were all in agreement, demonstrating a time-dependent increase in new bone formation in all groups. Analysis of the defect bridging as well as new bone density (i.e., new bone maturity) could reveal differences in the bone mineralization process between the groups analyzed. The present analysis revealed that bone regeneration in all TGF-β1 groups was lower than in the BMP-2 group, which itself was lower than that previously reported (5). This discrepancy with BMP-2 could be explained by differences in the source of fibrin sealant and the growth factor.

Overall, the present study showed that evaluation of fibrin gels supplemented with TGF-β1 or BMP-2 for bone tissue engineering applications warrants further investigation. In future studies, wider doses of growth factors, longer healing times, and different fibrin gel formulations could be investigated for enhanced bone regeneration.

References: 1. Cox et al., Tissue Eng 2004;10(5-6):942-54; 2. Wong et al., Thromb Haemost 2003;89(3): 573-582; 3. Catelas et al., Tissue Eng Part C 2008;14(2):119-128; 4. Janssens et al., Endocr Rev 2005;26(6):743-774; 5. Schmoekel et al., J Orthop Res 2004;22(2):376-381.