"Bone Tissue Engineering using BMP-2, IFN-γ, and IL-4 loaded in a Microporous Fibrin Scaffold"

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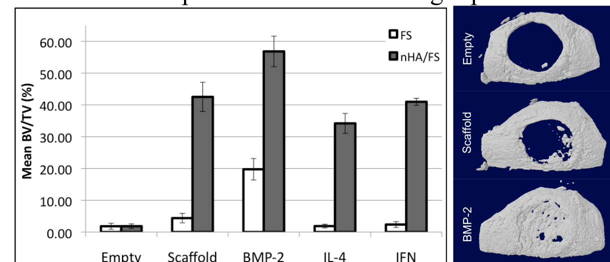
Statement of Purpose: Inflammation is a prominent feature of bone regeneration in craniofacial bone wounds. Macrophages, depending on their phenotype, have the capacity to direct a proinflammatory (M1) or prohealing (M2) response. We have observed robust bone regeneneration in fibrin scaffolds loaded with bone morphogenetic protein-2 (BMP-2). Inflammation in this context was characterized by an initial M1 macrophage activation phase, followed by an M2 macrophage activation stage. On the other hand, untreated fibrin scaffold showed little bone regeneration and was characterized by an accumulation of M1 macrophages. We hypothesize that exogenous control of M1/M2 macrophage phenotypic balance, mediated by, IFN-γ, an M1 activator, and IL-4, an M2 activator, may differentially affect bone healing in a porous fibrin scaffold. Specifically, the M1 and M2 phenotype would have catabolic and anabolic affects, respectively, on bone formation. To test this hypothesis, fibrin scaffolds alone (FS), or fibrin scaffolds loaded with either IFN-γ or IL-4 were tested for their ability to modulate bone regeneration in a murine cranial critical size defect model.

Methods: Microporous nanofibrous fibrin scaffolds (FS) (~200-250μm pore size, 5mm diameter, 0.5mm thick) were fabricated as previously described.[1] Poly(methyl methacrylate) (PMMA) beads were close-packed into molds and sintered at 145°C for 22h to form the scaffold template. 200mg/mL bovine fibrinogen in 0.9% NaCl was then infiltrated into the template pores. 267U of thrombin, 133μL of 2N $CaCl_2$ and 1% antibiotic/ antimycotic solution in DMEM was added to the fibrinogen and allowed to incubate for 24h to form fibrin. The PMMA template was then dissolved in acetone for 72h, and then resolvated in 70% ethanol overnight. nHA/FS were prepared by mixing 0.25 nHA in the fibrinogen solution before casting into PMMA templates, and processed similarly to the FS scaffolds. The cytokine-treated FS or nHA/FS scaffolds were immersed in 10μg/mL of rhBMP-2, 20ng/mL IFN-γ, or 60ng/mL IL-4 immediately prior to implantation. Animals were used under protocol approved by IACUC, Univ. Washington. Critical size defects (~4.2mm diameter) were drilled in the left and right parietal bones of 6-7 week old C57BL/6 mice (n=5 for each group) using a trephine burr. FS and nHA/FS were implanted in the left and right defects, respectively. Animals were sacrificed at 45 days and parietal bone specimens were explanted, fixed in 4% formalin, and imaged using microCT (Skyscan 1076) at 35μm resolution, 50 kV, 0.5 mm aluminium filter. Images were reconstructed, re-sliced, and analyzed using Dataviewer and CTAn software (Skyscan). Cylindrical regions of interest at the size of the empty defect were used to quantify bone volume per total volume (BV/TV) on manually thresholded binarized images. Statistical testing was done by a one-way ANOVA, assuming equal variance, using Tukey's HSD on SPSS v16.0 software.

Results: Gross inspection of the explanted tissue showed the nHA/FS samples showed more robust bone formation than the FS alone samples, as expected. In the FS, the BMP-2 treated animals showed significantly higher mean BV/TV than empty control ($p<0.01$) and scaffold control ($p<0.01$). The IFN-γ and IL-4 treated fibrin scaffold groups showed no significant difference compared with empty and scaffold control. Similarly, in nHA/FS, BMP-2 treated animals showed significantly higher mean BV/TV than empty control ($p<0.01$) and scaffold control ($p<0.05$). IFN-γ and IL-4 showed no difference from control. FS samples are shown in the right panel.



Conclusions: Bone healing is characterized by an inflammatory phase, which is controlled by a series of proinflammatory cytokines and the osteogenic cytokine BMP-2.[2] In this study, we confirmed that BMP-2 loaded in a microporous fibrin scaffold elicits robust bone regeneration. IFN-γ and IL-4 treatments showed no difference compared to scaffold control. Although we did not observe enhanced bone formation by IL-4, this result may not be surprising because IL-4 has pleiotropic effects on multiple cell types beyond the macrophage. Reports suggest IL-4 targets both osteoblasts and osteoclasts, inhibiting bone remodeling. Mice that overproduce IL-4 develop severe osteoporosis, reflecting a catabolic effect.[3] IL-4 may have both anabolic and catabolic effects on bone. Similarly, IFN-γ has been shown to be an inhibitor of osteoclastogenesis, an anabolic effect, yet has also been shown to stimulate bone resorption in patients with osteopetrosis.[4] In future studies, we are interested in the effect of IFN-γ and IL-4 on osteoblast and osteoclast formation by the markers, ALP and TRAP, in addition to M1/M2 macrophage markers, iNOS and mannose receptor, respectively. This will help to decouple the competing effects by osteoclast/osteoblast balance and macrophage activation on bone formation. Furthermore, the spatial and temporal effects of the cytokine action in bone healing may be particularly important. Future studies to temporally and spatially control cytokine release during bone regeneration will be needed to address this issue.

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