Effects of Co-Delivering Low-Dose VEGF With Osteogenic Growth Factors on Bone Formation and Revascularization of Critically-Sized Defects

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Introduction: Nonunion of large bone defects is a common clinical challenge. Standard allograft transplantation is limited by lack of graft revitalization and frequent refracture. Tissue-engineered alternatives may be viable solutions provided they enhance revitalization of the defect with functional bony tissue. Previously we have shown that treatment of critically-sized femoral defects with growth factor-augmented polymer scaffolds enhance bone formation and functional repair of the defect in a dose-dependent manner1. The goal of this study was to quantify the effects of combined BMP-2/TGF-β3 ± VEGF delivery on repair and revascularization of the defect.

Materials and Methods: Four treatment groups were included: scaffold, scaffold + 2000ng BMP-2/200ng TGF-β3, scaffold + 1000ng VEGF, and scaffold + 2000ng BMP-2/200ng TGF-β3/1000ng VEGF. Implants: Poly(L-lactide-co-D, L-lactide) cylinders were selected for their mechanical integrity and oriented macroporosity. PLDL scaffolds were prepared as previously described2 and fibronectin-coated. Growth factors were incorporated into RGD-alginate, infiltrated into the PLDL porosity, and crosslinked in place. Surgical procedure: Aged female rats were utilized. Hindlimbs were approached anteriorly. Custom modular fixation plates were secured to the femur. The plates provide radiolucent windows for in vivo micro-CT imaging. Bilateral diaphyseal femoral defects 8 mm long were created. PLDL scaffold ± growth factors were implanted and incisions closed. The hindlimbs were fully weight-bearing by day 3. Micro-CT analysis and mechanics: In vivo micro-computed tomography was conducted at 4 and 12 weeks post-op. A consistent central volume of interest was evaluated. Global thresholding isolated bony tissue from scaffold and soft tissue. Vascular volumes (VV) were assessed with contrast-enhanced post-mortem micro-CT. Functional integration was assessed by torsional testing after plate removal.

Results: Treatment groups receiving BMP-2/TGF-β3 ± VEGF had significantly higher bone volumes at 4 and 12 weeks post-op than scaffold or scaffold + VEGF groups. Delivery of VEGF did not impact bone volume over a scaffold alone. [Figure 1] VV evaluated at 3 weeks post-op was not enhanced by growth factor delivery although extensive vascularity in the defect site was apparent. [Figure 2] Consistent bony union (11/12 samples) was achieved in the BMP-2/TGF-β3 ± VEGF groups. Delivery of a scaffold alone ± VEGF did not promote bony union (union rates 2/7 and 0/5). [Figure 3] Torsion testing of samples at 12 weeks post-op revealed that treatment of defects with BMP-2/TGF-β3 ± VEGF significantly enhanced both stiffness and maximum torque over delivery of a scaffold ± VEGF.

Discussion: The use of osteo- and angiogenic agents in cooperation to generate bone has been explored using gene delivery and met with success in ectopic models3. In the critically-sized femoral defect model presented here, low dose VEGF did not improve the quantity of bone or vascularure generated in the defect. Although treatment with BMP-2/TGF-β3 significantly improved bone volume regardless of VEGF delivery, this therapy did not affect VV. Treatment of defects with BMP-2/TGF-β3 ± VEGF significantly improved maximum torque and stiffness. In this model, co-delivery of osteo- and angiogenic factors did not result in additive or synergistic benefits for defect revitalization. Interactions determined in other models may be masked by the acute physiologic response to the severe defect created here. The trauma of creating a large defect elicits biological and mechanical signals that could be innately maximizing the natural angiogenic response. It is also possible that the dose of VEGF applied here is too low to exert significant influence on defect revitalization. Future work focuses on cellular and gene-delivery therapies.


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