Bridging the Gap: Assessing an Engineered Biomimetic Periosteum on Bone Allografts for the Reconstruction of Large Segmental Bone Defects in Mice

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Statement of Purpose: Load bearing critical-sized bone defects pose a unique and challenging clinical problem. Clinical interventions consist of a bone graft capable of immediate mechanical support. Unfortunately, these types of bone grafts require a large amount of donor tissue. Autologous bone grafts, which typically have the best clinical outcomes, are limited by the amount of graftable tissue and potential donor site morbidity. On the other hand, bone allografts are not limited by the amount of graftable tissue yet suffer from suboptimal clinical performance. Periosteum removal from a bone allograft decreases an allograft's ability to reintegrate with host tissue as the periosteum has been shown to be crucial to the bone fracture healing response[1]. **We hypothesize that engineering a biomimetic periosteum on bone allografts will enhance bone allografts' osseointegration.** Allografts with an engineered periosteum were implanted into a critical sized femoral defect in C57BL/6 mice to evaluate this technology's potential for clinical translation.

Methods: Chitosan was acquired from Novamatrix (Sandvika, Norway). Heparin sodium from porcine intestinal mucosa was purchased from Celsus Laboratories (Cincinnati, OH). Chitosan was methylated to make *N*,*N*,*N*─trimethyl chitosan (TMC) following a method previously reported[2]. Mouse mid─diaphyseal femur allografts (4mm) were harvested from 8 week old BALB/c mice. The allografts were cleansed and frozen at −80 °C for at least 2 weeks prior to surface modification with an engineered periosteum[3]. Allografts' surfaces were coated with electrospun chitosan nanofibers (NF). The NF scaffolds were neutralized with an NH_4OH solution and were then subsequently modified with TMC and heparin polyelectrolyte multilayers (PEMs) using a layer─by─layer deposition procedure. rhFGF─2, rhTGF─β1, hFGF basic Duoset and hTGF─β1 Duoset ELISA kits were purchased from R&D Systems (Minneapolis, MN). Growth factors were reconstituted and adsorbed for 1 hour under gentle agitation onto PEM─modified NF scaffolds. Passage 3 mouse luciferase─expressing adipose─derived stem cells (Luc─ASCs) were seeded onto modified allografts at 500,000 cells in 30 μl^{-1} and allowed to attach for 2 hours at 37 °C and 5 % CO_2. Modified allografts were moved to new wells and cultured overnight. Uncoated allografts and modified allografts were implanted into a 4─mm mid─diaphyseal femoral defect in 6─8 week old C57BL/6 mice and stabilized with an intramedullary pin. Experimental groups consisted of uncoated allografts, allografts with NF+FGF─2+TGF─β1, allografts+ASCs, and allografts with NF+FGF─2+TGF─β1+ASCs. Mice were monitored for 6 weeks. Luc─ASCs were longitudinally tracked in mice to track persistence at defect site by a subcutaneous injection of D─luciferin. At post-operative week 6, mice were euthanized, femurs excised, formalin fixed, and prepared for microcomputed tomography (μCT) analysis to measure new bone formation. Bone tissue was then prepared for histological analysis by decalcification and paraffin embedding followed by subsequent sectioning and H&E staining. A blinded histological analysis was performed by a board-certified veterinary pathologist to assess graft incorporation. All animal experiments were approved by Colorado State University's Institutional Animal Care & Use Committee.

Results: Luc─ASCs were found to persist in the femoral defect site for up to 7 days. Luc─ASCs were found to initially proliferate as evidenced by increased bioluminescent signal at day 4. By day 7, Luc─ASCs bioluminescent signal decreased. Mice and biomimetic periosteum did not emit background signal.

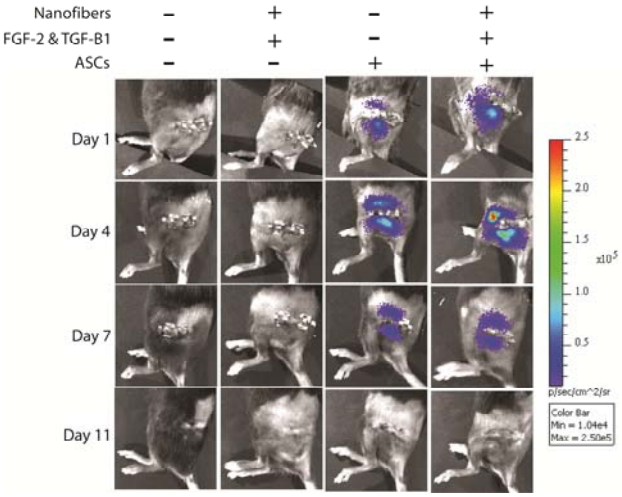

Figure 1. Luc─ASCs are longitudinally tracked over time in C57BL/6 mice.

Conclusions: Our engineered biomimetic periosteum was validated as a vehicle for cell transplantation. Luc─ASCs persistence in the femoral defect site was found to be 7 days, similar to work by Hoffman et al[4]. Inadequate vascular supply is the most likely cause for transplanted cell death. Effect of engineered biomimetic periosteum on allograft incorporation will be evaluated with μCT and histological analysis. This data will inform the design of future preclinical studies for potential clinical translation of an engineered biomimetic periosteum.

References:

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