Tethered Epidermal Growth Factor delivery for the repair of large segmental bone defects

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Statement of Purpose: Bone marrow connective tissue stem and progenitor cells (referred to herein as "CTPs") play an essential role in the regeneration of large segmental bone defects, evidenced by poor clinical outcomes in patients with CTP deficiencies. Autograft bone remains a gold standard source of CTPs, but use of autograft is associated with high morbidity at the harvest site. An emerging attractive alternative grafting approach involves combining freshly-aspirated marrow with a synthetic scaffold in the operating room. The relatively low prevalence of CTP in some patients, combined with inflammatory milieu post-grafting, presents challenges to the use of marrow CTPs as an intraoperative graft. In this work, we aim to improve outcomes of bone graft procedures by manipulating the pro-survival signaling pathways activated by the epidermal growth factor receptor (EGFR), as signaling through this receptor has been shown to favorably influence both early stages of CTP colony formation, as well as promote their survival and differentiation [1-3]. Specifically, we demonstrate that epidermal growth factor tethered to a commercial clinical beta tricalcium phosphate (BTCP) scaffold, has a positive influence on CTP phenotype with respect to CTP colony formation, transplantation, and progeny proliferation without interfering with osteogenic differentiation.

Methods: BTCPbp was discovered through the use of the 12-mer Phage Display Peptide Library kit sold by New England Biolabs. The BTCPbp-EGF fusion protein was expressed in *E. coli*. Binding of BTCPbp-EGF to BTCP materials (scaffolds, powder and coverslips) was achieved by simple incubation of the BTCP and protein solution in a well at 4°C for 24-36 hours. Quantification of bound protein was done by BCA assays. CTP colony forming unit assays using samples from 8 patients were performed on BTCP coverslips. Half a million nucleated cells from a bone marrow buffy coat of each patient was plated onto BTCP coverslips with and without BTCPbp-EGF. Analysis was performed after 9 days of culture. Proliferation experiments using CTP progeny (Passage 4) were performed *in vitro* using Therilok™ β-TCP 3mm scaffolds (Provided by Therics, 115 Campus Drive, Princeton, NJ 08540). Quantification of cells was done with the Alamar Blue assay. Mice experiments were performed using FVB mice and the BTCP-powder/CTP-progeny/tEGF composites were embedded in a 1:1 collagen/matrigel matrix and injected subcutaneously. The CTP progeny was labeled with CellTracker™ CM-Dil before xeno-transplantation. Explants were collected at Days 2, 7, and 21 post-implantation. Analysis of CTPs in explants was done by liberase treatment and subsequent flow cytometry (human CTP markers and CM-Dil stain).

Results: The target dose of 6 ug tEGF/10 mg BTCP scaffold, based on pilot studies with cell proliferation as an outcome, was achieved and remained bound for more than 7 days at 37°C. This dose promoted a 2-3 fold expansion of CTP progeny cultured onto BTCP scaffolds for 7 days. Using primary human marrow aspirates to investigate the effect of tEGF on progenitors in marrow, we observed a 60% increase in CTP colony forming efficiency across 8 different human bone-marrow samples. In a subcutaneous implantation mice model, tEGF was able to increase the survival of xeno-transplanted CTPs by a factor of 15 three weeks post-implantation (Figure 1).

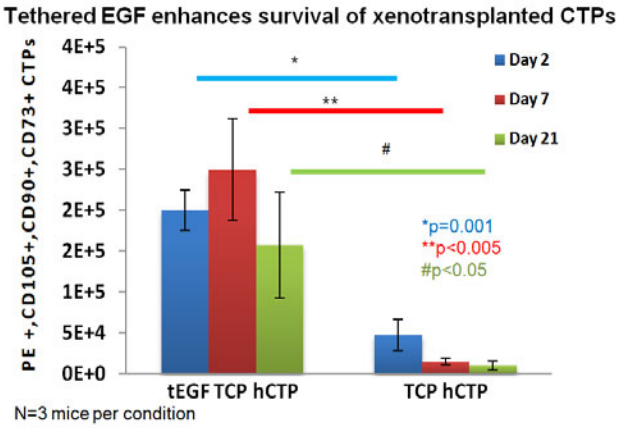

Figure 1. Tethered EGF delivery increases the number of viable, xenotransplanted CTPs in a mice implantation model. Results show a 4-fold increase at Day 2 ($p=0.001$); 16-fold at Day 7 ($p<0.005$); and, 15-fold at Day 21 ($p=0.05$).

Conclusions: Using our BTCP binding strategy we were able to successfully non-covalently tether EGF onto BTCP materials with high affinity. Tethered EGF presented on BTCP substrates was able to improve CTP transplantation and, CTP colony formation across multiple patient samples. This demonstrates the clinical value of tEGF beyond *in vitro* proliferation assays. Our current efforts are focused on pre-clinical testing of this technology on a Canine Femoral Multi-Defect (CFMD) model. BTCP surface modification using BTCPbp-EGF is a versatile strategy that could be used to augment the performance of these implantable biomaterials.

References: [1] Marcantonio NA. Biomaterials. 2009;30:4628-4638 [2] Platt MO. J Cell Physiol. 2009;221:306-317 [3] Fan VH. Stem Cells. 2007;25:1241-1251