Novel Bioactive Coatings to Improve Allograft Incorporation Evaluated in eGFP Chimeric Rats

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Statement of Purpose: Even after aggressive surgical management with bone allografts, complications from battlefield trauma arise in the form of non-union, infection, and reduced functional capacity. Particularly challenging is the integration of massive structural allografts that are commonly used for limb salvage. These allografts can provide vastly superior mechanical stability relative to demineralized allograft, but also suffer particularly poor long-term performance [1-4]. This study employs a rat tibial defect model in a wild type Sprague Daley (WTSD) rat chimera with bone marrow cells heterozygous for enhanced green fluorescent protein (eGFP). Treatment with local, sustained delivery of FTY720 results in improved allograft incorporation in critical size defects in long bones. Exploring the mechanisms of this therapy will give particular focus on the role played by bone marrow derived stem cells.

Methods: Colony forming assays and osteogenic differentiation assays were performed on bone marrow cells isolated from eGFP -/- SD rats (eGFP negative rats obtained after breeding eGFP rats and WT rats), eGFP -/+ SD rats (eGFP positive rats obtained after breeding eGFP rats and WT rats) and WTSD animals 2 weeks after seeding on 6 well plates.

SDTg(GFP)2BalRrrc rats were obtained from the colony maintained at the Rat Resource & Research Center of the University of Missouri. The genetic alteration was performed by infection of embryos with a lentivirus transgene containing the ubiquitin promoter driving eGFP with a fixed insertion site on chromosome 14. eGFP+/- bone marrow rats were created by lethally irradiating SD-eGFP-/- female rats with 10 grays and 20e6 bone marrow cells were immediately injected intravenously from an eGFP+/- donor. Complete characterization of the chimeras was done with hemavet, flow cytometry (eGFP, CD90, CD45, and CD11b markers) and histology. 8 weeks after chimera creation, 4 mm tibial defects were created in these rats and were treated with allografts coated with PLAGA loaded FTY720 (1:200, drug: polymer). X-rays were taken at weeks 2, 4, 6 and 8 after treatment. MicroCT imaging and Mason's trichrome histology was done at week 8. GFP staining of the graft region (ongoing) at week 8 allows for the identification of host cell integration in the graft.

Results: Crystal violet staining cells (**Figure 1**) after incubation for two weeks showed no marked differences in the colony forming ability of eGFP-/+ SD rats and eGFP -/- SD rats. The ability of these cells to mineralize was measured via alizarin red staining. The cells isolated from eGFP+/- rats had slightly better differentiation capability than those from eGFP-/- animals.

eGFP rat chimeras (1005 survival rate) were created with cells from eGFP+/- animals and showed ~50% chimerism by week 4 as measured by flow cytometry (**Figure 2**). Critical sized defects were created in the tibia of the chimeric rats at week 8 after they had achieved complete reconstitution. Treatment with FTY720 coated tibial allografts resulted in good graft-host integration as discerned from the bi-weekly x-ray images (**Figure 3**) and Mason's trichrome staining (data not shown here). GFP staining is currently underway to measure the contribution of host bone marrow cells towards allograft integration and bone regeneration at the defect site.

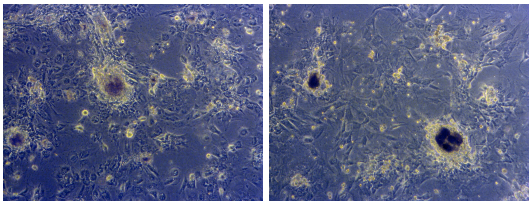

Figure 1: Crystal violet staining shows that the colony forming capability of the bone marrow mesenchymal stem cells is not affected after breeding WT SD and eGFP rats.

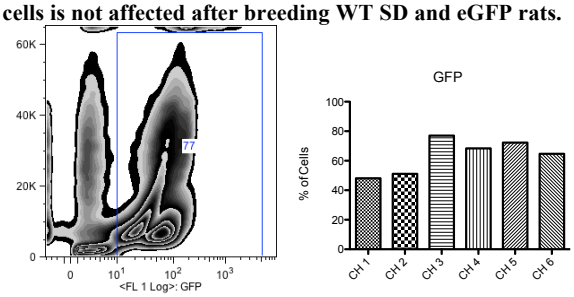

Figure 2: eGFP rat chimeras (CH) created from breeding WT SD and eGFP rats show ~50% chimerism in their peripheral blood as early as 4 weeks after creation as measured by flow cytometry. (A) Gating strategy. (B) The % of GFP+ cells for 6 different chimeras at week 4.

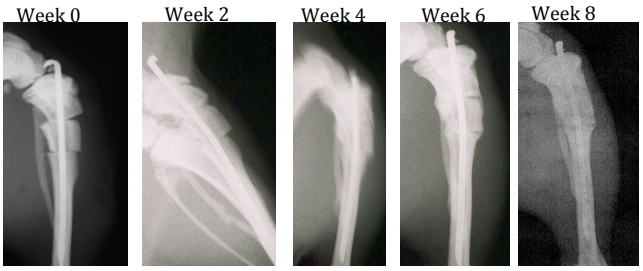

Figure 3: X-ray of the defect region after treatment with FTY720 coated allograft taken biweekly.

Conclusions: In this study, a small molecule drug delivery therapy is being utilized to provide local, sustained cues for endothelial, mural, inflammatory, osteoblastic, and stem cells aimed to improve allograft integration while healing critical size skeletal injury in eGFP chimeric rats. A tibial defect study in the eGFP rat chimera model will allow exploration of mechanisms during bone healing.

References: [1]Delloye C et al. *J Bone Joint Surg Br* 2007; 89:574 -579. [2]Enneking WF et al. *J Bone Joint Surg Am*. 2001; 83-A: 971-986. [3]Mankin HJ et al. *Clin Orthop Relat Res*. 2005; 432:210-216. [4]Thompson RC, et al. *Joint Surg Am*. 1993; 75:1663-1673.