A Controlled Delivery Method to Localize Stem Cells *in Vivo*

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Statement of Purpose: One notable disadvantage of cell based therapies is that the injected cells tend to disperse away from the intended site (1). The aim of this project was to develop a system of injectable hydrogels to deliver stem cells for the purpose of tissue regeneration, thereby allowing the cells to remain at the area of injury, in order to proliferate and secrete soluble factors that will facilitate tissue regeneration. The hydrogel employed is alginate; however; it does not readily degrade *in vivo* for six months. We previously demonstrated the controlled release of viable cells from degradable alginate microbeads via alginate-lyase mediated degradation *in vitro*. In addition we showed that microbeads were able to degrade *in vivo* and that their by-products did not elicit an inflammatory response (Fig. 1) (2). In this study, we investigated the ability of the degradable alginate microbeads to localize cells at the delivery site *in vivo*.

Methods: ASCs were isolated from inguinal fat pads of green fluorescent protein positive Sprague-Dawley rats. First passage GFP-rASCs were plated at 5000 cells/cm^2 and labeled with XenoLight DiR fluorescent dye. The optimal dye concentration for labeling GFP-rASCs was determined over a two week period. Labeled GFP-rASCs were microencapsulated (10x10^6 cells/ml alginate) in low viscosity, high mannuronate (LVM) alginate in 75mM calcium crosslinker solution containing glucose as an osmolyte. Degradable alginate microbeads were made by combining equal volumes of LVM alginate and alginate-lyase solution to form alginate microbeads of various ratios of alginate-lyase to alginate (1.75 U/g to 0.06 U/g). 100µl of microencapsulated cells were injected into the right gastrocnemius and left deltoid of athymic nude mice for 2 weeks. Cell suspensions of labeled cells were also injected as a control. The migration of the cells away from the initial site was followed with the aid of the Maestro 2 Imaging System by taking images on days 0, 1, 2, 4, 6, 8, 10, 12 and 14. On the final day animals were euthanized and organs imaged to identify the presence of labeled GFP-rASCs. The fluorescence was quantified and normalized to the fluorescence measured on day 0 for each animal's right gastrocnemius and left deltoid. Statistical significance was determined by multi-way ANOVA with Bonferroni's post test (n=4, per variable).

Results: The fluorescence of the labeled GFP cells that were delivered as a suspension significantly decreased at by the end of the two week period indicating cell migration away from the delivery site. These cells seemed to have migrated to inguinal lymph node and spleen of the animals that received cell suspension. Cells delivered using alginate microbeads (0U/g, 0.06U/g, 0.22U/g, and 1.75U/g) remained at the delivery site over the two week period.

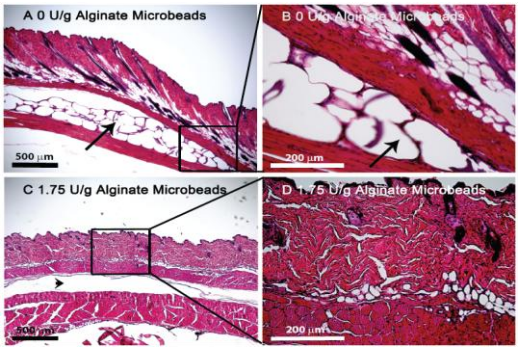

Figure 1 Figure 1: H&E staining of subcutaneous site of alginate microbead implantation after 2 weeks: (A,B) alginate microbeads (0 U/g), arrows indicate alginate microbeads; and (C,D) alginate microbeads incorporating alginate-lyase (1.75 U/g), the arrow head indicates the implantation site. (2)

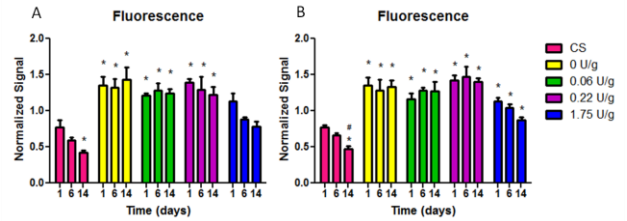

Figure 2. Normalized fluorescence of cells delivered *in vivo* over 2 weeks in (A) left deltoid and (B) right gastrocnemius. (CS-cell suspension) *p < 0.05 v. CS or day 1, # v. day 6.

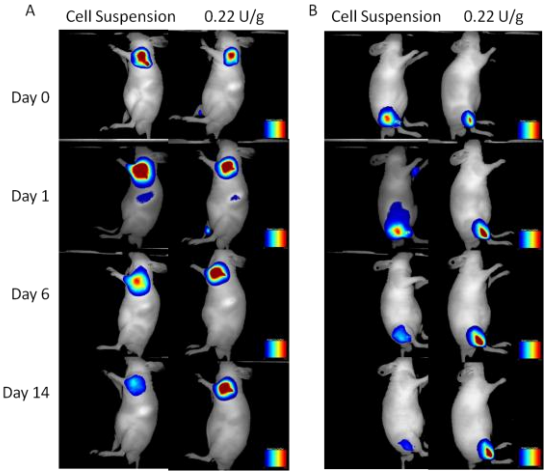

Figure 3. Fluorescent images indicating the location of the delivered cells in (A) left deltoid and (B) right gastrocnemius on days 0, 1, 6, and 14.

Conclusions: Cells delivered with degradable alginate microbeads remained at the injected site.

References:

1. Li F, Stem Cells 2007;25:3183-93
2. Leslie. S. Biomaterials 2013;34(33): 8172-84