

## Background

The overall goal of this research is to develop an implementation strategy for overcoming barriers to the widespread use of wound healing treatments for pressure ulcers, a secondary complication in the spinal cord injured (SCI). Specifically this requires reducing recovery time while at the same time making the treatment accessible in a home-health environment. This project is a step in the process to develop the optimized wound healing treatment.

Currently two treatment options exist for SCI patients with pressure ulcers: non-surgical treatment and six months bedrest, or surgery and six weeks bedrest. Though surgery results in less bedrest, the rate of recurrence and cost is high [1]. In order to overcome the limitations preventing widespread use of non-surgical treatments, bedrest time must be reduced to match that of surgery; approximately double the current healing rate.

In order to develop a clinically viable non-surgical treatment, this project combines three experimental approaches: cellular, growth factor, and scaffold based treatments. Mesenchymal stem cells (MSCs) were used as a vehicle for growth factor delivery as well as a means of alleviating the cellular deficit associated with non-healing wounds. TGF-β3 was overexpressed in MSCs using an adenovirus to promote regenerative healing and improve ECM production. Albumin was crosslinked with modified poly(ethylene-glycol) (PEG-SG) to produce a tissue adhesive scaffold and provide a provisional matrix within the wound bed. These three approaches produced positive results alone, but not enough to be clinically significant. We hypothesize the treatments will work synergistically producing clinically significant results.

## Methods

The vectors used in this study are adenoviruses containing cytomegalo virus (CMV) promoter stably transfected with the genetic sequence for TGFβ-3. The efficiency of adenovirus infection is dependent on coxsackie-adenovirus -receptor, which is present in low amounts on the surface of MSCs. To compensate for this, the fiber sequence was also altered in the vector AdCMVpK7RGDTGFβ3 to express 7 lysine residues (pK7) followed by arg-gly-asp (RGD) giving the virus a greater ability to bind to the cell surface and infect the cell (Figure 1). The wild type vector, AdCMVTGFβ3, was also tested. Both assays described below were performed 3 separate times (n=3).

### Vector Testing

### Coculture using Mink Lung Epithelial Cells

MSCs were cocultured with mink lung epithelial cells (MLECs) (provided by Daniel Rifkin, PhD, New York University) that were permanently transfected with the construct p800neoLUC fused to the human plasminogen activator inhibitor-1 (PAI-1) gene as previously described [2]. PAI-1 is regulated positively by activation of the TGFβ pathway, enabling indirect detection of TGFβ-3 activity via luciferase activity. Briefly, MSCs were plated in a 24 well plate at a seeding density of 2x10<sup>5</sup> cells/well and allowed to adhere for 24 hours. Cells were then infected with AdCMVTGFβ3 or AdCMVRGDpK7TGFβ3 at 5000, 1000, 100, 10, or 0 MOI. 24 hours post infection, MSCs were trypsinized and seeded in

duplicate at 1x10<sup>5</sup> cells/well in a separate 24 well plate. MLECs were then immediately seeded with the MSCs at a density of 1x10<sup>5</sup> cells/well. 24 hours after starting the coculture the media was removed, each well washed with PBS twice, 100µl cell lysate was applied, and a luciferase assay was performed.

### Albumin Scaffold Preparation

Albumin scaffolds were prepared by mixing a buffered difunctional PEG-SG solution with lyophilized rabbit albumin dissolved in 0.85% NaCl H<sub>2</sub>O using a dual syringe system. Unfunctionalized PEG (200-300 Mw, or ~1000 Mw) was added to the PEG solution, which dissolved out ~24 hours post mixing creating a porous structure. Mixed solutions solidified within 5 minutes. 25/15, 25/10, and 30/10 albumin/PEG ratios were prepared.

### Mechanical Testing

Ultimate tensile strength of albumin scaffolds was measured using an MTS axial loading machine until fracture. Fibrin, an FDA approved tissue sealant, was used as a control. Data was analyze using Microsoft Excel.

## Results

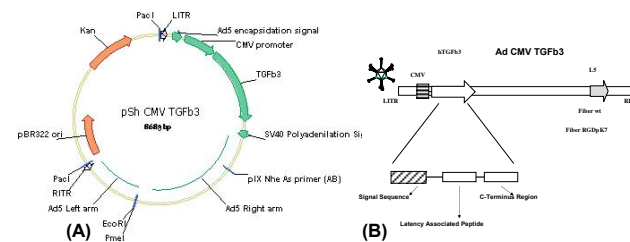


Figure 1. (A) Schematic of the shuttle vector used during the cloning process. (B) Schematic of the vector backbone used.

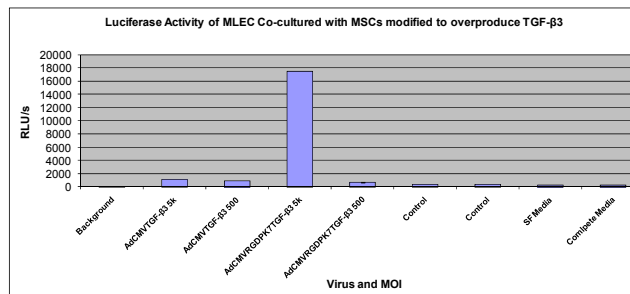


Figure 2. Luciferase activity of MLECs cocultured with MSCs infected with viruses causing overexpression of TGFβ-3. AdCMVRGDpK7TGFβ3 produced the most RLU/s, which is directly proportional to TGFβ-3 binding to TGFβ receptors, indicating that AdCMVRGDpK7TGFβ3 at 5000 MOI caused the most TGFβ-3 expression.

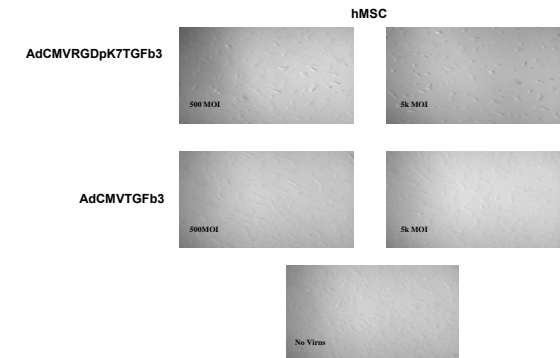


Figure 4. Human MSCs cells 24 hours post infection. Note the morphological differences present between the 5000 (5k) MOI and no virus.

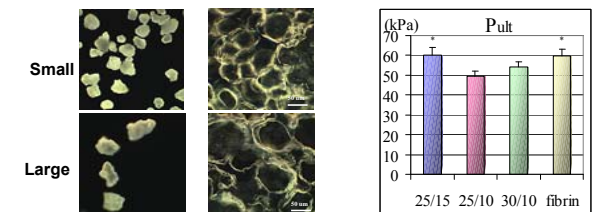


Figure 5. (A) Stereoscopic image of porous albumin scaffold produced by (top) small unfunctionalized PEG and (bottom) large unfunctionalized PEG. Pore sizes ranged from 50-200µm. (B) Mechanical testing of varied albumin/peg ratios compared to fibrin. Statistical significance occurred only between fibrin and 25/10 ratio and 25/15 and 25/10.

## Conclusions

- Adenovirus modified with the gene sequence for TGFβ-3 is capable of infecting MSCs
- AdCMVRGDpK7TGFβ3 infects cells with greater efficiency than AdCMVTGFβ3
- Greater sample number is needed
- Highly porous albumin scaffolds are obtainable
- Tensile strength is comparable to fibrin
- Investigation of interactions between MSCs infected with AdCMVRGDpK7TGFβ-3 and albumin scaffold are needed
- Before development of a clinical system *in vivo* optimization is needed

## References

1. Mast, B., 1992 The skin, in Wound Healing: Biochemical & Clinical Aspects, ed. by Cohen I., Diegelman, R., and Lindblad, A., W. B. Saunders, Philadelphia, pp. 344-355.
2. Jurukovski, et al. Methods for Measuring TGFβ3 Using Antibodies, Cells, and Mice. Meth Mol Med. 117 161-175. 2005.