Nanoparticle-mediated gene transfer modulating equine corneal wound repair

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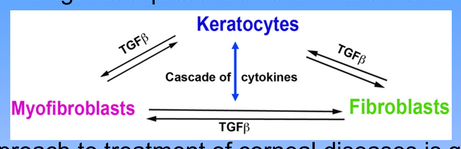
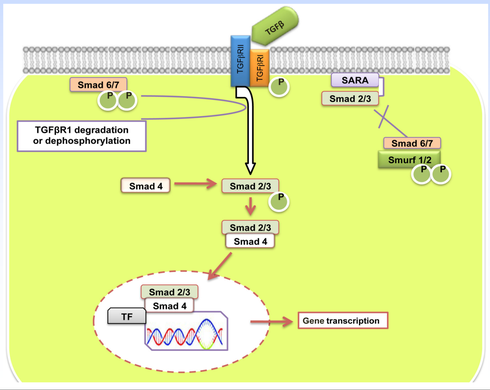
Introduction

Corneal diseases and injuries commonly lead to fibrosis and subsequent loss of transparency. During fibrosis corneal fibroblasts transform into myofibroblasts under the influence of TGFβ1 signaling and begin to express α smooth muscle actin (SMA).



A novel approach to treatment of corneal diseases is gene therapy based on genes involved in TGFβ1 signaling. This study focuses on one of the anti-fibrotic genes, Smad7. We delivered Smad7 gene into corneal fibroblasts (ECF) with PEI nanoparticles and examined its effects on equine fibrosis treatment by measuring fibrotic changes.



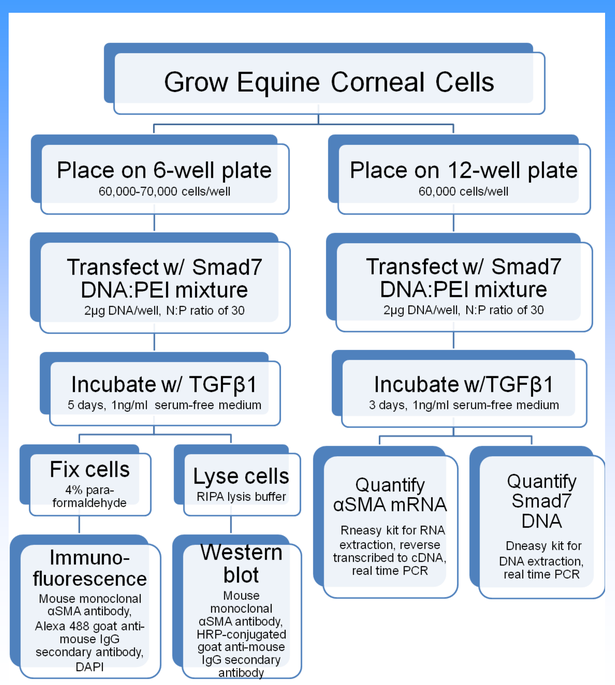
Objectives and Hypothesis

Hypothesis: *In vitro* delivery of Smad7 gene into equine corneal fibroblasts using PEI nanoparticles will lead to decreased α-smooth muscle actin expression.

Objectives:

1. To optimize gene delivery parameters for delivering anti-fibrotic Smad7 gene in ECF using an *in vitro* model.
2. To characterize changes in expression of α-smooth muscle actin (SMA), a fibrosis biomarker, following Smad7 delivery.

Methods



Results

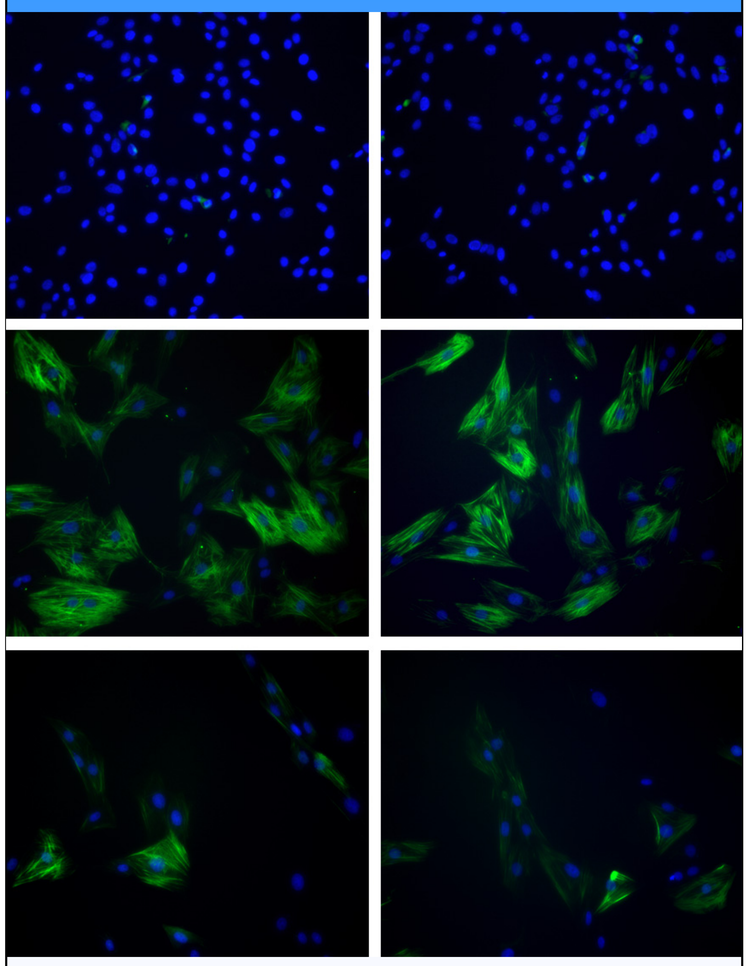


Fig 1: Representative immunocytochemistry images showing levels of αSMA in non-treated (A, B), TGFβ1 transformed (C, D) and TGFβ1 transformed following Smad7 transfection (E, F) corneal fibroblasts. Nuclei are stained blue with DAPI. Fibroblasts transfected with Smad7 (E, F) showed lowered αSMA expression and a significant decrease in the number of SMA positive cells compared to cells treated with TGFβ1 without transfection (C, D).

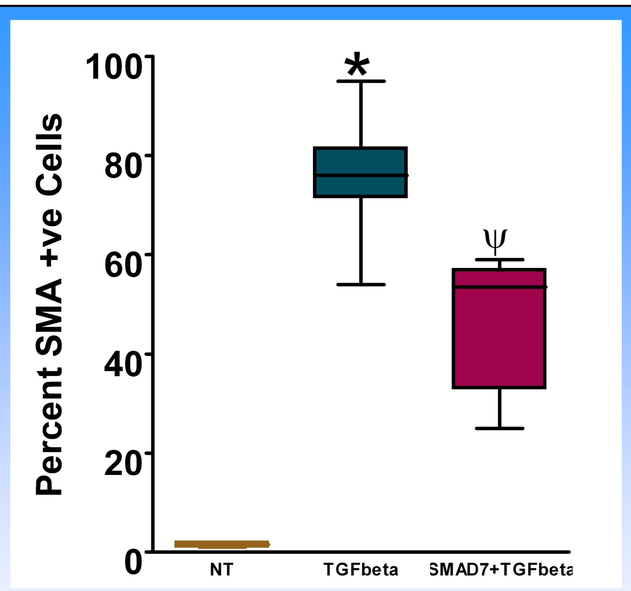


Fig 2: Quantification of αSMA-positive cells in non-treated, TGFβ1 transformed, and TGFβ1 transformed following transfection with Smad7 fibroblasts. Treatment with Smad7 caused a significant decrease in the percentage of αSMA-positive cells.

* P<0.001

Ψ P<0.001

One-way ANOVA and Tukey's multiple comparison test were used for statistical analysis.

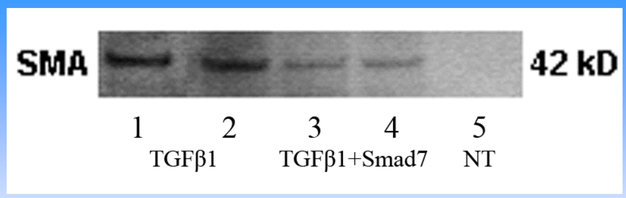


Fig 3: Western blotting comparing levels of SMA in ECFs treated with TGFβ1 (Lanes 1, 2), TGFβ1 and Smad7 gene transfer (3, 4), and non-treated (5). Non-treated cells have non-detectable levels of SMA, and the weaker bands in lanes 3 and 4, compared to lanes 1 and 2 represent a decrease in SMA expression after Smad7 gene therapy.

Ongoing and Future Studies

1. An ongoing study will quantify the gene copy number of Smad7 gene in ECFs 3 days following transfection to determine the efficiency of gene transfer.
2. Another ongoing study will quantify and compare the levels of SMA and wound healing genes (collagen, fibronectin, MMPs etc.) mRNA in Smad7-transfected and non-transfected ECFs gowin +/- of TGFβ1.
3. A future immunofluorescence study will improve on the previous experiment (Fig. 1) by measuring the levels of fluorescence to quantify the decrease in SMA expression following Smad7 gene transfer.

Conclusions

- PEI nanoparticles are potent vector for gene transfer into ECFs.
- Transfection of ECFs with Smad7 gene decreases SMA expression in the presence of TGFβ1 reflects decreased transformation of ECFs into equine corneal myofibroblasts.
- Gene therapy based on anti-fibrotic genes such as Smad7 has potential to inhibit or prevent fibrosis in equine cornea.
- Toxicity studies are warranted.

Acknowledgements

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