Trabecular Meshwork Engineering and Live Tracking in Perfused Porcine Anterior Segments

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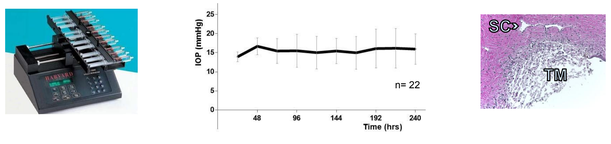
Purpose

To establish a trabecular meshwork (TM) engineering model using porcine anterior segments of consistent high quality in a physiological, fixed perfusion system.

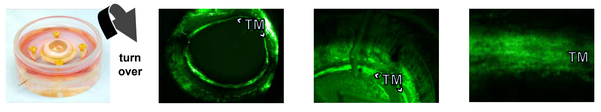
Methods

Within 4 hours of death, porcine anterior segments were mounted after removal of lens and uvea. (1) Baseline parameters were determined in 22 eyes with constant flow rate (CF) and compared to 8 gravity perfused eyes at constant pressure (CP) of 15 mmHg. (2) Gene delivery targeted to the TM was established using lentiviral FIV vectors expressing eGFP (GREEN, n=8). Additional eyes (ABLATED, n=8) were transduced with a conditionally cytotoxic (ganciclovir) eGFP FIV vector. Effects of targeted TM ablation were compared to those of non-selective cell lysis with saponin (n=8). (3) TMs were seeded with either fibroblasts or adipose tissue derived stem cells that had been permanently labeled with an FIV vector and enriched by flow cytometry. eGFP was visualized through the bottom of a culture dish. Eyes were analyzed histologically at the conclusion of experiments.

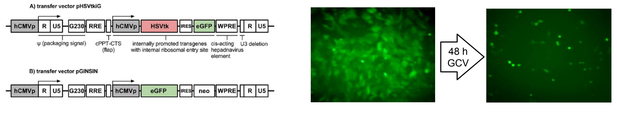
Porcine Anterior Chamber Perfusion System



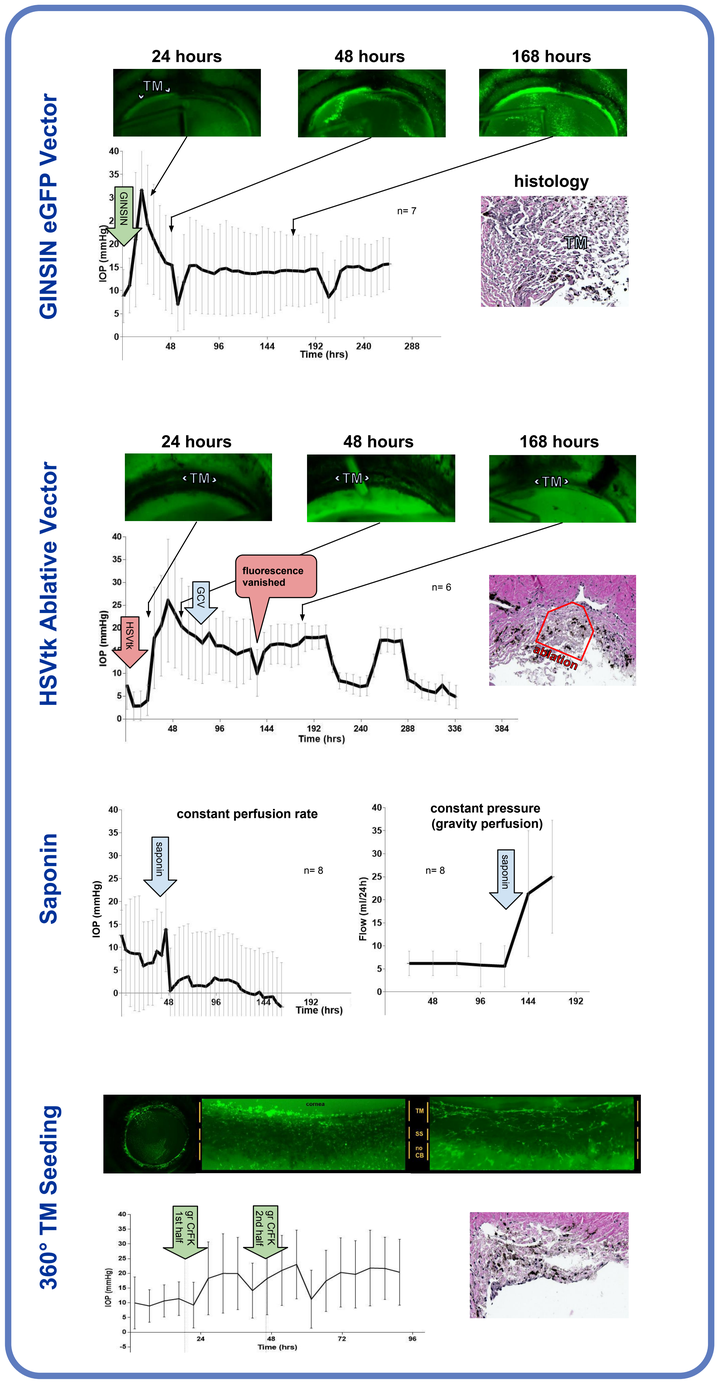
Direct Visualization through Bottom of Redesigned Chamber



Inducible Cytoablation with Herpes Simplex Thymidine Kinase and Ganciclovir



Results



Porcine eyes were a reliable source for consistent and high quality anterior segment cultures with a low failure rate of 12%. (1) CO had a physiological IOP of 15.8±1.9 mmHg at fixed pump perfusion with 3 microliters/min compared to gravity perfused COG with imputed 3.7 ±1.6 microliters/min. (2) Some GREEN and ABLATED experienced a transient post-transduction IOP increase by 44% that resolved at 48h. Ganciclovir in ABLATED reduced IOP by 32% (3.2 fold facility increase) compared to a 20 fold increase of facility with 0.01% saponin. (3) Fibroblasts and adipose tissue derived stem cells populated the TM and exposed sclera but not the corneal endothelium. Increase in cell density ceased at 3 days. Histology indicated that compared to prior to ablation, transduction alone did not change TM cellularity. Cells seeded into TM were seen in the corneo- and uveoscleral TM.

Discussion

Compared to previously used human donor eyes, this inexpensive porcine anterior segment perfusion model is of sufficient, repeatable high quality to develop strategies to genetically modify, ablate and repopulate the TM. Despite significant anatomic differences, effects of transduction and ablation in the porcine model presented here replicate the main aspects of previously explored human, feline and rodent models.

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