Development of rAAV technologies to facilitate topical gene delivery to the cornea

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Background

Intracameral administration of recombinant adeno-associated virus (**rAAV**) vector is known to be an effective method for delivering genetic material to cells of the anterior chamber, including corneal endothelium and trabecular meshwork, but problematically, the injection causes a transient spike in intraocular pressure (**IOP**) and increases the risk of developing post-operative complications that could exacerbate glaucoma progression, including endophthalmitis and uveitis. The high level of invasiveness, coupled with the potential for sudden vision loss in an otherwise slowly progressive disease due to a severe adverse reaction, poses a significant barrier to the translation of any primary open angle glaucoma gene therapy.

The objective of this Shaffer Award supported project was to establish whether topical application of rAAV is able to effectively transduce cells of the cornea. As rAAV vectors administered directly to the corneal surface are swiftly eluted from the tear film before they have the opportunity to bind the epithelium, resulting an absence of transduction, we hypothesized that delivery of rAAV immobilized on the anterior surface of a custom contact lens would increase retention time and consequently improve transduction efficiency.

Methods

rAAV contact lens deposition
7μl rAAV vector (1x10^{12} vector genomes (vg)/ml) was pipetted into the anterior surface of a 3mm aclar contact lens (Ocuscience, Henderson, NV) and either dried at room temperature overnight in a desiccating cabinet (SPI supplies) or freeze dried in a lyophilizier (Virtis Freezemobile 12XL, SP Scientific, Warminster, PA) at -80C for 20 minutes. All contact lenses were used within 24 hours of preparation and were stored at 4ºC.

rAAV vector elution and infectivity assay
Preparations of rAAV vector expressing green fluorescent protein (GFP) from a ubiquitous promoter (chicken beta actin) were manufactured in multiple serotypes (rAAV2/1, rAAV2/2, rAAV2/6, rAAV2/9 and rAAV2/2[MAX]) via triple transfection in HEK293T cells followed by iodixanol gradient certification and buffer exchange, with the titre calculated using a picogreen assay, as described previously.[1-2] Following desiccation/lyophilization onto the contact lens, it was placed in 100μl Hank's buffered saline solution (HBSS) and the amount of vector recovered compared to 100μl HBSS spiked with 7μl rAAV vector (1x10^{12} vg)/ml from the same preparation using a picogreen assay. To determine infectivity, HEK293T cells were seeded in 6-well plates and dosed with either a rAAV coated contact lens or an equivalent volume of free rAAV, with infectivity assessed qualitatively via fluorescence microscopy.

Ex vivo corneal culture
Corneas were dissected post-mortem from C57BL/6J mice and placed directly into 24-well transwell inserts (BD Falcon) containing 700μl media and stored at 4ºC and cultured for up to 1 month. Purified rAAV vectors (~7x10^{11} vg/well) were added directly to the media and incubated for a period of 14 days before the tissue was fixed in 4% paraformaldehyde (PFA) for 24ºC for 24 hours before being flat mounted and GFP expression assessed via confocal microscopy (Nikon Eclipse, Japan).

In vivo corneal transduction assay
C57Bl/6j mice (N=3-5 per serotype underwent baseline reflectance and fluorescent imaging of the cornea using a custom multiline spectralis confocal scanning laser ophthalmoscope (cSLO; Heidelberg Engineering, Germany). rAAV coated contact lenses were places on the corneas of each mice whilst under isoflurane (2% in oxygen) anesthesia for a period of up to 30 minutes prior to the contact lens being removed. cSLO imaging was repeated in all animals four weeks post application to assess for signs of GFP fluorescence within cells of the cornea.

References

1 Reid, C.A. & Lipinski, D.M. (2018) **'Small and micro-scale recombinant adeno-associated virus production and purification for ocular gene therapy applications.'** Methods Mol. Bio. 1715:19-31

2 Piedra, J., Ontiveros, M., Miravet, S., Penalva, C., Monfar, M. & Chillon, M. (2015) **'Development of a rapid robust and universal picogreen-based method to titre adeno-associated vectors.'** Hum. Gene. Ther. Methods. 26(1):35-42

Results

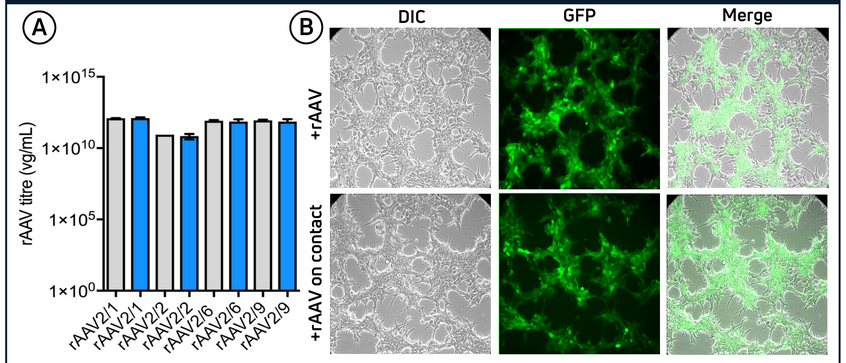

Figure 1 – rAAV can be dried onto contact lenses without loss of titre or infectivity. (A) ~7x10^9 vg of purified rAAV vector of several serotype (rAAV2/1, 2/2, 2/6 and 2/9) was desiccated overnight on the anterior surface of mouse aclar contact lenses (n=3 per group) before being eluted and the amount of vector recovered quantified via picogreen assay. There was no significant decrease in viral titre for any serotype evaluated compared following elution, indicating that desiccated vector can be recovered with high efficiency. **(B)** Representative images of HEK293T cells administered either rAAV directly to the media (top row) or desiccated on an aclar contact lens placed in the media (bottom row) indicates that eluted vector remains capable of transduction, resulting in equivalent levels of GFP expression in both groups.

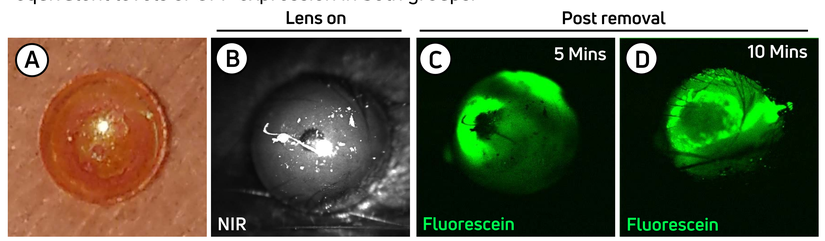

Figure 2 – Materials desiccated onto contact lenses transfer efficiently to the cornea and are retained for several minutes. (A) Fluorescein desiccated overnight on the anterior surface of a mouse-sized aclar contact lens prior to application. **(B)** Near infrared reflectance (NIR) image of the anterior chamber with the lens positioned over the central cornea. After a period of 30 minutes the contact lens was removed and the eye imaged using cSLO imaging with a 486nm excitation laser (emission: 502 – 537nm) revealing widespread fluorescence 5- **(C)** and 10–minutes **(D)** post-removal, indicating retention of fluorescein. No adverse effects (e.g. abrasions, ulceration) were observed following long-term contact lens placement and removal

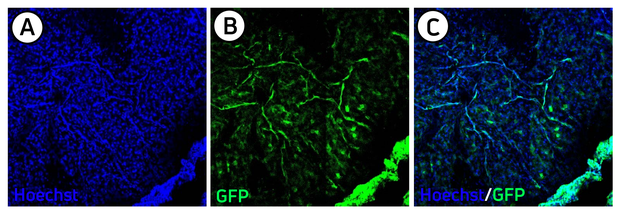

Figure 3 – rAAV[MAX] administered ex vivo to corneal explant cultures results in widespread GFP expression. 14 days post-administration of rAAV2/2[MAX], a capsid mutant vector with strong tropism for neuronal cells, explanted corneal tissue was fixed and imaged using a confocal microscope. GFP expression was observed throughout the tissue, but appeared to be restricted to corneal nerves. The tissue was counted stained with Hoechst to highlight the nuclei.

Section Title

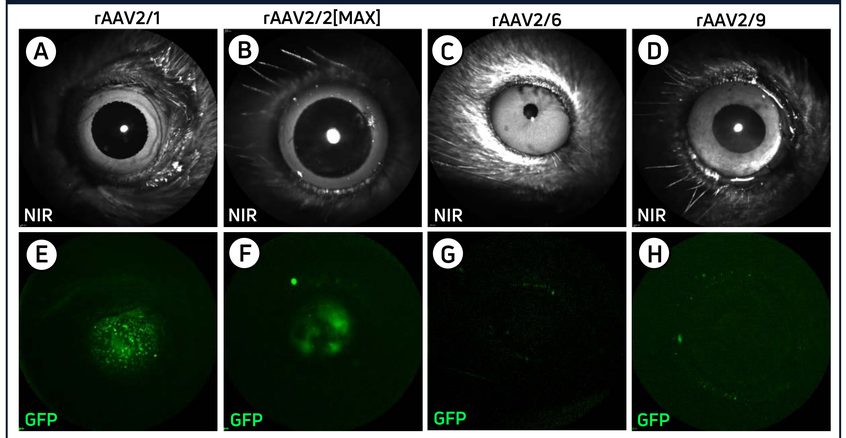

Figure 4 – Contact lens-mediated topical delivery of rAAV results in GFP expression within the cornea in a serotype dependent manner. Near infrared reflectance (NIR) **(A-D)** and in vivo fluorescence **(E-H)** image of the anterior chamber of C57Bl/6j mice four weeks post-application of a contact lenses containing desiccated rAAV2/1, rAAV2/2[MAX], rAAV2/6 or rAAV2/9 vector (~7x10^9 vg/lens). GFP signal was observed in eyes receiving rAAV2/1 **(E)** and rAAV2/2[MAX] **(F)**, but interestingly not in rAAV2/6 **(G)** or rAAV2/9 **(H)**, indicating that increasing retention time is not the only factor limiting topical transduction of the cornea by rAAV.

Conclusions

- rAAV can be easily immobilized on to the anterior surface of an aclar contact lens either through drying at room temperature over night (desiccation) or drying rapidly at cold temperatures (lyophilization).
- Immobilized rAAV vector is recoverable via elution and does not demonstrate any reduction in infectivity compared to vector that has not been immobilized.
- Contact lens placement on the corneal surface for 30 minutes is well tolerated and resulted in increased retention of fluorescein compared to dropwise application that was visible up to 10 minutes following removal.
- Explanted corneal tissue could be maintained over a period of weeks and may serve as a useful screening tool for evaluating the effectiveness of rAAV vector transduction.
- Contact lens-mediated delivery of immobilized rAAV to the corneal surface of C57Bl/6j mice resulted in observable GFP expression in rAAV2/1 and rAAV2/2[MAX], but not rAAV2/6 or rAAV2/9 treated corneas, indicating that increasing retention time is sufficient to increase transduction efficiency following topical delivery, but that serotype selection is also important.
- Critically, using a contact lens-based delivery strategy, gene delivery was achieved without abrading, scarifying or otherwise damaging the corneal surface or tear film in order to faciality viral entry into the cornea.

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