# Development of Hydrogel Implants for the Sustained Delivery of Adeno-Associated Viruses in Ocular Gene Therapy

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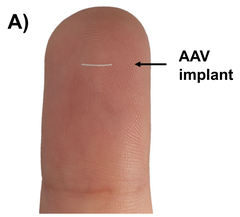
## BACKGROUND

- Ocular gene therapy studies have demonstrated dose-dependent inflammation that can reduce efficacy and lead to dose-limiting toxicity[1-4]
- A sustained-release modality of AAVs in the eye could maintain lower vector concentration over time, leading to reduced inflammation and improved safety outcomes[5], while still providing a high total dose
- In the present work, we evaluated a novel, hydrogel-based, biodegradable implant for sustained release of encapsulated AAVs **(Figure A)**
  - AAV implants are small, solid rods designed to be injected through a needle into the vitreous
  - Designed to deliver AAV for up to 1 month




## PURPOSE

**1) To assess the compatibility of AAVs following hydrogel processing**

**2) To assess the feasibility for AAV implants to transduce in vivo in a rat model**

## METHODS

### Cell Infectivity Assay

- Serotypes commonly used in ocular gene therapies were lyophilized and mixed with hydrogel precursors to simulate gel encapsulation conditions and compared to stock AAV solutions as a (+) control
- AAV2-, AAV2.7m8-, and AAV8-CMV-eGFP were cultured with HEK293T cells at an MOI of 1E+5, 1E+4, and 1E+6, respectively, for 48 hours
- To assess AAV infectivity, % GFP+ cells and MFI were measured via flow cytometry

### In Vitro Release Kinetics

- Gold nanoparticles (AuNPs) (26-30nm) were utilized as surrogates to screen hydrogel formulations due to a similar size to AAVs (25nm)
- Degradation rate of hydrogel implants were modulated to tune AuNP release kinetics
- Implants were submersed in PBS (pH 7.2) and sampled over 28 days to assess release via UV/Vis (AuNP) or an ELISA kit (AAV)

### Rodent Study Design

- Sprague-Dawley rat eyes were injected bilaterally with 10uL bolus of AAV2-CMV-Luc or with a single AAV implant (**Table 1**)
- Luminescence intensity was measured over 1 month using an In Vivo Imaging System

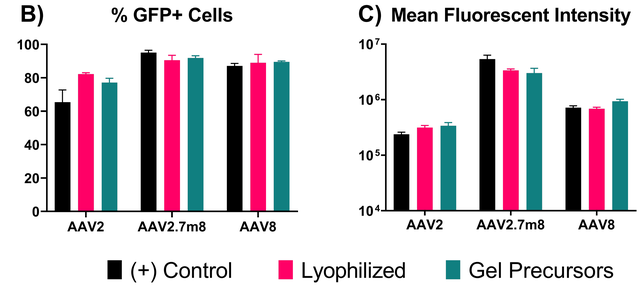
**Table 1. Experimental Groups**

| Group | Dose (GC/eye) | Number of Animals |
|---|---|---|
| AAV Bolus – Low Dose | 2.5E+09 | N=3 |
| AAV Bolus – High Dose | 1.2E+10 | N=3 |
| AAV Implant – Low Dose | 6.2E+09 | N=4 |
| AAV Implant – High Dose | 2.5E+10 | N=4 |

## RESULTS

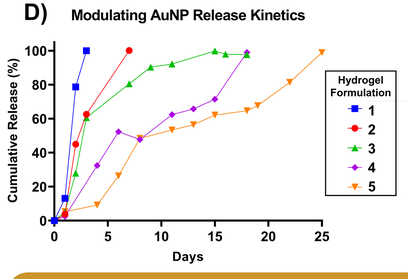
### AAVs Retained Infectivity Following Hydrogel Processing Conditions

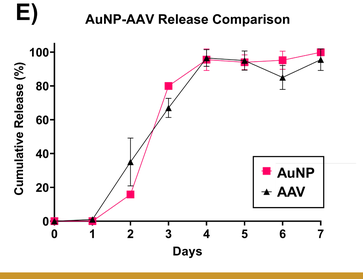
- Percentage of GFP+ cells after hydrogel processing conditions was comparable to (+) control for each AAV serotype **(Figure B)**
- MFI log difference from the (+) control was ≤0.25 for each serotype and processing condition demonstrating minimal change in AAV infectivity **(Figure C)**




### Release of AAVs from Hydrogel Implant Can Be Controlled Through Formulation Parameters

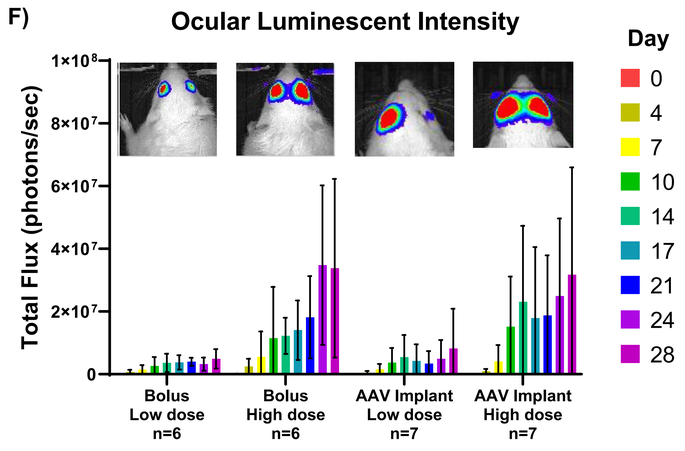
- AAV implant release kinetics was dictated by rate of hydrogel degradation and can be tuned so complete 100% release of AAV is achieved from 4 to 25 days **(Figure D)**
- In all AAV implants, initial burst of release at Day 1 was minimal
- Release profile of AAVs was comparable to AuNPs from the same hydrogel formulation **(Figure E)**



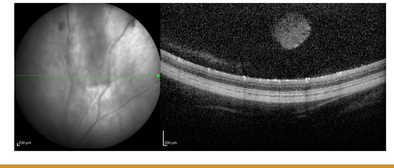




### Transduction in Rodent Eyes from AAV Hydrogel Implants was Comparable to AAV Bolus

- Luminescent intensity was comparable between AAV bolus and AAV implants at the same dose **(Figure F)**
- Larger animal models will be needed to better assess intravitreal AAV implants
  - Small size of rat eyes presented difficulty in accurate placement of AAV implants
  - Out of 8 eyes for AAV implants groups, implants were subconjunctival (n=1), subretinal (n=2), or intravitreal (n=5). Eyes with subconjunctival implants were excluded from analysis




### Post-dose Visualization of IVT Hydrogel Implant via OCT



## CONCLUSIONS

- **AAVs incorporated into hydrogels retained infectivity and were capable of transducing ocular tissue in vivo**
- **Release of AAVs from hydrogels can be controlled via rate of hydrogel degradation**
- **These data suggest that the use of a hydrogel platform for controlled delivery of AAVs in ocular gene therapy is feasible**

**See Oral Presentation "Reduced Ocular Inflammation and Improved GFP Expression in Rabbits with Controlled Release of AAV from Degradable Hydrogel implants" (Thu, May 19 | Abstract # 1232)**

**Disclosures:** All authors are employees of Ocular Therapeutix, Inc.

**Abbreviations:** AAV, Adeno-associated virus; AuNP, gold nanoparticle; CMV, cytomegalovirus; HEK293T, Human Embryonic Kidney; GC, genome copies; eGFP, enhanced green fluorescent protein; IVT, intravitreal; MFI, mean fluorescent intensity; MOI, multiplicity of infection; OCT, Optical Coherence Tomography;

**References: 1.** Cukras C, et al. *Mol Ther*. 2018;26:2282-2294. **2.** Timmers AM et al. *Human Gene Therapy*. 2020;31:80-89. **3.** Bainbridge JWB et al. *N Engl J Med*. 2015; 372:1887-1897. **4.** Dimopoulos IS et al. *Am J Ophthalmol*. 2018;193:130-142. **5.** Verdera HC et al. *Mol Ther*. 2020;28(3):723-746.

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