Electronic Supplementary Information

Synthesis and characterization of an injectable telechelic material for the epiretinal delivery of retinal gene therapies

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Table of Contents

1. Calibration Curves

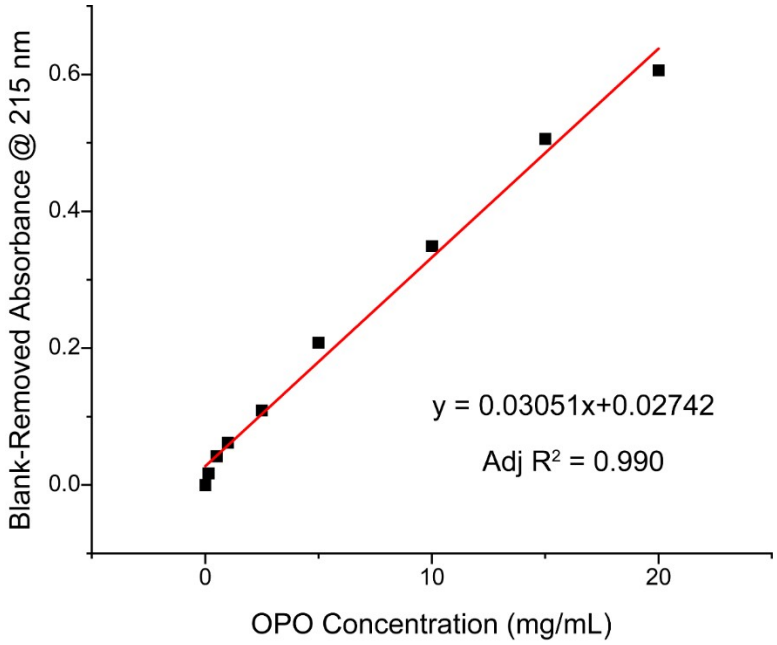

Figure S1. Calibration curve of absorbance at 215 nm vs. OPO concentration in 1X PBS. The software OriginLab was used to generate a linear fit.

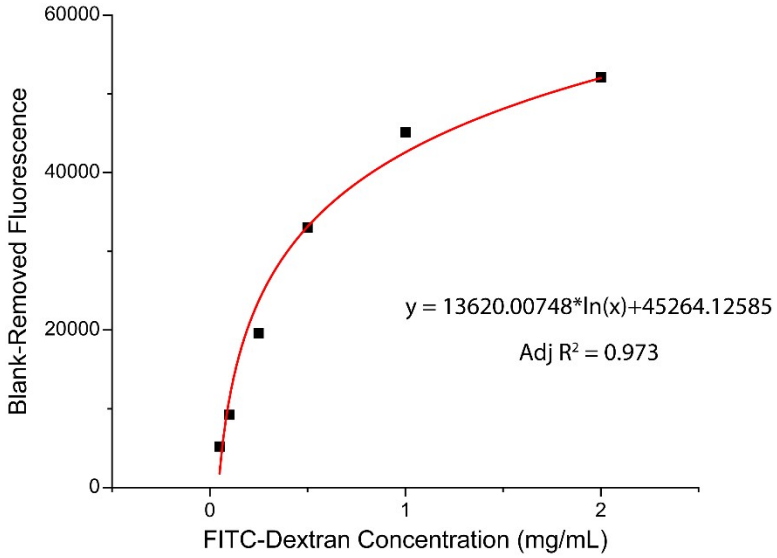

Figure S2. Calibration curve of fluorescence (ex = 490 nm, em = 520 nm) vs. FITC-dextran concentration in 1X PBS used in release assay studies. The software OriginLab was used to generate a logarithmic fit.

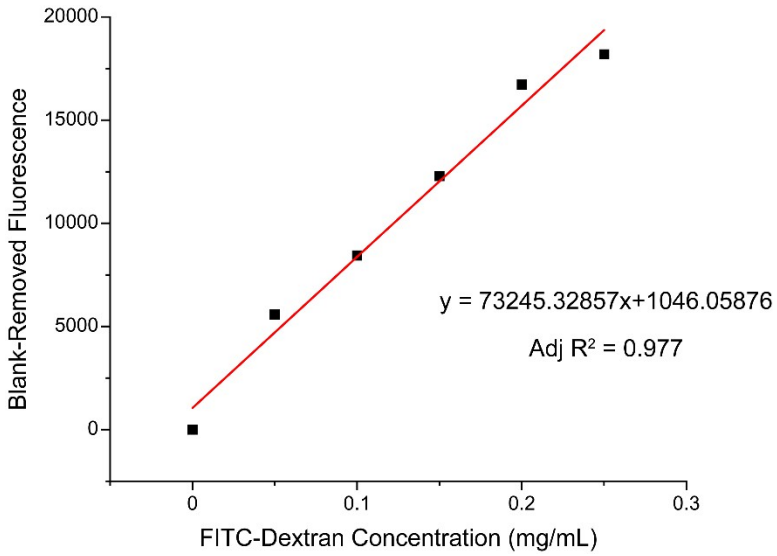

Figure S3. Calibration curve of fluorescence (ex = 490 nm, em = 520 nm) vs. FITC-dextran concentration in 1X PBS used in backing layer studies. The software OriginLab was used to generate a linear fit.

2. ^{1}H NMR Spectra

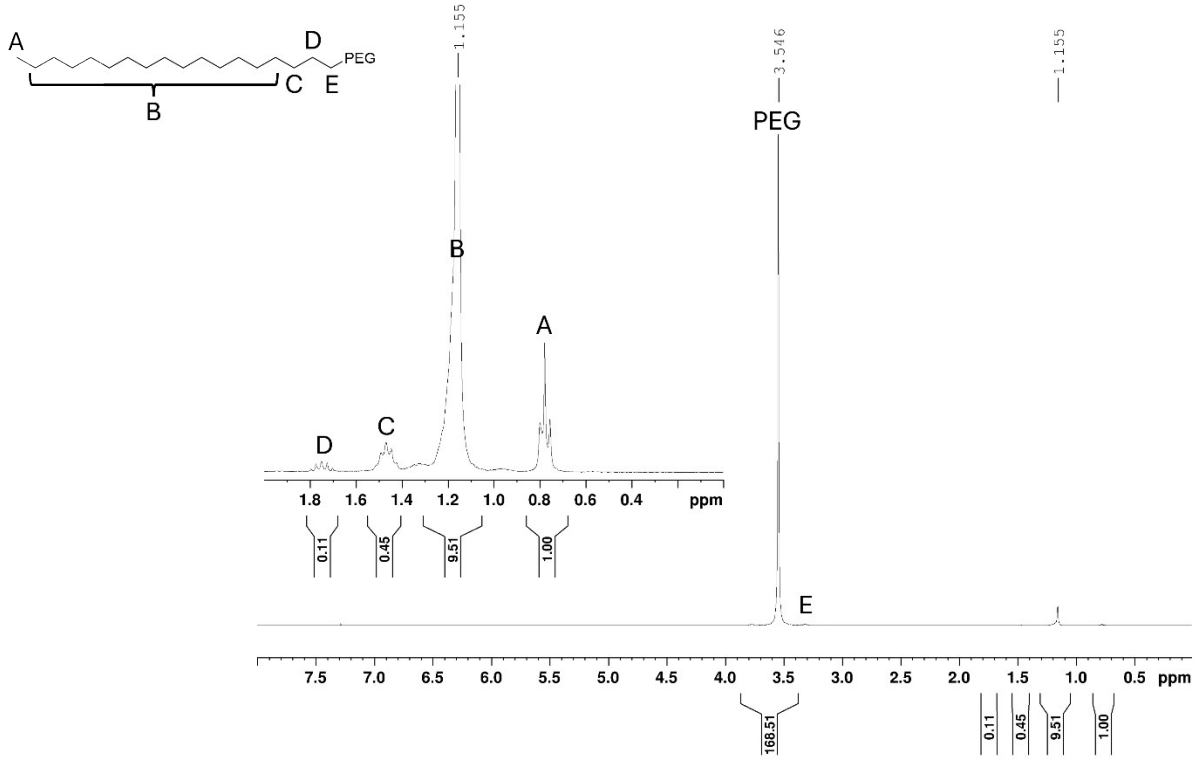

Figure S4. NMR spectra of OPO (~10 mg/mL in $CDCl_3$). M_n was estimated using peaks labeled “PEG” and “B”.

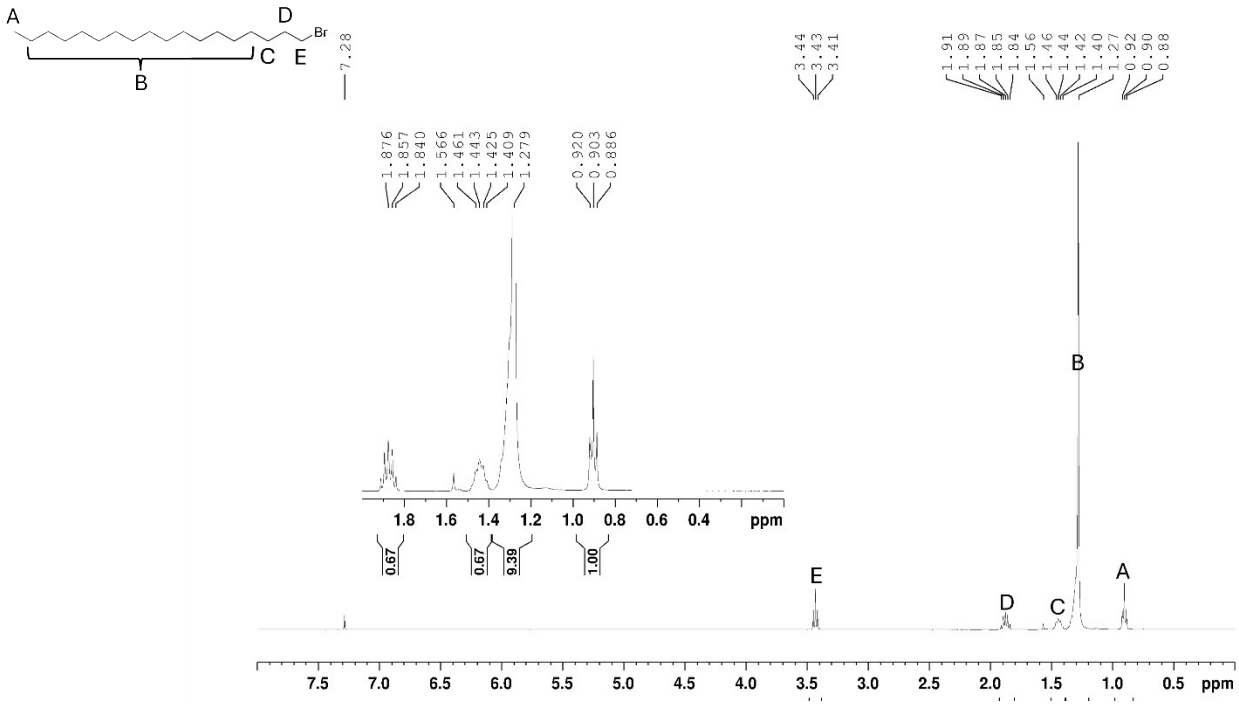

Figure S5. NMR spectra of 1-bromooctadecane (~10 mg/mL in $CDCl_3$).

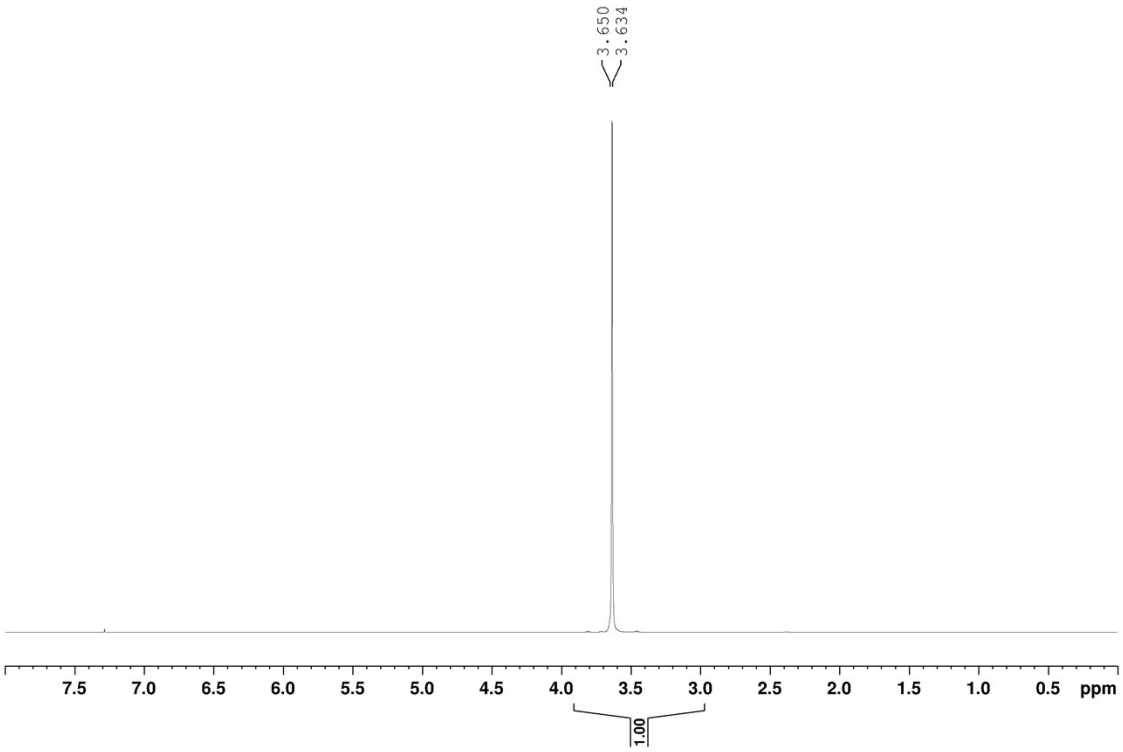

Figure S6. NMR spectra of poly(ethylene glycol) (10 kDa, ~10 mg/mL in $CDCl_3$).

3. SEC Chromatogram

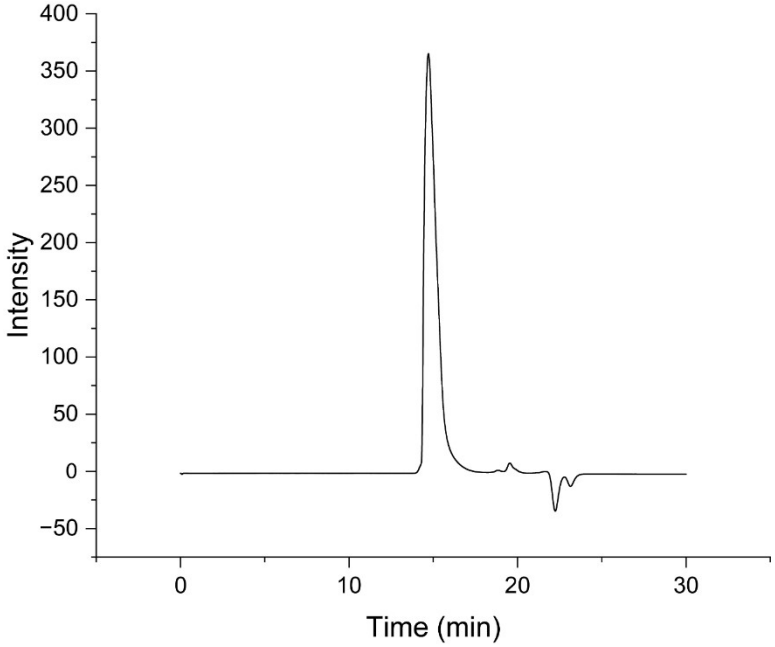

Figure S7. Size-exclusion chromatogram of OPO (10 mg/mL in THF).

4. MALDI-MS Spectrum and Discussion

Mass spectra were collected using a Bruker Daltonics UltrafleXtreme MALDI TOF-TOF spectrometer. Dihydroxybenzoic acid was used as the MALDI matrix, and NaCl as the salt solution. Samples were analysed in a 1:1:1 volume ratio of polymer (5 mg/mL in THF), matrix (11 mg/mL in water), and salt solution (10 mg/mL in water), respectively.

From the nominal mass of the expected product, we anticipated the measured molecular weight of our synthesized OPO to be ~11 kDa. However, the molecular weight measured by MALDI-MS was unexpectedly small (~2 kDa). We hypothesize that this discrepancy was caused by ineffective ionization of the large OPO polymers in the selected MALDI matrix. Nevertheless, we confirmed the presence of octadecane end groups and observed minimal changes in measurements between batches (<2% difference in measured molecular weights) that indicate our reported procedure can reproducibly synthesize the desired OPO product.

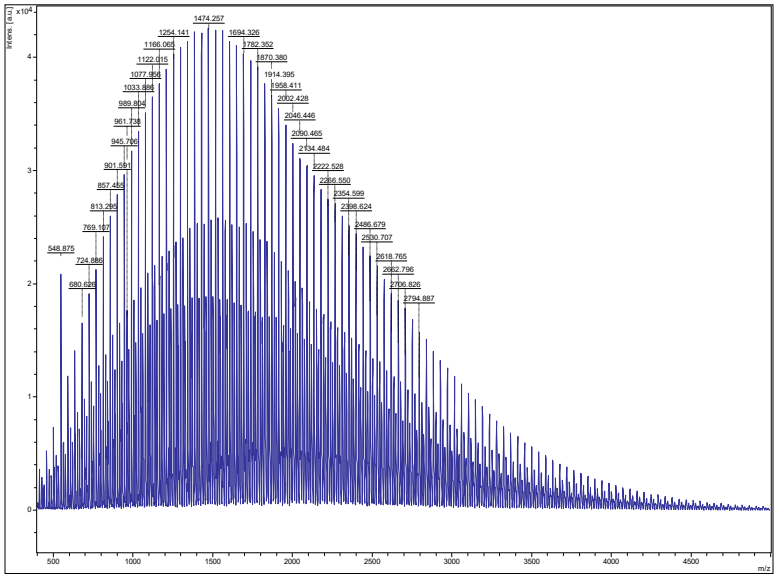

Figure S8. MALDI-MS spectra of OPO (1:1:1 ratio of 5 mg/mL in THF:11 mg/mL dihydroxybenzoic acid in water:10 mg/mL NaCl in water).

5. Impact of Material Volume on Release

Figure S9 shows the release of FITC-dextran dye from OPO material prepared at two different volumes. Dye in 100 µL material was released more quickly than dye in 500 µL material. This further supports our understanding that dissolution of OPO drives release of loaded agents, since a smaller amount of material is expected to dissolve more quickly than a larger amount of material in an equivalent volume of bulk solution. We note that even at this reduced volume, the OPO material stays intact for >30 min, which is sufficient time to administer the material *in vivo*. Future work will investigate the optimal volume to administer *in vivo* for effective treatment.

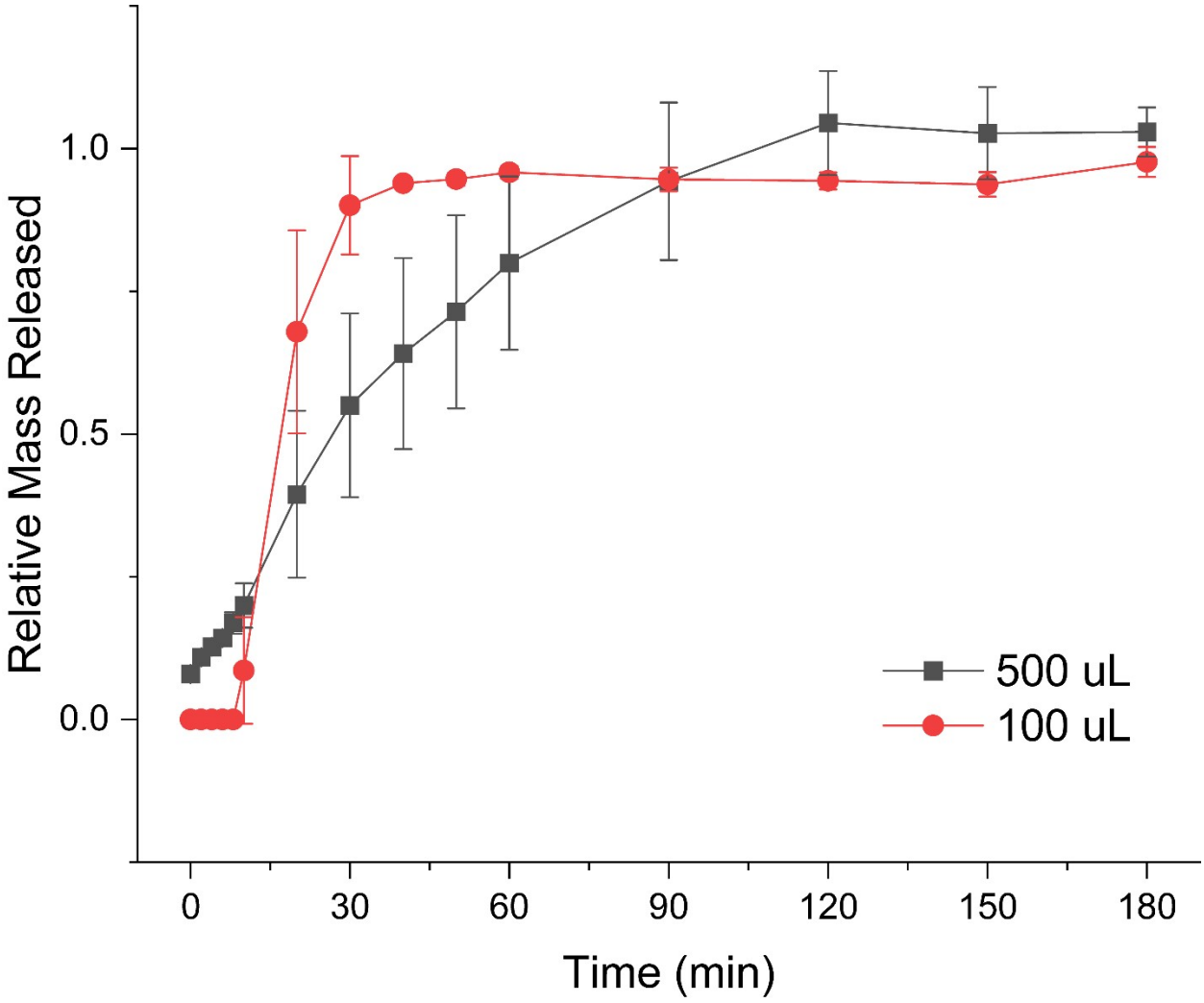

Figure S9. Dependence of release on material volume. OPO loaded with FITC-dextran was prepared at two different volumes: 500 µL (black) and 100 µL (red). Release of dye from the material was evaluated as described in Section 2.4.2 in the main text. Relative mass was calculated by dividing the mass released at each time point by the mass released after 24 h. Error bars represent standard deviations.

6. AAV-Loaded OPO Release Assay

OPO material (14%, 500 μL) was loaded with AAVs (~$5x10^{11}$ GC, AAV2-tomato, purchased from PackGene), injected onto the bottom of a DNA LoBind tube, incubated in PBS (5 mL), and gently agitated on a mixer (250 rpm). At various time points (t=0, 10, 30, 60, 150, 180 min), an aliquot was removed from the bulk solution. AAV titer was measured in each aliquot using a titer kit (Applied Biological Materials Inc, Cat. No. G931). Figure S10 shows the cumulative release of AAVs from the material. The results show a similar trend as those presented in Fig. 8 in the main text and reinforce that FITC-dextran is an appropriate simulant for the release of AAVs in preliminary investigations.

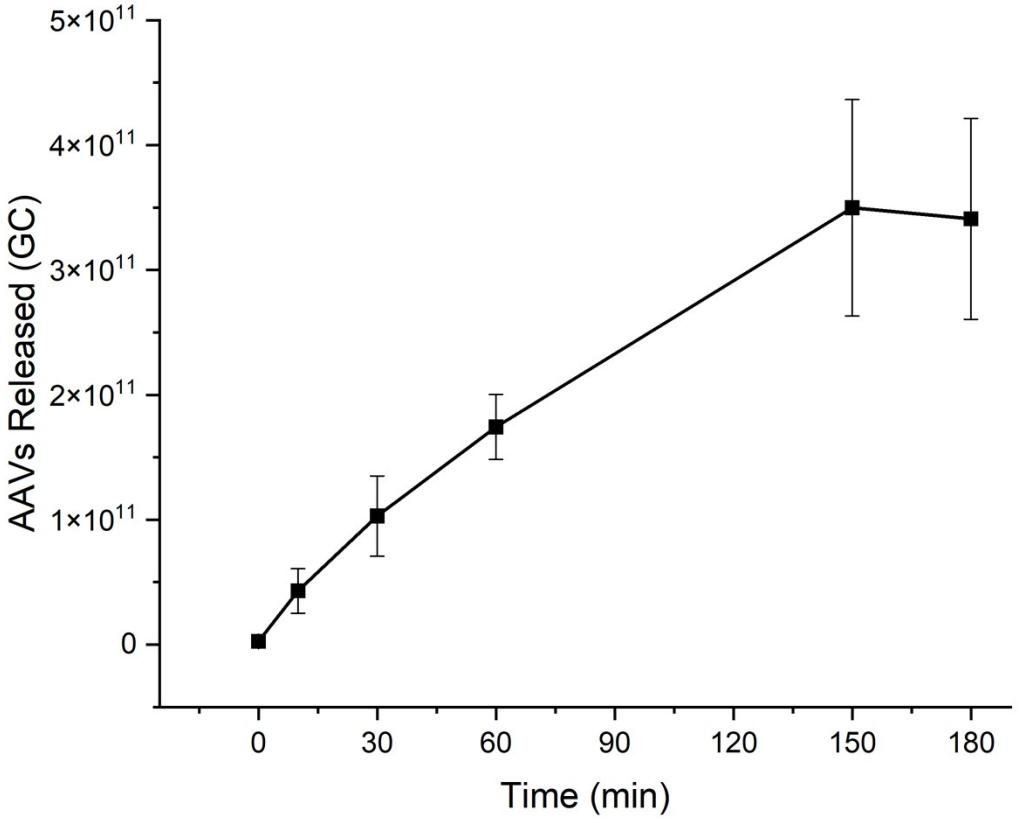

Figure S10. Release of AAVs from OPO material.

7. Supplementary Video Descriptions

SI Video 1_Inversion: This video shows OPO material prepared at four concentrations—26%, 20%, 14%, and 8%. On inversion, the material remains in place. After ~20 mins, the 8% OPO material begins to flow, demonstrating the viscoelastic properties of the material. Note that the video starts after the materials have been inverted for 15 min, and the playback speed is 10x. The contrast and brightness of the video were adjusted using Adobe Express to enable better visualization of the material. The 8% OPO material begins to flow at the 35 second mark.

SI Video 2_Injection_and_Spreadability: This video shows OPO material loaded with FITC-dextran being injected into buffer. The video demonstrates the injectability of the material, that loaded agents are retained on injection, and that the material can be spread on a surface during injection.

SI Video 3_Agitation: This video shows OPO material loaded with FITC-dextran in buffer being agitated. The video demonstrates the adhesive properties of the material and that loaded agents are retained despite external agitation.

SI Video 4_Injection on Retina: This video shows OPO material loaded with FITC-dextran being injected onto an *ex-vivo* bovine retina (obtained from a local abattoir), inverted, and agitated on a shaker that is ramped up to 1000 rpm for 1 min. The material remains attached to the retina for the duration of the video. After the period of agitation, the retina is inverted again, and the OPO material remains in place where it was initially injected. The video demonstrates the adhesion of OPO material to retinal tissue.

8. Manual Count of Fluorescent Cells

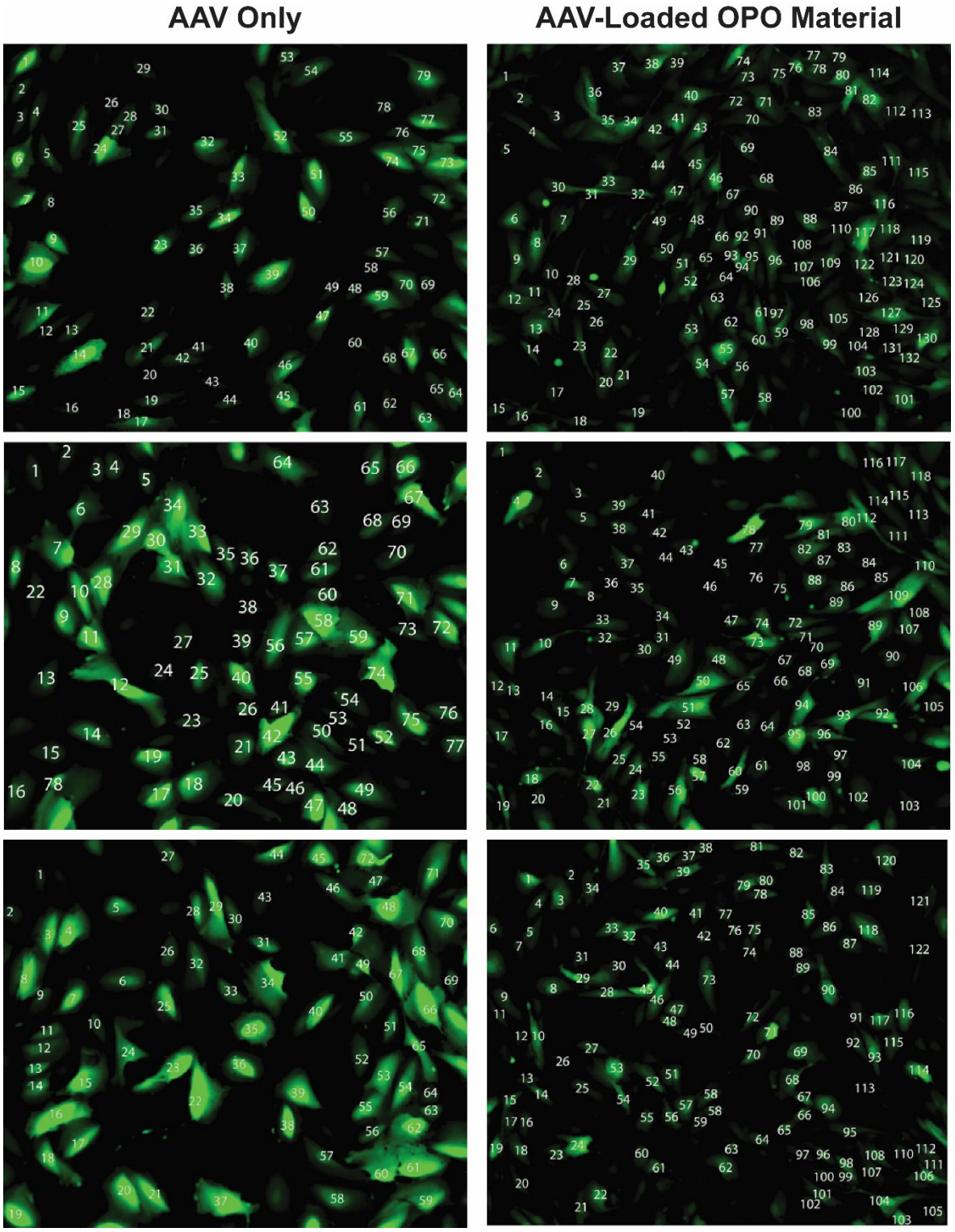

Figure S11. Manual count of fluorescent cells in images found in Fig. 11 of the main text. Cells were counted by visual inspection in Adobe Illustrator. Cells that were partially cut off on the edges in each image were excluded from the count.