Cones Will Survive: Affinity-Controlled Release of Rod-derived Cone Viability Factor from an Injectible Hydrogel Drug Delivery Vehicle

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Retinitis pigmentosa (RP) is a genetic disease that causes rod photoreceptor cell degeneration, which subsequently leads to cone photoreceptor cell death, impaired vision and eventually blindness. Rod-derived cone viability factor (RdCVF) is a paracrine factor, which is expressed as two isoforms through alternative splicing. The full length isoform, composed of 212 amino acids (RdCVFL or RdCVF212) contains a putative thioredoxin fold that could protect photoreceptors by relieving hypoxia in the retina. However, sustained delivery of RdCVFL remains a challenge.

We developed a controlled release strategy for RdCVFL to overcome this challenge: we covalently modified an injectable physical blend of hyaluronan and methylcellulose (HAMC) with a peptide binding partner of the Src homology 3 (SH3) domain and expressed RdCVFL as a fusion protein with an SH3 domain. To assess bioactivity, chick retinal dissociates were harvested and treated with the recombinant protein. Cone cell viability, quantified after 6 days in culture, increased with the addition of RdCVFL-SH3 relative to controls. Importantly, sustained release of RdCVFL-SH3 was achieved from HAMC-binding peptide for 7 days. We developed an in vivo mimetic assay where RdCVFL-SH3 diffused from our delivery vehicle and maintained the viability of the cone photoreceptors, in contrast to other delivery vehicles which can degrade loaded therapeutic protein.

Our affinity-based system constitutes a versatile delivery platform for ultimate intraocular injection in the treatment of retinal degenerative diseases.

Figure 1. Schematic demonstrating controlled release of RdCVF-SH3 from the hydrogel and bioactivity. (A) HAMC hydrogel drug delivery vehicle functionalized with SH3 binding peptides on the methylcellulose polymer chains that slow the release of the RdCVF-SH3 fusion protein from the delivery vehicle. (B) Architecture of the chick retina, and the process of dissociating and demonstrating the bioactivity of the RdCVFL-SH3 on primary cone photoreceptor cells. Cone photoreceptors treated with RdCVFL-SH3 specifically showed increased viability after culture for 6 days in vitro.

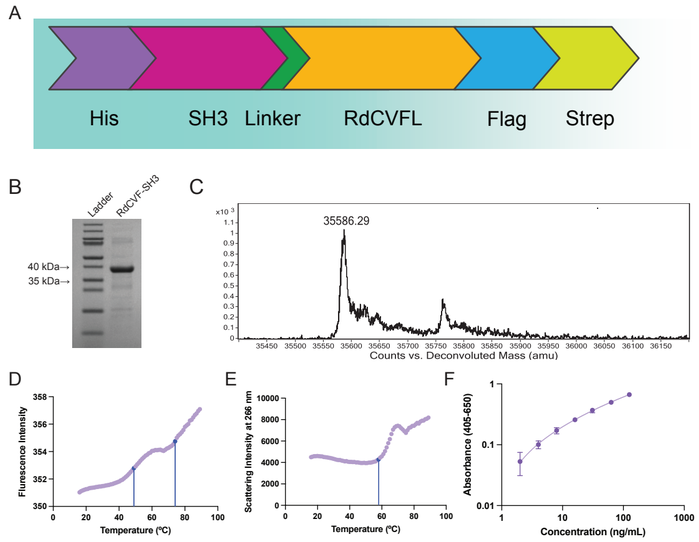

Figure 2. Purification and characterization of RdCVF-SH3. (A) Schematic of the RdCVF-SH3 fusion protein organized from N-terminus to C-terminus. (B) SDS-Page gel showing the RdCVF-SH3 fusion protein between 37 kDa and 49 kDa. (C) Mass spectrometry trace showing RdCVF-SH3 fusion protein present at 35,586 g/mol which is similar to the theoretical of 35,528.42 g/mol. (D) Differential scanning fluometry showing an increase in fluorescence with increasing temperature, and melting temperatures (Tm) of the RdCVFL and SH3 domains indicated at the by the drop lines at 49.8°C and 74.4°C, respectively. (E) Scattering intensity at 266 nm showing the aggregation temperature (Tagg) at 55.7°C. (F) Standard curve using a custom ELISA and detecting the FLAG tag of HIS-RdCVF-SH3-FLAG (n=4, mean ± standard deviation)

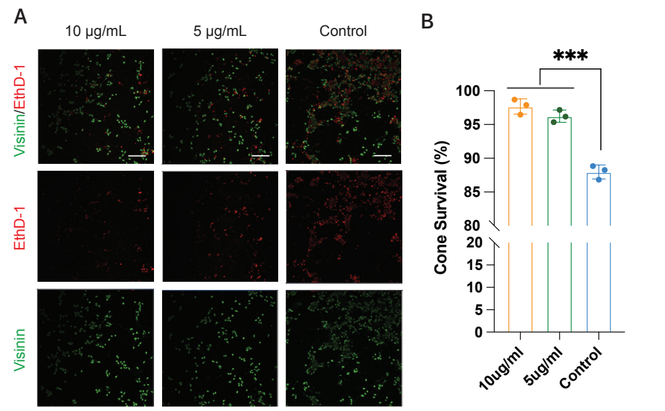

Figure 3. Bioactivity of RdCVF-SH3 with chick retinal dissociates. (A) Representative image showing photoreceptor dissociates. Cells were stained for visinin (cone photoreceptor marker) and ethidium homodimer (for dead cells). Only visinin-positive and ethidium-homodimer negative cells were quantified as viable cells. (B) Percent viability of the cone photoreceptor cells after culture with or without RdCVF-SH3 fusion protein. Cells treated with 10 µg/mL and 5 ug/mL concentrations of RdCVFL-SH3 showed significantly improved viability compared to untreated cells. (n = 3, mean ± SEM, one-way ANOVA with Tukey's post-hoc test, *** P ≤ 0.001).

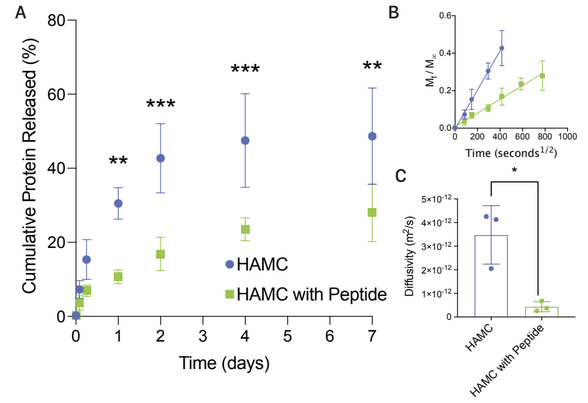

Figure 4. Release of RdCVF-SH3 from a hydrogel drug delivery vehicle. (A) Cumulative release of RdCVF-SH3 fusion protein from the HAMC hydrogel with or without 100x molar excess of binding peptide. (B) Fractional mass release of protein from the HAMC hydrogel plotted against the square root of time. The gel modified with SH3-binding peptide extended Fickian diffusion for 7 d whereas plain HAMC released protein for approximately 2 d, after which it mostly plateaued. (C) Relative diffusivity of the RdCVF-SH3 fusion protein in the HAMC hydrogel functionalized with or without binding peptide. (n = 3, mean ± standard deviation, two-way ANOVA with Bonferroni's post-hoc test for (A) and unpaired two-tailed Student's t-test for (C): *p < 0.05, **p < 0.01, ***p < 0.001.)

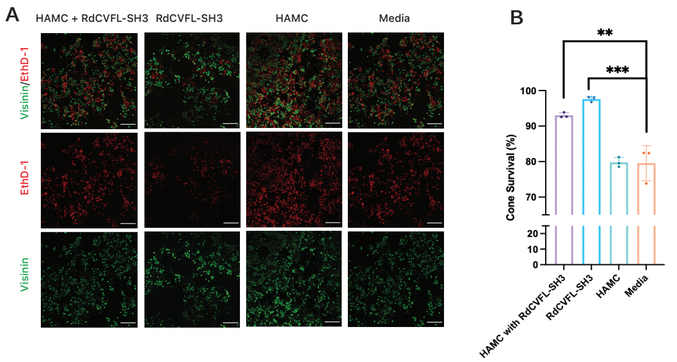

Figure 5. Bioactivity of RdCVFL-SH3 released from the hydrogel drug delivery vehicle. (A) Representative image showing photoreceptor dissociates. Only visinin-positive and ethidium-homodimer negative cells were quantified as viable cells. (B) Percent viability of the cone photoreceptor cells after culture with HAMC with RdCVFL-SH3, RdCVFL-SH3, HAMC, or media alone. Conditions with RdCVFL-SH3 showed significantly improved viability compared to untreated cells. (n = 3, mean ± SEM, one-way ANOVA with Dunnet's post-hoc test, **p < 0.01, *** p<0.001).

Acknowledgements

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