Affinity-Controlled Release of Rod-derived Cone Viability Factor for Cone Photoreceptor Survival

Carter J. Teal[1,2], Margaret Ho [1,2], Lia Huo [2,3], Hidekiyo Harada [4], Laura C. Bahlmann [1,2], Thierry Léveillard [5], Philippe Monnier [4,6], Molly S. Shoichet [1,2,7]

[1] Institute of Biomedical Engineering, University of Toronto; [2] Donnelly Centre, University of Toronto; [3] Institute of Medical Science, University of Toronto; [4] Vision Division, Krembil Research Institute;
[5] Department of Genetics, Institut de la vision, Inserm, Université Pierre et Marie Curie Paris 6; [6] Department of Physiology, Faculty of Medicine, University of Toronto;
[7] Department of Chemical Engineering and Applied Chemistry, University of Toronto

Retinitis pigmentosa (RP) is the most common inherited retinal degenerative disease, affecting 1 in 4000 people worldwide. The disease is characterized by mutations that disrupt the function of rod photoreceptors causing them to degenerate, which subsequently leads to the degeneration of cone photoreceptors. Administration of rod derived cone viability factor (RdCVF) has been implicated in the survival of cone photoreceptors after rod photoreceptor cell death. We developed an affinity-controlled release strategy for the long isoform of RdCVF (RdCVFL). An injectable hydrogel composed of hyaluronan and methyl cellulose (HAMC) was covalently modified with a peptide binding partner of the Src homology 3 (SH3) domain. An SH3 domain was expressed as a fusion protein with RdCVFL, enabling its controlled release from the peptide modified hydrogel. Bioactivity of the fusion protein was demonstrated through culture with ex vivo retinas harvested from embryonic chickens, demonstrating an increase in total number of viable cone photoreceptors when compared to controls. The fusion protein diffused from the delivery vehicle modified with binding peptide over 7 days in vitro, and the released protein was quantified by ELISA. The vehicle modified with binding peptide demonstrated significantly less protein released, and reduced its overall effective diffusivity. To ensure bioactivity after incorporation into the hydrogel, transwells were prepared with the peptide modified hydrogel loaded with RdCVFL-SH3 and demonstrated no significant decrease in bioactivity. Our affinity-based system constitutes a versatile delivery platform for the treatment of retinal degenerative diseases.

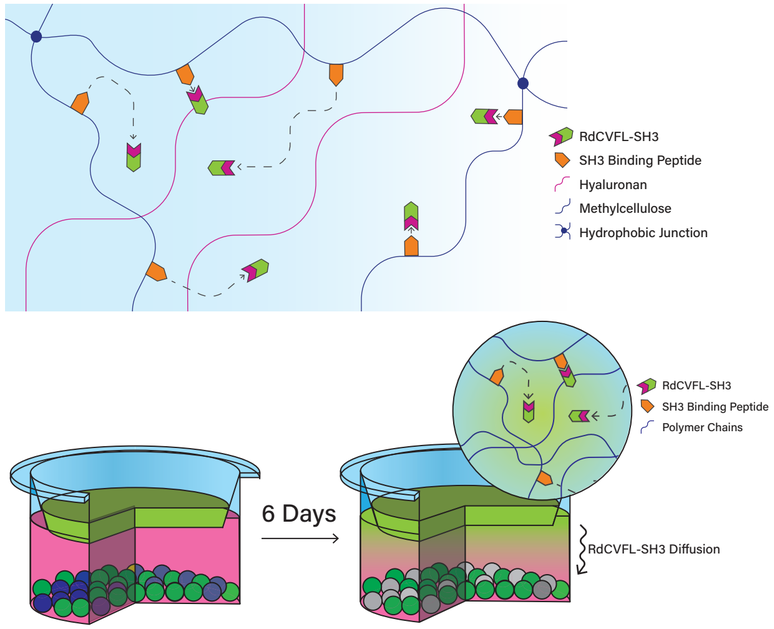

Figure 1. Schematic demonstrating controlled release from the hydrogel and bioactivity of RdCVFL-SH3. Chick retinas are dissociated and seeded into a 12-well plate. Transwells are loaded with the HAMC hydrogel delivery vehicle. The HAMC hydrogel is functionalized with SH3 binding peptides on the methylcellulose polymer chains that slow the release of the RdCVFL-SH3 fusion protein. Cone cell viability is quantified after 6 days of in vitro culture.

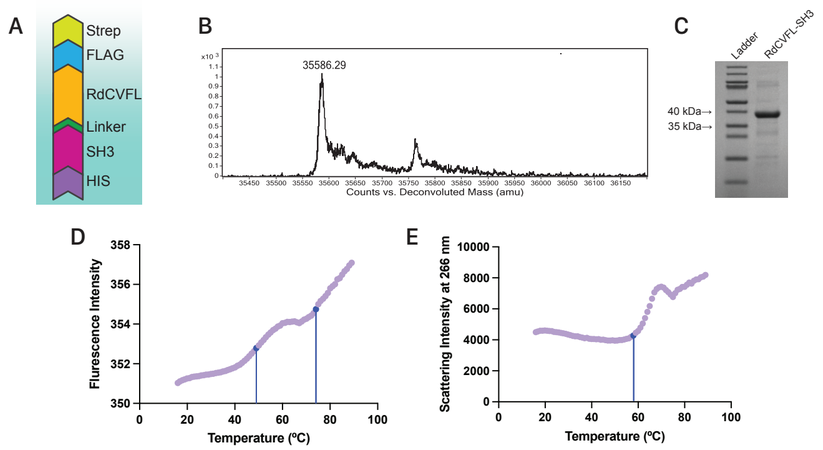

Figure 2. Purification and characterization of RdCVFL-SH3. (A) Schematic of the RdCVFLSH3 fusion protein from N-terminus (HIS) to C-terminus (Strep-tag II). (B) Mass spectrometry trace showing RdCVFL-SH3 fusion protein present at 35,586.42 g/mol. (C) SDS-Page gel of purified RdCVFL-SH3. (D) Differential Scanning Fluorimetry shows an increase in fluorescence with increasing temperature: the melting temperatures (Tm) of the RdCVFL and SH3 domains are indicated in blue. (E) Scattering intensity at 266 nm shows the aggregation temperature in blue for RdCVFL-SH3.

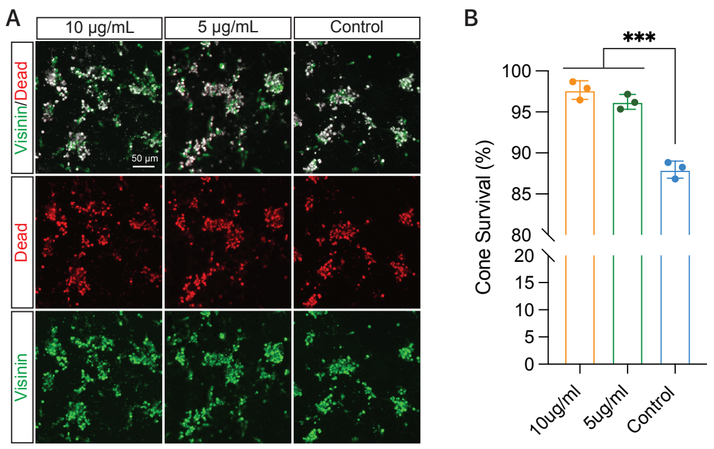

Figure 3. RdCVFL-SH3 increases viability of chick retinal dissociates. (A) Representative images showing photoreceptor dissociates. Cells were stained for visinin (green, cone photoreceptor marker) and a fixable dead-cell dye (red). (B) Percent viability of the cone photoreceptor cells after culture with or without RdCVFL-SH3 fusion protein. Cells treated with 5 or 10 µg/mL RdCVFL-SH3 showed significantly improved viability compared to untreated cells (n = 3 separate cone isolations, mean ± SEM, one-way ANOVA with Tukey's post-hoc test, ***p < 0.001).

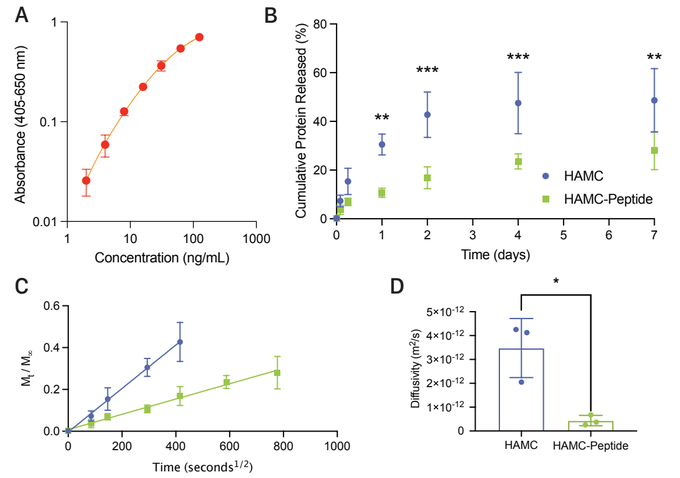

Figure 4. Release of RdCVFL-SH3 from a hydrogel drug delivery vehicle. (A) Standard curve of the HIS/FLAG tag ELISA, depicting the range of detection from 125 ng/mL to 2 ng/mL (3.5 nM to 28.15 pM). (B) Cumulative release of RdCVFL-SH3 fusion protein from the HAMC hydrogel with or without 100x molar excess of the binding peptide. (C) Fractional mass release of protein from the HAMC hydrogel plotted against the square root of time. (D) Relative diffusivity of the RdCVFL-SH3 fusion protein in the HAMC hydrogel functionalized with or without binding peptide. (n = 3 separate release experiments, mean ± standard deviation, two-way ANOVA with Bonferroni's post-hoc test for (B) and unpaired two-tailed Student's t-test for (D): *p < 0.05, **p < 0.01, ***p < 0.001.)

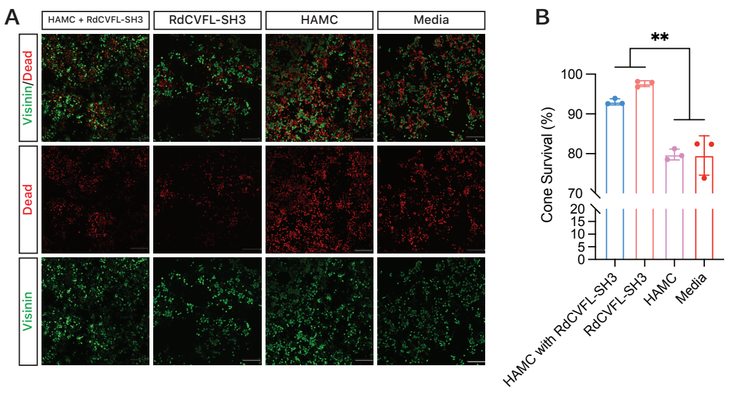

Figure 5. Bioactivity of affinity-released RdCVFL-SH3 released from the hydrogel delivery vehicle. (A) Representative images showing photoreceptor dissociates. Only visinin-positive (green, cone photoreceptors) and dead-negative (red, fixable dead cell dye) cells were quantified as viable cells. (B) Quantification of viable cone photoreceptor cells after culture with: HAMC with RdCVFL-SH3; RdCVFL-SH3, HAMC, or media alone. (n = 3 separate cell studies, mean ± SEM, one-way ANOVA with Tukey's post-hoc test, **p < 0.01).

Acknowledgements

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