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Sustained Release of Sh3-RdCVF to Rescue Cone Photoreceptor Death

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Retinitis Pigmentosa (RP) is an incurable disease that can lead to blindness. Though rod-derived cone viability factor (RdCVF) is a promising therapeutic that improves cone survival in rodent models of RP, it cannot be translated to patient care because of its rapid clearance and limited bioavailability. To overcome these challenges, we designed a biocompatible slow-release formulation of RdCVF. Here, we investigated the release profile of RdCVF and its bioactivity *in vitro*.

We expressed RdCVF with a Src homology 3 (Sh3) domain and modified an oxime-crosslinked hyaluronan (HA-oxime) hydrogel with Sh3 binding peptides to slow protein release through Sh3 and Sh3-binding peptide interactions. To investigate tunability of release, the hydrogel was made with increasing molar excesses of Sh3-binding peptide to Sh3-RdCVF, and released protein was quantified using an ELISA at different time points for 14 days. To assess if the protein would retain its function after release, a transwell containing our HA-oxime gel loaded with Sh3-RdCVF was placed above chick retinal dissociates. After 6 days in culture, cone survival was quantified with cone-specific marker visinin.

As the molar excess of Sh3 binding peptide increases with respect to the Sh3-RdCVF protein, the slower the protein release. In addition, linear diffusion was extended, and relative diffusivity was decreased with increasing amounts of binding peptide, indicating that the release can be controlled with the addition of Sh3-binding peptide. Furthermore, the released protein was bioactive as shown by an increase in cone viability in chick retinal dissociates as compared to the hydrogel alone ($p=0.0018$) or Sh3-RdCVF mutant ($p=0.0162$). Therefore, the released RdCVF remains functional and is able to activate basigin1 to mediate cone survival.

The sustained release of RdCVF is not only controllable with our drug delivery system, but also bioactive, thereby laying the foundation for *in vivo* studies in disease models of RP.