Ocular delivery of a model biopharmaceutical, lacritin, using protein polymers

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ABSTRACT SUMMARY
The delivery of protein and peptide therapeutics to the ocular surface remains a challenge. Due to the rapid turnover of the ocular tear film, sustained release may be necessary to maintain therapeutic concentrations. To approach this problem, we have explored the fusion of thermally-responsive protein polymers directly to therapeutic proteins. As a candidate biopharmaceutical, we have focused on lacritin, which is under evaluation as a treatment for dry eye disease (DED). Lacritin is found in human tears and has both prosecretory and mitogenic functions in the anterior segment of the eye. It is deficient in the tears of DED patients, and lacritin replacement enhances the quality of the tear film in animal models. To modulate the ocular residence time of lacritin, we have expressed it in fusion to a library of elastin-like polypeptides (ELPs). ELPs are thermally responsive protein polymers that can be tuned to phase separate when they reach the temperature of the ocular surface. Lac-ELP fusions have thermally-responsive properties similar to those of the plain ELP and also have activity in a variety of in vitro and in vivo models, including the induction of tear secretion from the lacrimal glands of non-obese diabetic (NOD) mice.

INTRODUCTION
The lacrimal gland-cornea axis plays a critical role in maintaining ocular health. While avascular cornea serves as both a protective barrier and main refractive element of the visual system, the lacrimal gland is the major organ secreting key proteins and electrolytes into the tear film that overspreads the cornea and conjunctiva [1]. Beyond a hydrating effect, tear proteins contribute to anti-microbial and anti-inflammatory defense of the exposed ocular surface [2]. DED is a multifactorial disease of the tears and ocular surface causing visual disturbance and tear film instability [3]. Accordingly to reports, severe DED affects approximately 5 million Americans above age 50 and its global prevalence ranges from 5% to 35% [3]. Traditional approaches to treat DED include lubricating the ocular surface with artificial tears, conserving the secreted tears using tear plugs and eye-shields, or treating the associated ocular surface inflammation with eye drops containing anti-inflammatory medications [4]. Nevertheless, these therapies are generally regarded as ineffective and there remains a demand for novel DED therapy.

EXPERIMENTAL METHODS
Lac-ELP genes were expressed in E. coli and purified by inducing ELP-mediated phase separation. This was followed by size exclusion chromatography to obtain pure material (Fig. 1b). Phase behavior of fusion proteins was characterized by optical density (Fig. 1C). In vitro activities were tested using rabbit lacrimal gland acinar cells and SV40-immortalized human corneal epithelial cells. In vivo prosecretory potential of Lac-ELPs were tested on 12-week non-obese diabetic (NOD) mice via single bonus intra-lacrimal gland injection.

RESULTS AND DISCUSSION
A novel fusion protein library based on recombinant human lacritin and elastin-like-polypeptides (ELPs) was designed as a potential therapeutic for the ocular surface. The Lacritin-ELP fusion proteins imparted thermo-sensitive assembly of viscous coacervates that may promote retention in the lacrimal gland, on the
cornea, or on contact lenses. Lac-ELP induced tear secretion from male NOD mice, which have inflammation in their lacrimal gland resulting in low tear flow (Fig. 2). Viscous coacervates Lac-V96 formed local drug depot in murine lacrimal gland near injection site (not shown). Using human corneal epithelial cells, Lac-ELPs evoked both a Ca$^{2+}$ wave and closure of a scratch (not shown).

**CONCLUSION**

Exploration of ELP-mediated retention of protein drugs in the anterior segment of the eye opens new possibilities for functionalizing peptide and protein-based therapeutics. In this abstract, we demonstrate that lacritin fusions retain both tear secretion potential and also the temperature dependence needed to enhance their retention upon administration to the eye.

**REFERENCES**


**ACKNOWLEDGMENTS**

This work was facilitated by support from the University of Southern California School of Pharmacy, The National Institutes of Health and National Eye Institute grant EY011386, the USC Whittier foundation and the USAMRAA/TATRC VRP HAD 11262019.