Hydrogel delivery device for the in-vitro sustained release of active rhGALNS enzyme

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Statement of Purpose: Mucopolysaccharidosis IVA (Morquio A disease) is a genetic disorder caused by deficiency of N-acetylgalactosamine-6-sulfate-sulfatase (GALNS), leading to accumulation of keratan sulfate and chondroitin-6-sulfate in lysosomes. Many patients become wheelchair-dependent as teens, and their life span is 20-30 years. Currently, enzyme replacement therapy (ERT) is the treatment of choice. Although it alleviates some symptoms, replacing GALNS enzyme poses several challenges including very fast clearance from circulation and easy denaturation. These constraints affect frequency and cost of enzyme infusion, and ability to reach all tissues. In this study, we developed injectable and biodegradable polyethylene glycol (PEG) hydrogels, loaded with recombinant human GALNS (rhGALNS) to improve enzyme stability and bioavailability, and to sustain release [1][unpublished data]. The developed hydrogel delivery device could overcome current limits of rhGALNS replacement and improve quality of life for Morquio A patients.

Methods: rhGALNS was produced in Chinese Hamster Ovary cells and purified by chromatography. Hydrogels at 10% w/v were prepared by combining 4-arm PEG-acrylate (4-arm PEGAc) and PEG-dithiol (PEG-diSH) crosslinker in an equimolar ratio of Ac:SH in 0.1 M HEPES buffer pH 7.4 and allowing to gel for 1 h. GALNS interaction with individual hydrogel components (macromer, crosslinker, buffer, etc) was tested through an enzyme activity assay and non-reduced SDS PAGE. GALNS was encapsulated by mixing in the gel solution. Hydrogel gelation time and mechanical properties were characterized via rheology. GALNS release from the hydrogels was measured via bulk release experiments and diffusivity through fluorescence correlation spectroscopy (FCS). GALNS uptake was tested in anonymous human primary GALNS-deficient fibroblasts.

Results: We first tested the effect of the various gel components on GALNS activity and determined that lowering the pH of the HEPES buffer from 7.4 to 6.9 led to better enzyme activity (80% vs 100%) and that the 4-arm PEGAc and PEG-diSH decreased enzyme activity to about 70% and 90%, respectively. Based on our SDS page results GALNS did not react with PEG-diSH crosslinker when incubated for 1 hr but reacted with the PEGAc, where larger species formed with longer incubation times up to 24 hr. However, since the hydrogel gelation time was ~30 min, we suggest that such interactions were minimal upon hydrogel encapsulation. The hydrogel degraded via hydrolytic degradation in about 1 month, had a storage modulus of ~2.5 kPa and a mesh size of 12 nm. The GALNS enzyme has a hydrodynamic radius of 4 nm, so we expected GALNS release via passive diffusion. Via FCS measurements we confirmed that the hydrogel obstructed GALNS diffusivity to 1.2×10^{-7} cm^2/s compared to 4.8×10^{-7} cm^2/s in buffer. We showed that the hydrogel was able to provide sustained release of GALNS as well as retain over 70% bioactivity up to 7 days (**Fig. 1A**). Note that in buffer the enzyme lost activity in about 3 hr. We further showed that ~5% bioactivity could be measured even at 28 days of release (at gel degradation), even though the most activity was observed in the first week. To confirm that the enzyme encapsulated in the hydrogels was released in active form and could be taken up by cells, we measured patient fibroblasts' uptake of GALNS. Deficient fibroblasts were cultured in media alone (control) or in media with either a bolus dose of GALNS or GALNS-loaded PEG hydrogels. Under both conditions there was an overall increase in enzyme activity from 5 hours to 24 hours, indicating cells were continuously uptaking GALNS (**Fig. 1B**).

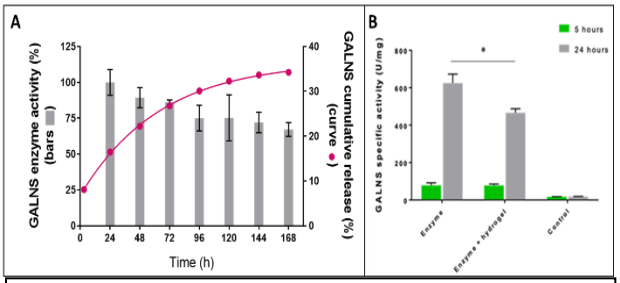

Fig. 1: A) Enzyme activity (gray) and cumulative release (magenta) of rhGALNS encapsulated in a PEG hydrogel over 7 days. **B)** Specific activity of lysate from cells cultured with media only (control), media containing a bolus dose of rhGALNS enzyme, and media containing rhGALNS enzyme released from a PEG hydrogel. *p<0.05, n=6.

Conclusions: We developed injectable and biodegradable PEG hydrogels for rhGALNS sustain release. PEG hydrogels preserved enzyme activity during sustained release for up to 28 days. Enzyme activity was also confirmed by measuring its uptake in patient fibroblasts.

References: Jain E, Drug Delivery and Translational Research 2020;10:1341-1352.