Uricase Functionalized Hydrogel for the Localized Treatment of Gout

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Statement of Purpose: Gout is a type of inflammatory arthritis caused by uric acid crystallization within the joint space, often accompanied by hyperuricemia. Conventional urate lowering therapies (ULTs) often fail due to patient noncompliance and comorbidities limiting tolerability. Additionally, it has been shown that it may take years of chronic exposure to ULTs to completely dissolve urate tophi, the deposits of urate crystals within the joint space[1]. Patients with chronic gout refractory to ULT experience debilitating pain, which can lead to irreversible joint damage. There is a need for a therapy that acts quickly to dissolve urate tophi and prevent reoccurrence of gouty flare in this patient population. Uricase is an enzyme that converts uric acid into hydrogen peroxide and allantoin, which is a more soluble end product, making it an attractive therapeutic option for gout. In this work, this uricase enzyme has been fused to a CATCH (Co-Assembly Tags based on CHarge complementarity) peptide tag. The CATCH system consists of a pair of oppositely charged peptides that, when combined, co-assemble into nanofiber-based hydrogels[2]. This work aims to show the efficacy of a CATCH-uricase (CATCH-U) functionalized hydrogel for the localized treatment of gout.

Materials and Methods: *Protein Expression and Purification:* The CATCH-U fusion protein was expressed in an E. coli host system and purified via nickel affinity chromatography. *CATCH Hydrogel Fabrication:* CATCH peptides were dissolved in aqueous buffer and combined in equimolar ratios, with the addition of CATCH-U, to final gel volumes of 10-40uL. The gels were left to incubate at 4ºC for 1 hour and then washed three times with PBS to remove any free protein or peptide not incorporated into the gel. *Activity with Soluble Uric Acid:* Depletion of uric acid substrate (0.05-0.4mM) by soluble CATCH-U and CATCH-U gels was measured overtime via absorbance at 293nm and 37ºC. *Activity with Crystallized Uric Acid*: Uricase activity was also tested with monosodium urate (MSU) crystals as the substrate. Wells containing soluble or gelled enzyme were imaged in a 96-well plate and the rate of crystal dissolution was determined by optical density at 600nm over time. *Enzyme Retention within the Gel:* Sets of gels were incubated in PBS for 1, 24, and 48 hours. The PBS was removed from the gels at these timepoints and tested for activity with uric acid substrate. *In vivo retention:* CATCH-U was labeled with Cy5.5 and fabricated into 40uL gels that were injected into female C57BL/6 mice. The mice were then fluorescently imaged using the IVIS. *In vivo gout model:* Female C57BL/6 mice were treated with a 20uL hydrogel with or without uricase injected subcutaneously (SQ) into the top of the hind paw. Two days later, 0.5mg MSU was subcutaneously injected into the same paw. Paw inflammation was subsequently assessed with digital calipers.

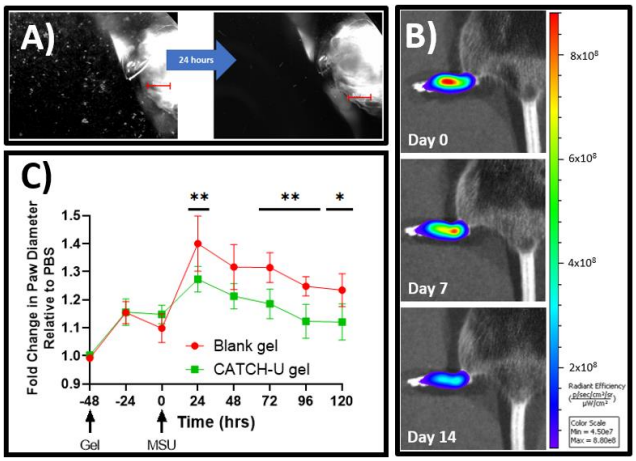

Figure 1: A) CATCH-U hydrogels incubated with MSU crystals for 24 hours. MSU appear as white flakes and the gel is the white mass on the right. Scale bar = 500µm, B) IVIS imaging of CATCH-U-Cy5.5 hydrogels injected SQ into the top of the hind paw, C) Fold change in paw diameter relative to contralateral paw for blank vs uricase-loaded hydrogels injected at -48 hours and MSU crystals at 0 hours SQ into the top of the hind paw (2-way ANOVA, Bonferroni post-hoc, * $p < 0.05$, ** $p < 0.01$)

Results and Discussion: The CATCH-U enzyme remains active immobilized within the hydrogel as determined by uric acid depletion assays. We have also shown that the enzyme is retained and remains active within the hydrogel for 4 days at 37ºC *in vitro*. This is important for translation *in vivo* to enable complete, rapid dissolution of gouty tophi. Moreover, the CATCH-U hydrogel is able to establish a concentration gradient capable of dissolving crystalized uric acid, the pathological component of gout (Figure 1A). Next, we were able to show that Cy5.5 labeled CATCH-U in a gel formulation is retained at the site of injection for over 14 days *in vivo* (Figure 1B). This long-lasting retention shows promise for not only rapidly dissolving dense tophi, but also for preventing new crystallization events within the joint space. Finally, in a mouse model of gout, the functionalized hydrogel was able to decrease the inflammation associated with uric acid crystal insult as determined by caliper measurements.

Conclusion: These data demonstrate preliminary efficacy of CATCH-U hydrogels for the localized treatment of gout. Future work includes determining if the decreased inflammation due to the therapy is also associated with decreased cell infiltration and cytokine production, as well as decreased thermal and mechanical sensitivity in the paw.

References:

(1) *Seroski, D.T. Cell Mol Bioeng. 2016. 9, 335-350.*
(2) *Reinders, M. K. Ther Clin Risk Manag. 2010. 6, 543.*