Immunomodulatory Enzyme Immobilized in Co-assembling Peptide Hydrogels

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Statement of Purpose: Hydrogels formed from peptides that co-assemble into nanofibers are finding increasing use in applications such as drug delivery, tissue engineering, and immune engineering. Co-assembling peptides, such as the CATCH pair developed by our lab[1], can be used as recombinant handles to integrate proteins into hydrogels. The physically-crosslinked hydrogels can be delivered via minimally-invasive injection without loss of protein activity. The enzyme, Adenosine Synthase A (AdsA), is found in S. aureus and dephosphorylates ATP to Adenosine[2]. AdsA is an attractive therapeutic to downregulate the immunostimulatory activity of extracellular adenosine triphosphate (eATP). Here we will present co-assembled peptide hydrogels with immobilized AdsA as biomaterials that can down-regulate pro-inflammatory cytokine secretion and neutrophil activity. In particular, we report the activity of AdsA immobilized in CATCH hydrogels, the efficacy of CATCH-AdsA hydrogels to down-regulate IL-1β secretion and diminish myeloperoxidase (MPO) activity of neutrophils.

Methods: AdsA was fused to the CATCH(6-) peptide. Stock solutions of CATCH4(+) [QQKFKFKFKQQ] and CATCH(6-) [EQEFEFEFEQE] peptides are mixed in equal volumes in saline and cured for one hour to generate the supramolecular hydrogel (total peptide = 12 mM). Soluble AdsA and CATCH-AdsA hydrogels activity was assessed incubating the protein with 40 µM ATP for 1 hour. Free phosphates in solution were quantified at the endpoint using the malachite green assay. THP-1 cells were differentiated to macrophages using phorobol-12-myristate-13-acetate and exposed to lipopolysaccharide (LPS), an inflammatory stimulus, to promote expression of pro-IL-1β. The cells were then incubated with soluble AdsA or CATCH(4+/6-)-AdsA hydrogels and ATP, and the expression of IL-1β was evaluated using an ELISA kit. C57BL/6J mice received subcutaneous injections of 40 µL of CATCH(4+/6-) or CATCH-AdsSA hydrogel followed by LPS injection. Neutrophil MPO activity was quantified using luminol non-invasive imaging method. Mice received an intraperitoneal (i.p.) injection of luminol solution (10 mg/mL in 0.9% saline solution) and after 10 minutes the injection site was imaged using bioluminescence imaging technique with the in vivo imaging system (IVIS). All protocols involving animals were approved by the UF IACUC.

Results: CATCH(4+) and CATCH(6-) co-assemble into self-supporting hydrogels with a storage modulus close to 1 kPa. AdsA immobilized in CATCH hydrogels dephosphorylates ATP (Figure 1 A). CATCH-AdsA hydrogels decreased the amount of IL-1β secreted by macrophages (Figure 1 B). CATCH-AdsA hydrogels, with 9 µM AdsA, diminish MPO neutrophil activity after inflammatory stimulus (Figure 1 C-D).

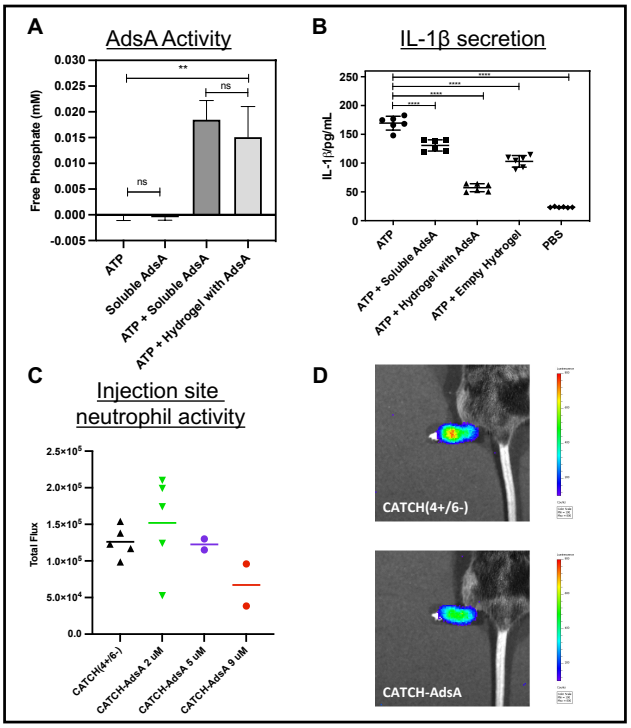

Figure 1. (A) CATCH-AdsA hydrogels can dephosphorylate ATP. **(B)** CATCH-AdsA hydrogels decreased the amount of IL-1β secreted by macrophages. **(C)** At day 3 post injection, CATCH-AdsA hydrogels decreased the neutrophil MPO activity in the acute phase of inflammation. **(D)** Representative images of luminol bioluminescence used to quantify neutrophil MPO activity.

Conclusions: CATCH(4+) and CATCH(6-) co-assemble into hydrogels that undergo shear thinning and recovery in response to applied strain. CATCH(4+/6-) hydrogels injected into subcutaneous tissue via a fine-gauge needle do not induce significant local inflammation. AdsA immobilized in CATCH(4+/6-) can downregulate inflammatory cytokine secretion by macrophages, and diminish neutrophil degranulation in response to an inflammatory stimulus. Together, these data establish CATCH hydrogels as an attractive candidate for injectable biomaterials for biomedical applications, such as local drug delivery.

References:

[1] Seroski DS. Cell Mol Bioeng. 2016;9:335-350.
[2] Thammavongsa V. J. Exp. Med. 2009;206:2417-2427

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