Utilizing Microporous Annealed Particle Scaffold as a Platform for IL-33-Mediated Immunomodulation

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Statement of Purpose: Over 6.5 million patients in the United States are affected by chronic wounds [CDC], and effective wound treatment is a growing and unmet clinical need. Wound healing is partially directed by immune cells that secrete cytokines, chemokines, and growth factors that modulate the healing response. IL-33 plays an important role in wound healing, and binds to ST2 receptors of immune cells (*e.g.,* macrophages) which leads to activation of MAPK and NFκB pathways. A recent study has shown that the delivery of soluble IL-33 to wounds in diabetic mice promoted increased re-epithelization, angiogenesis, and pro-resolutory (M2) macrophage development[1]. Therefore, IL-33 may be a useful target to resolve inflammation and promote healing in chronic wounds. In this study, we used a novel injectable biomaterial, microporous annealed particle (MAP) scaffold, as a platform for IL-33 delivery. MAP scaffold is an injectable biomaterial that is composed of hydrogel microspheres which are annealed *in situ* to provide a microporous environment[2]. MAP scaffold elicits a very low, baseline immune response and has no discernable foreign body response, which renders it a "blank canvas" for IL33-mediated immunomodulation. We hypothesize that immobilizing IL-33 proteins to MAP scaffold will enhance MAP immunogenicity *in vitro* and activate macrophages through IL33-ST2 signaling pathway.

Methods: MAP microgels were synthesized using a high-throughput microfluidics technique as previously described[3]. The MAP formulation consisted of 5 wt% poly(ethylene) glycol backbone (2 mM excess for ligand attachment) and MMP degradable crosslinker. The excess maleimide groups were either quenched via hydrolysis with a strong base (triethylamine) or non-quenched and stored in PBS buffer with pH 3.5. Traut's reagent, which react with primary amines to introduce thiol (-SH) groups, was used to thiolate recombinant mouse IL-33 protein (Abcam). Protein thiolation was confirmed by Ellman's assay (Fig. 1A). To test for maleimide-mediated covalent attachment, thiolated IL-33 (IL33-SH) was incubated with MAP microgels for 1 hour at room temperature. Microgels were then washed in infinite PBS sink conditions for at least 30 minutes. After excess protein was removed, o-Phthalaldehyde (OPA), which fluorescently reacts with free amines, was added to quenched (Q) and non-quenched (NQ) microgels and the fluorescent signal was compared to a standard curve of known IL33-SH protein concentrations (Fig. 1B). To determine bioactivity of IL33-MAP compared to soluble IL-33, used an NFκB luciferase reporter RAW 264.7 cell line (murine macrophages). After 6 hour incubation, the cells were lysed and luminescence (indicative of NFκB activation) was measured with a plate reader and normalized to control groups (cells only and MAP+cells only). ST2 KO RAW 264.7 cells were generated with the CRISPR Cas9 lentiviral system. IL33-MAP was incubated with WT RAW cells or ST2 KO RAW cells, and TNFα cytokine secretion was measured by ELISA (data not shown) after 12 hr to confirm NFκB activation through the ST2 receptor.

Results: Ellman's assay quantified about 2-fold increase in thiol group concentration in thiolated IL-33 compared to unmodified IL-33. The OPA assay detected no protein on quenched MAP, which had no free maleimides available to reacted with thiolated IL33. About ~70% of initial protein concentration (20 µg/mL) was quantified in the non-quenched MAP condition after the washing step. The NFκB reporter RAWs produced luciferase in response to IL-33. There was no difference between soluble IL-33 and IL33-SH conditions, suggesting thiolation doesn't affect IL-33 bioactivity. IL33-MAP also caused a significant fold change in luciferase signal compared to the MAP only condition, indicating that IL-33 remains bioactive when tethered to MAP scaffold. Additionally, TNFα cytokine secretion by WT RAW cells increased in response to IL33-MAP (data not shown), but not in ST2 KO cells, indicating that IL33-MAP cell activation is ST2 pathway dependent.

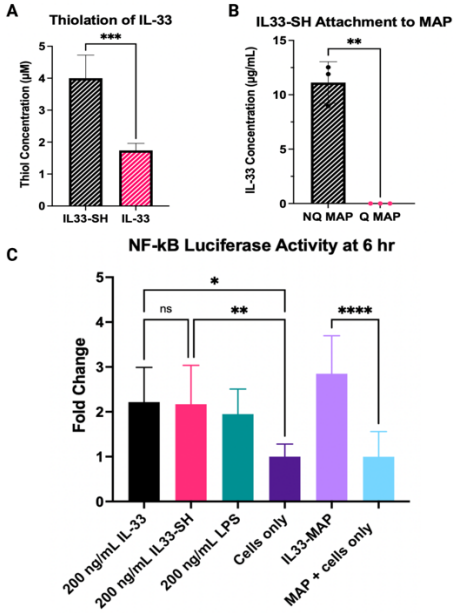

Figure 1: A) Ellman's assay thiol concentration of thiol-modified IL-33 and unmodified IL-33. B) OPA assay data determined IL-33 protein concentration tethered to non-quenched MAP vs. quenched MAP. C) Luciferase measurements of NFκB RAW reporter cells incubated for 6 hours. LPS is positive control. Soluble IL-33 normalized to cells only; IL33-MAP normalized to MAP + cells only.

Conclusions: We have successfully generated an immunomodulatory biomaterial that has the ability to activate macrophages through the IL33-ST2 pathway. We are currently analyzing a wound healing study, in which IL33-MAP was administered to wounds on diabetic mice.

References: [1] Yin H. Mol. Immuno. 2013;56:347-353 [2] Griffin D. Nat Materials. 2015;14:737-744. [3] de Rutte J. Adv. Func. Materials. 2019;29(25).