

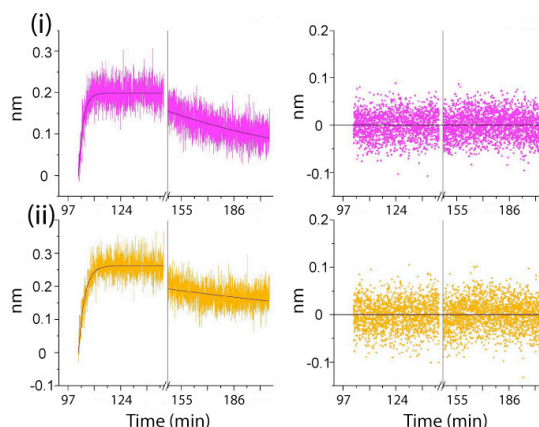
## Multi-Functional Bioscaffolds to Modulate Local Inflammation

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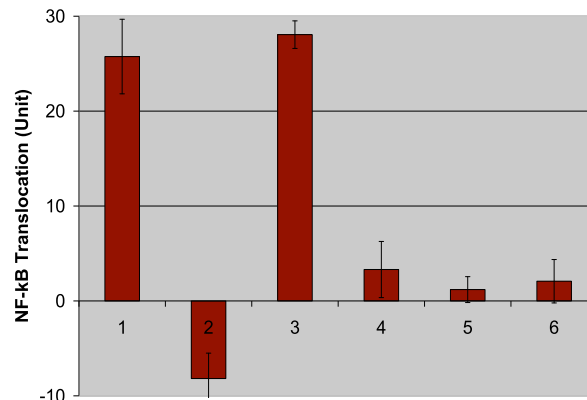
**Statement of Purpose:** Inflammation is a natural biological response to harmful stimuli such as pathogens and cell debris. Its purpose is to clean up the wound site for subsequent healing to take place. However, inflammation can also be tuned to promote disease states, such as chronic inflammatory diseases, some of the autoimmune diseases, and cancer<sup>1</sup>. Recent works toward understanding wound healing have suggested that perhaps modulating inflammation at an injury site could lead to better wound healing response<sup>2</sup>. For regenerative medicine purpose, modulating inflammation in the microenvironment by inhibiting soluble mediators of inflammation can protect therapeutic stem cells<sup>3</sup> from initiating cell death program and promote macrophages to adopt M2 phenotype<sup>4</sup>. Hyaluronic acid (HA) was chosen as our materials for the scaffold construct, because of biocompatibility and bioactivity profile and its potential for chemical modifications. We covalently attached anti-interleukin-1b monoclonal antibodies (mAb) to high-molecular weight HA, and in-vitro characteristics of the conjugates were evaluated.

**Method:** Hyaluronic acid (HA,  $\sim 1.6 \times 10^6$  g/mol), *N*-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), and 4-(Dimethylamino)pyridine (4-DMAP) were purchased from Sigma-Aldrich and used as received. Acryloyl-PEG-*N*-hydroxysuccinimide (ARCL-PEG-NHS, 3.4 kDa) was purchased from Nectar Therapeutics. Monoclonal anti-human IL-1b antibody and IL-1b (IL-1F2) were purchased from R&D Systems Inc (Minneapolis, MN). HA-mAb conjugates were synthesized by a two-step reaction. HA-*N*-hydroxysulfosuccinimide was first synthesized followed by the carbodiimide-mediated coupling of mAb to HA. The conjugates were purified by saturated ammonium sulfate solution followed by dialysis. The conjugates were characterized by polyacrylamide gel electrophoresis to quantify HA concentration and fluorescence immunosorbent assay to measure antibody concentrations. The Octet system (ForteBio Corp.) was utilized to measure HA-mAb binding affinity for IL-1b. The results were analyzed by the ForteBio analysis program that generated the best-fit binding isotherm and the association rate  $k_{on}$  and dissociation rate  $k_{off}$  were calculated from the isotherm. The biological activities of the conjugates were measured using ArrayScan VTI imaging cytometer (Cellomics, Pittsburgh, PA). PMA-differentiated THP-1 macrophages were stimulated according to specified conditions, and NF- $\kappa$ B translocation was quantified by compartmental analysis software.

**Results:** The degrees of functionalization of the conjugate is analyzed to be one antibody for every seven HA chain. The binding affinity of the conjugates were measured to be comparable to that of the native antibodies. (Fig 1.) The conjugated antibodies were still biologically active after the coupling chemistry. (Fig 2.)



**Fig 1.** Left to right: Association, dissociation, and residuals.  $kD \sim 100pM$ .



**Fig. 2.** (1) 100ng/ml IL-1b, (2) fresh media (3) 0.1%wt HA+100ng/ml IL-1b, (4) 0.1%wt HA (5) 10 $\mu$ g/ml mAb+100ng/ml IL-1b, and (6) 0.1%wt HA-mAb conjugate+100ng/ml IL-1b.

**Conclusion:** Hybrid biopolymers composed of hyaluronic acid and monoclonal antibodies against IL-1b were successfully synthesized. The HA-mAb conjugates are equally capable of binding to IL-1b and inhibiting IL-1b activity as unconjugated mAb in vitro. This material will next be examined in vivo.

### Reference:

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