Local Regulation of Cytokines with Antibody-Functionalized Materials

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Statement of Purpose: Fetal wounds are capable to regenerate without forming scars, while scar formation is the inevitable result of adult wounds. Inflammation plays a critical role in wound healing process, as it prepares the wound site for the subsequent repair mechanism to begin. In order to promote better healing outcome, we hypothesize that native healing trajectory can be altered in favor of tissue regeneration by modulating inflammation early in the wound healing process. Furthermore, recent studies[1,2] have discovered that macrophages, a major player in immune system, are capable to adopt M1 or M2 phenotypes in response to stimuli in the microenvironment. M1 macrophages secrete cytokines that promote inflammation, while M2 macrophages release growth factors to promote repair mechanism. Increasing presence of M2 macrophages could potentially be beneficial to tissue regeneration by removing factors that stimulate M1 phenotype. In this study, we functionalized hyaluronic acid (HA) with monoclonal antibodies that are capable of neutralizing the activities of pro-inflammatory cytokines, including TNFa and IL-1b. *In vitro* characterizations confirmed that the biological activities of these antibodies were retained following conjugation. Preliminary *in vivo* results suggested that the mAb-HA conjugate was biologically active. It inhibited macrophage infiltration to the wound site and promoted M2 macrophage phenotype.

Methods: Anti-IL1b and anti-TNFa monoclonal antibodies were purchased from R&D systems. Antibodies were covalently attached onto 1.6MDa hyaluronic acid (Aldrich) through amide bond formation. The conjugated material was precipitated with saturated potassium sulfate solution followed by dialysis against water. Binding affinity of the product against specific target was measured using Octet system manufactured by ForteBio. Biological activity of the conjugate was determined using imaging cytometry technique that measures the amount of NF-kB nuclear translocation following cytokine stimulation. In vivo studies were performed on Sprague Dawley rats using incision wound models. The rats were sacrificed four days after the surgery, and the tissues were stained with hematoxylin and eosin stain, CD68, CD163, and CCR7 specific antibodies. The number of cells stained positive with specific antibodies was counted in specified regions of the wounds under optical microscope.

Results: *In vitro* imaging cytometry assay demonstrated the same level of IL-1b activity inhibition by the conjugate as that by the unmodified antibodies. *In vivo* results showed less CD68+ cells in the wound environment with HA-mAb conjugate treated wounds comparing to pure HA and saline treated wounds. (Fig 1 & 2) About same 10 percent of CD68+ cells were CCR7+ in both HA-mAb and pure HA treated wounds, while saline treated wounds had much higher percent (around 40%) of CCR7+ cells among CD68+ population. (Fig 2)

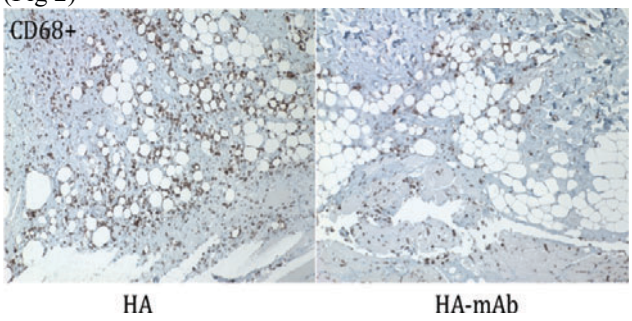

Figure 1. Images of tissue stained by CD68 antibodies.

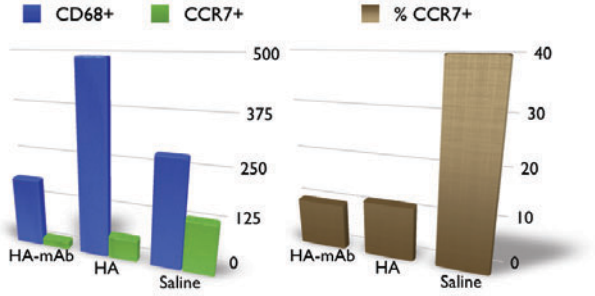

Figure 2. CD68+ and CCR7+ cells were counted to quantitatively analyze the wound condition.

Conclusions: *In vitro* results suggested that neutralizing activity of the antibodies was retained after conjugation chemistry and the conjugate would be active *in vivo*. The *in vivo* data demonstrated a reduction of the amount of macrophages at the wound site treated with the conjugate comparing to saline and HA treated wounds. This suggested that the antibodies were able to inhibit factors that direct macrophage infiltration to the wound site. HA and the conjugate were both able to inhibit macrophages to adopt M1 phenotype, CCR7+ cells, in contrast to saline treated wounds. This observation suggested that HA was able to promote M2 phenotype of macrophages, and this supports the observations that HA is a material that promotes healing. Further *in vivo* experiments will be performed to show repeatability and significance of these results. Physical evidence of better healing outcome will also be identified in the follow-up experiments.

Reference:

1. Gordon S. *Alternative activation of macrophages.* **Nat Rev Immunol.** 2003;3: 23-35
2. Mantovani A. *Macrophage diversity and polarization: in vivo veritas.* **Blood.** 2006;108(2): 408-409