The Effects of Removing Unwanted Cytokines from PRP on Human Articular Cartilage

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INTRODUCTION**:** Platelet Rich Plasma (PRP) is an orthobiologic that is commonly used to treat musculoskeletal injuries. Due to PRP's high concentration of platelets and proteins involved in the wound healing process, it has the potential to enhance repair. However, PRP also contains a significant amount of pro-inflammatory cytokines which could impair wound healing. Our group developed an antibody functionalized microsphere technology that can specifically bind target cytokines. Through *in vitro* studies we have demonstrated the capabilities and specificity of this technology for use in complex protein solutions such as PRP. This study aims to evaluate the effect of pro-inflammatory molecules on human articular cartilage and understand whether the removal of these pro-inflammatory molecules from the culture environment could improve tissue health.

METHODS**:** Polystyrene microspheres (Bang Laboratories) were functionalized with antibodies specific to inflammatory proteins (IL-1β and TNF-α), using carbodiimide coupling. Functionalized microspheres were then added to a single protein solution (1% BSA in PBS) or complex protein solution such as platelet lysate, spiked with target protein, and allowed to react for 1 hour at room temperature. Supernatant was analyzed with ELISA to determine the concentration of remaining cytokine. Immunofluorescent staining and imaging were used to ensure the removed cytokine was bound to the surface of the microspheres.

To evaluate the effect of pro-inflammatory cytokines on musculoskeletal tissue an *ex vivo* culture model was used. This model included a 2mm biopsy of human articular cartilage obtained from total knee arthroplasties. Tissue was cultured with 5ng, 10ng, 15ng or without IL-1β or TNF-α for 2 weeks. Samples were analyzed through histology.

RESULTS**:** 15ug of IL-1β monoclonal antibody functionalized microspheres successfully removed 1ng of IL-1β from both a simple saline solution and platelet lysate. 15ug of TNF-α polyclonal antibody functionalized microspheres successfully removed ~600pg of TNF-α from a simple saline solution (Fig. 1). Immunofluorescence confirmed the cytokines were bound to the surface of the microspheres (Fig. 2).

The addition of IL-1β or TNF-α to our *ex vivo* culture system showed detrimental effects on tissue health. This was demonstrated through glycosaminoglycan and proteoglycan loss, visualized with Safranin-O histological staining (Fig. 3).

DISCUSSION: The results of this study confirm the effectiveness of our functionalized microsphere technology, and that pro-inflammatory cytokines have a detrimental effect on human articular cartilage. We are currently conducting studies to understand whether the removal of cytokines from culture will allow tissue to recover back to a healthy state.

SIGNIFICANCE AND CLINICAL RELEVANCE: PRP therapy is currently being used in the clinic to treat musculoskeletal injuries such as osteoarthritis and tendinopathies. However, the efficacy of this technology is debated as the clinical outcome varies significantly between patients. This variability is believed to be partly due to the lack of consistency in composition between patients. Our group has demonstrated the capabilities of our functionalized microsphere technology to specifically bind and remove unwanted pro-inflammatory cytokines from complex protein solutions such as PRP. In addition, we have demonstrated the detrimental effects these cytokines have on the health of musculoskeletal tissue. This technology has the potential to reduce compositional variability between patients and increase efficacy and the regenerative potential of PRP.

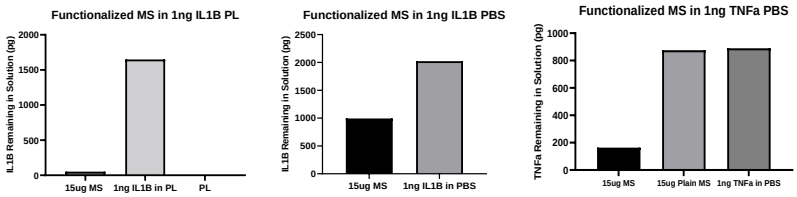

Fig. 1: ELISA supernatant analysis after microsphere incubation in 1ng IL-1β solution (left and middle) or 1ng TNF-α solution (right). 1ng spiked solutions were used as controls. Non-functionalized (plain) MS were used as an additional control for TNF-α.

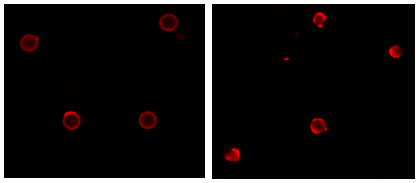

Fig. 2: Red fluorescence confirming presence of IL-1β (left) and TNF-α (right) on surface of microspheres following incubation.

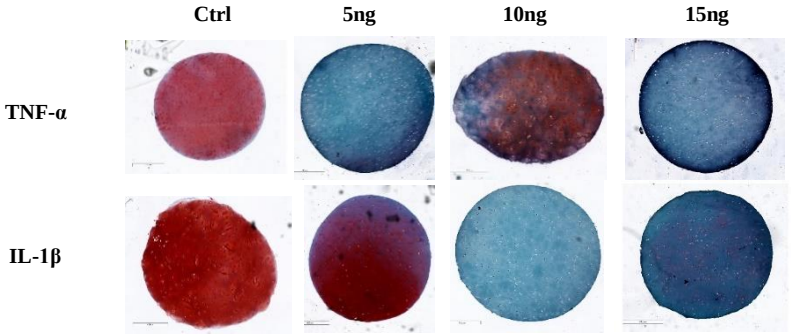

Fig. 3: Histological images of articular cartilage tissue cultured in the presence of IL-1β or TNF-α at various concentrations. Sections are stained with Safranin-O (reds staining for proteoglycans and Fast Green with a Hematoxylin counterstain.