Red Blood Cell Interaction with Hyaluronan is Modulated by Molecular Weight in a Hemarthrosis Model

Neo Esinathi Nyoni[1], Courtney Wright[1], Gerard A. Ateshian[3,1], Roshan P. Shah[2], Clark T. Hung[1,2]
[1]Department of Biomedical Engineering, [2]Department of Orthopedic Surgery, [3]Department of Mechanical Engineering, New York, NY
nen2122@columbia.edu

Disclosures: . NN (N), CW (N), GAA (N), CTH (Editor, ORR; Associate Editor JOR, MTFBiologics)

INTRODUCTION: Intra-articular bleeding of the diarthrodial joint, or hemarthrosis, accompanying joint trauma has deleterious effects on synovial joint tissues (**Figure 1**)[1]. While extravasated whole blood is rapidly cleared out by the barrier function of the synovium, even a singular bleeding episode has been demonstrated to initiate the inflammatory cascade leading to downstream tissue changes such as hyperplasia and neo-angiogenesis. Furthermore, *in vivo* models of acute joint bleeds as well as *ex vivo* analyses of blood-affected human cartilage have revealed rapid and irreversible changes to cartilage and synovium that are representative of early-stage osteoarthritis (OA). Blood-induced tissue changes include collagen loss and proteoglycan release from cartilage, hemosiderin deposits, and ferroptosis. In addition, the pro-inflammatory changes to synovial tissue architecture include pannus formation, hypertrophy, and villous proliferation as evidenced by histopathological staining[3,4]. Hyaluronic acid (HA), a major component of synovial fluid (SF), contributes to joint lubrication and homeostasis. In healthy joints, HA exists primarily as high molecular weight (HMWHA, >1000 kDa), whereas OA SF contains higher concentrations of low molecular weight HA fragments (LMWHA, <500 kDa). This shift may influence cell–surface interactions, including those involving red blood cells (RBCs) following trauma-induced hemarthrosis. RBC adhesion is carried out, in part, by fibroblast-like synoviocyte (FLS) membrane-bound CD44 receptors binding HA, (**Figure 1,2)**. RBC adhesion to intra-articular tissue has been shown to contribute to inflammation, joint degeneration, and OA progression. This suggests that changes to synovial fluid during OA progression may play a role in RBC adhesion to synovial tissue, however, these adhesive interactions remain understudied. Here, we investigate the adhesive response of RBC in suspension culture under conditions that mimic the native and OA synovial joint environment. We hypothesize that HA degradation, as observed in osteoarthritic and inflammatory states, alters the synovial environment to favor RBC adherence.

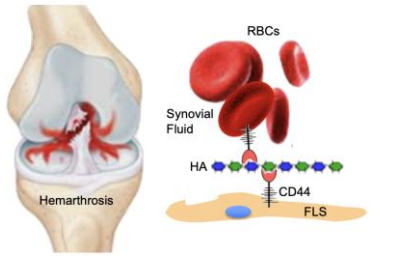

Figure 1. Schematic of hemarthrosis and CD44-HA binding in synovial fluid; potential of HA also binding to CD44 on FLS.

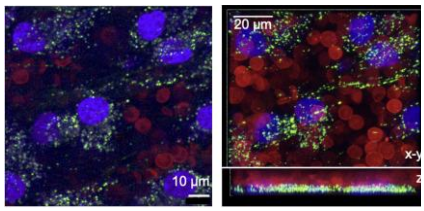

Figure 2. Immunocytochemistry of 0.4% v/v RBC (PKH26-red) parachuted over FLS- CD44 (green) and Hoechst (blue) for 1 hour before imaging at 63X.

METHODS: RBC Adhesion: Engineered SF (eSF) was prepared using four different HA molecular weights (3000 kDa, 1000KDa, 250 kDa, 10-25 kDa) resuspended in aMEM to reflect healthy (3000 – 1000k Da) and diseased conditions (250 – 10kDa). eSF was used to pre-treat glass slides for 1 hr. Then 0.001% v/v RBC (New York Blood Center) were plated onto the glass slides and incubated for 15 min. Parallel-plate flow chamber unidirectional fluid shear was performed by flowing Hank's Buffered Salt Solution over the RBC monolayer for a 15 sec timelapse at 20 dyne/cm^2 to assess the number of adherent cells (n = 3). Immunocytochemistry: Healthy FLS were isolated from synovium explants (MTFBiologics) and were plated onto glass bottom dishes and grown to confluency. The FLS monolayer was then stained for CD44 and Hoechst. 0.4 % v/v RBC were stained with PKH26 and then parachuted over the monolayer to assess for CD44-mediated adhesion to the FLS. Statistics: One-way ANOVA with Tukey's HSD post-hoc test ($\alpha = 0.05$) was used to determine significant differences in the percentage of RBCs adhered.

RESULTS: RBC Bind to FLS via CD44: Imaging of RBCs parachuted onto an FLS monolayer shows that CD44 is expressed on the surface of FLS. This provides a possible mechanism for RBC adhesion, mediated by HA found in synovium fluid, (**Figure 2**). HA MW Mediates RBC Adhesive Properties: RBC adhesive properties differ depending on the MW of the HA treatment. Highest rates of adhesion were seen with the 250 kDa HA treatment (55.06% adhesion), followed by the 10-25 kDa HA treatment (32.68% adhesion), and the 1000 kDa HA treatment (3.17% adhesion). The untreated group and largest MW treatment (3000KDa) showed no adherence to the plate, (**Figure 3**).

DISCUSSION: Under fluid shear conditions, RBC adhesion was significantly greater in the presence of LMWHA (250 kDa - 10 kDa) compared to HMWHA (3000 – 1000 kDa). These findings are consistent with the hypothesis that HA degradation, as observed in osteoarthritic and inflammatory states, alters the synovial environment to favor RBC retention. Reduced HA size may diminish steric hindrance, or increase HA-CD44 binding affinity, collectively promoting RBC attachment. The highest adhesion occurred at 250 kDa rather than the smallest HA fragments (10–25 kDa), suggesting an optimal HA size that facilitates RBC interactions. These results align with previous reports showing that hyaluronan fragments promote distinct cell–matrix interactions and amplify inflammatory responses in reactive extracellular matrix environments[2,5]. Once adhered, RBCs can undergo lysis, releasing hemoglobin and iron derivatives that perpetuate oxidative stress and ferroptosis in cartilage. This data supports a model in which trauma-induced hemarthrosis or HA fragmentation during OA progression creates a permissive environment for RBC adhesion, triggering downstream inflammatory cascades that exacerbate joint degeneration. Future work will investigate the molecular mechanisms of RBC–HA interaction, including the role of CD44 signaling, and evaluate whether HA supplementation or viscosity-modifying therapies can mitigate RBC adhesion in post-injury or OA joints.

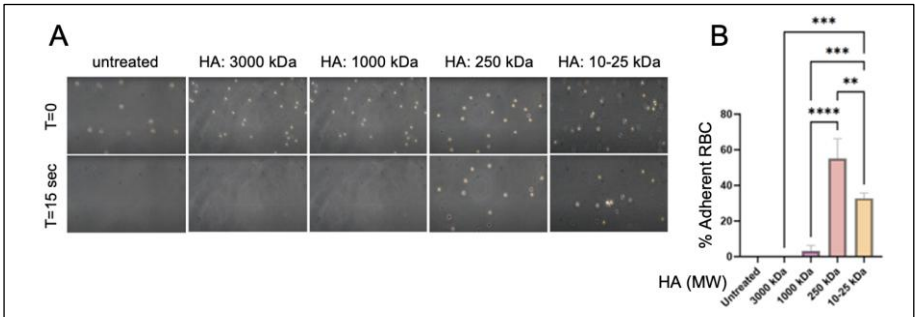

Figure 3. A) RBC cultured on HA of varying MW before and 15 seconds after applied fluid shear (20 dynes/cm2). B) Proportion of RBCs remaining attached per HA molecular weight (MW). **p<0.01, ***p<0.001, ****p<0.0001, n=3 slides.

CLINICAL RELEVANCE: This study underscores the importance of preserving HA integrity after joint injury and supports therapeutic strategies aimed at restoring HMWHA to reduce the deleterious effects of hemarthrosis.

REFERENCES: [1]Hardaker+Evaluation of acute traumatic hemarthrosis of the knee joint. South Med J, 1990. 83(6): p. 640-4. [2]Monslow J +Hyaluronan 2015. [3]Nieuwenhuizen+ Haemophilia, 2013. 19(4): p. e218-e227. [4]Roosendaal, G.+ The Journal of Bone & Joint Surgery British Volume, 1998. [5]Petrey & de la Motte. Hyaluronan, a crucial regulator of inflammation 2014.