ABSTRACT

Aging is strongly related to the development and progression of cardiovascular disease, and its associated thrombotic complications. While age-related increases in stiffness of the vasculature and their effects on flow shear stress have been extensively studied, little attention has been directed towards the effect of aging on the preeminent cell in thrombosis: the platelet. Changes in platelet mechanical properties and subsequent shear stress mechanotransduction over one's lifetime are overlooked but crucial processes that become prominent in an aging population. Regrettably, this oversight has led to development of antiplatelet therapy that is not specifically targeted to counter elevated pathological shear stresses associated with age-associated stiffer vasculature, featuring more prominently in cardiovascular diseases and implanted blood recirculating devices, and may introduce the other nemesis of hemostasis: bleeding. Therefore, there exists a ***critical need*** to identify platelet mechanical properties and mechanisms for healthy individuals across the lifespan which may then lead to development of safe and effective targets for antiplatelet therapy.

Our ***overall objective in this proposal*** is to study age-related changes in platelet mechanical properties that directly affect their function and how they are regulated by phosphoinositide 3-kinase (PI3K), a well-known mediator in mechanotransduction and promising target for antithrombotic therapies. We hypothesize that age-related changes in PI3K activity promote downstream signaling events, increasing platelet stiffness and shear-mediated platelet activation (SMPA). We formulated this hypothesis in part, based on several observations: (1) PI3K activity was elevated in adult platelets stimulated with thrombin receptor activator peptide 6 (TRAP-6) or high shear stress, as compared to umbilical cord platelets; and (2) phosphatidylserine scrambling, a thrombogenic marker, in sheared cord platelets was intracellular calcium (Ca^{2+})-independent compared to adult platelets, suggesting a calcium-regulated developmental disparity in SMPA, which is potentially regulated by PI3K. Our team, including Drs. Jawaad Sheriff (PI, BME), Wadie Bahou (Co-PI, Medicine/Hematology), Danny Bluestein (Co-PI, BME), and Yuefan Deng (Co-PI, AMS), is particularly ***well prepared*** to undertake the proposed research because of our extensive research track record in the fields of shear-mediated thrombosis, platelet regulatory mechanisms, and machine learning (ML). We will attain the overall objective by pursuing the following ***aims***:

Aim1: Characterize age-specific platelet mechanical properties. We ***hypothesize*** that platelet stiffness increases with subject age and is correlated with an increase in structural protein content. Stiffness of platelets from healthy representatives of four age groups (0, 18-39, 40-65, >65 years) will be calculated from their deformation in a microfluidic extensional flow device and correlated with structural actin and tubulin content quantified using fluorescence microscopy. Stiffness measurements repeated with inhibition of PI3K and intracellular Ca^{2+} will elucidate their role in age-specific platelet stiffness. An ML framework using a recurrent neural network (RNN), will be trained to identify relationships between age, protein content, stiffness, and PI3K activity.

Aim 2: Identify and characterize age-specific shear-mediated platelet function. We ***hypothesize*** that shear-mediated platelet activation, aggregation, and adhesion increases with age and in a PI3K-dependent manner. Platelets, with and without PI3K and intracellular Ca^{2+} inhibition, will be exposed to physiological and pathological shear stresses. Morphology, SMPA, PI3K signaling, and aggregation will be quantified using scanning electron microscopy, a prothrombinase assay, and flow cytometry. Adhesion kinematics in microchannels will be measured using ML-guided image segmentation. An RNN-based network will be trained to identify relationships between age, function, adhesion kinematics, and PI3K activity.

The proposed research addresses a key mechanotransduction pathway driving changes in platelet response to pathological flow conditions across the lifespan, highlighting PI3K as a key regulatory driver. Upon successful completion, we ***expect*** to fill a critical void in our understanding of age-specific shear-mediated platelet response. The results of this seed grant will form the basis of two NIH R01 proposals to the NHLBI and NICHD, as well as a proposal to the NSF, to address our ***overarching vision:*** to aid in the development of age-specific antiplatelet therapy that mitigates thrombotic complications arising from pathological conditions characterizing cardiovascular diseases and blood recirculating devices.