CELLULAR BIOMECHANICAL SYSTEMS FOR STUDYING AND DIAGNOSING HEMATOLOGIC DISEASES

David R. Myers, PhD[*,**] and Wilbur A. Lam, MD, PhD[*,**]
**Department of Pediatrics, Division of Pediatric Hematology/Oncology, Emory University School of Medicine, Georgia, USA*
***Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, USA*

State-of-the-art engineering and bionanotechnology techniques have enabled the development of novel *in vitro* platforms for the study and diagnosis of disease. Here we discuss how micromechanical systems can be applied to basic and translational research in clinical hematology. Specifically, we have developed microsystems to investigate platelet biomechanics at the single cell level and blood cell mechanics in microvascular hematologic diseases.

MICROMECHANICS OF PLATELET CONTRACTION

Blood clots are composed of fibrin, platelets, and other blood cells and proteins, which interact to prevent bleeding. Previous studies on clot formation have shown that the mechanical properties of clots have direct effects on patient health and that alterations of those clot mechanics are associated with disease. For example, clots are 50% stiffer and more resistant to dissolution in young patients with post-myocardial infarction than clots from healthy controls. Conversely, clots are softer and more prone to dissolution in patients with bleeding disorders [1,2]. As such, understanding the mechanical properties of clots is vital to understand hemostasis and thrombosis. As platelets drive this contraction phenomenon, single platelet measurements are required to obtain a mechanistic understanding of the retraction process and to identify specific therapeutic targets for disease states in which platelet/clot retraction is pathologically altered. In addition, as fibrin has recently been shown to have extremely complex material and mechanical properties [3], single platelet studies would decouple the effects of fibrin from platelets when examining clot mechanics. However, few studies have focused on the biomechanical role of platelets in clot formation and clot mechanics, especially at the single cell level. The key barrier which has prevented the study of single platelets has been the lack of technology with the sufficient precision and sensitivity to both manipulate and measure individual platelets. To that end, we recently published the first study investigating platelet contractility at the single cell level using a custom-built sideview atomic force microscope (AFM) [4]. In our system, a mechanically well-defined, fibrinogen-coated cantilever is brought into contact with a platelet and then brought to a fibrinogen-coated surface (Figure 1A). As the platelet contracts, the resulting deflection of the cantilever is measured with high accuracy to determine the force applied by the platelet. We observed that both the loading rate (Figure 1B) and maximum contraction force exerted by single platelets (Figure 1C) were a function of the mechanical stiffness of the cantilever. Ours is the first reported data measuring platelet contraction at the single cell level and reveals that platelets are extremely "strong" contractile machines, especially when taking account their small size. In addition, we discovered that platelets can "sense" their mechanical microenvironment, adjusting their contractility accordingly. This work provides new insight into platelet physiology form a mechanical perspective and has profound implications for understanding, diagnosing, and treatment of hematologic and cardiovascular disease.

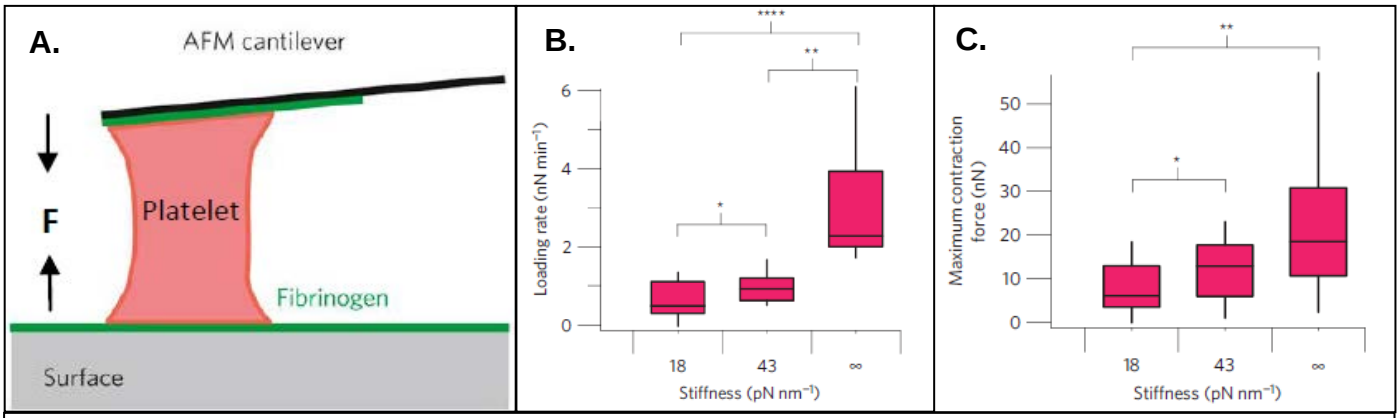

Figure 1. (A) Measuring platelet contractility in a cantilever-based system. F = direction of contractile force. (B,C) Distribution of contraction rates (B) and stall forces (C) for platelets pulling against cantilevers with stiffnesses of 18 and 43 pN/nm, and in an isometric clamp. Medians, quartiles and 90/10 percentile levels are shown, and the *,**,*** represent significant differences with *P* values of <0.05, <0.01, <0.001, respectively. (from Lam et al, *Nature Materials*, 2011)

MICROENVIRONMENTAL GEORMETRY GUIDES PLATELET ADHESION AND SPREADING: A QUANTITATIVE ANALYSIS AT THE SINGLE CELL LEVEL

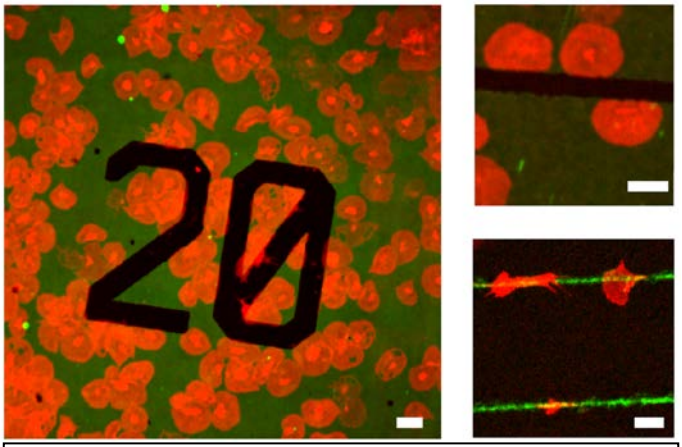

Figure 2. Platelets (red) spread on either collagen or fibrinogen-adsorbed micropatterns (green) on glass surfaces and follow the geometric boundaries of the protein micropattern with high fidelity. Non-patterned areas of glass are blocked with BSA and appear dark. (Left panel) Platelets spread along the edges of an arbitrary geometric protein micropattern. (Upper right) Platelets spread and conform to the boundaries of a wider micropatterned protein "stripe". (Lower right) On thinner micropatterns, the fidelity of platelet spreading along the micropattern boundaries is decreased.

To activate clot formation and prevent massive bleeding, platelets adhere and spread onto sites of vascular injury. Although this process is well-characterized biochemically, how the physical and spatial cues in the microenvironment affect platelet adhesion and spreading remain unclear. We recently published a study in which we applied deep UV photolithography and protein micro/nanostamping to quantitatively investigate and characterize the spatial guidance of platelet spreading at the single cell level and with nanoscale resolution [5]. Platelets adhered to and spread only onto micropatterned collagen or fibrinogen surfaces and followed the microenvironmental geometry with high fidelity and with single micron precision (Figure 2). Using micropatterned lines of different widths, we determined that platelets are able to conform to micropatterned stripes as thin as 0.6 μm and adopt a maximum aspect ratio of 19 on those protein patterns. Interestingly, platelets were also able to span and spread over non-patterned regions of up to 5 μm, a length consistent with that of maximally extended filopodia. This process appears to be mediated by platelet filopodia that are sensitive to spatial cues. Finally, we observed that microenvironmental geometry directly affects platelet biology, such as the spatial organization and distribution of the platelet actin cytoskeleton. Our data demonstrate that platelet spreading is a finely-tuned and spatially-guided process in which spatial cues directly influence the biological aspects of how clot formation is regulated and represent a new paradigm in the physiology of hemostasis.

IN VITRO MODELS OF MICROVASCULAR OCCLUSION AND THROMBOSIS IN HEMATOLOGIC DISEASES USING MICROFLUIDIC TECHNOLOGY

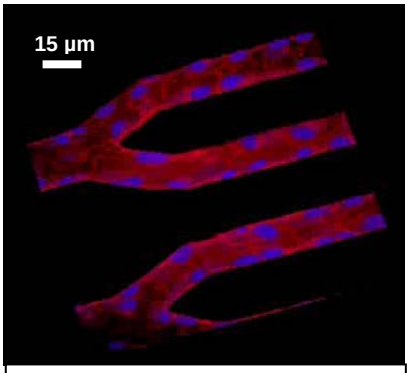

Figure 3. microvasculature-on-a-chip: 3-D culture of human endothelial cells in microfluidic channels (cell membrane – red, nuclei – blue)

In hematologic diseases, such as sickle cell disease (SCD) and hemolytic uremic syndrome (HUS), pathological biophysical interactions among blood cells, endothelial cells, and soluble factors lead to microvascular occlusion and thrombosis. We have recently developed an *in vitro* "endothelialized" microfluidic microvasculature model that recapitulates and integrates this ensemble of pathophysiological processes (Figure 3) [6]. Under controlled flow conditions, the model enabled quantitative investigation of how biophysical alterations in hematologic disease collectively lead to microvascular occlusion and thrombosis. Using blood samples from patients with SCD, we investigated how the drug hydroxyurea quantitatively affects microvascular obstruction in SCD, an unresolved issue pivotal to understanding its clinical efficacy in such patients. In addition, we demonstrated that our microsystem can function as an in vitro model of HUS and showed that shear stress influences microvascular thrombosis/obstruction and the efficacy of the drug eptifibatide, which decreases platelet aggregation, in the context of HUS. These experiments establish the versatility and clinical relevance of our microvasculature-on-a-chip model as a biophysical assay of hematologic pathophysiology as well as a drug discovery platform.

Overall, our combined expertise in clinical hematology and cellular mechanics provide our laboratory with a unique perspective that will advance the fields of both engineering and clinical medicine. Our interdisciplinary work provides the basis for novel assays and therapeutic strategies for patients with hematologic and cardiovascular disorders.

References

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