Project Summary/Abstract

The broad, long-term objective of the proposed research is to determine the mechanisms regulating fibrin and fibrinogen function, with the goal of improving the diagnosis and treatment of cardiovascular disease. In so doing, we plan to train the next generation of scientists with an interdisciplinary skill set. In this specific proposal, we will study the polymerization, structural properties, and enzymatic digestion of fibrin fibers. Fibrin fibers polymerize into a 3-D gel after thrombin converts fibrinogen to fibrin. The structural properties of fibrin are regulated by many factors such as enzyme concentrations, and altered fibrin structures have been linked to pathologies such as diabetes and COVID-19. Although fibrin polymerization has been studied for decades, there remains a crucial gap in our understanding of how early fibrin polymers transition into a fully-formed gel. Because the transitional period likely determines the final gel structure, this gap in knowledge prevents a direct association between polymerization processes and the biochemical and pathological conditions that result in altered clot structures. In this research project, we will utilize recently developed approaches to determine mechanisms of fibrin polymerization. We hypothesize that these polymerization processes propagate throughout the entire coagulation process, affecting both the final gel structure and fibrin's resistance to enzymatic digestion. Testing this hypothesis will rely heavily on undergraduate student researchers, providing training in molecular biology, biochemistry, biophysics, mathematical modeling, and blood coagulation.

Specific Aim 1: Make direct observations of fibrin polymerization and quantify polymerization processes and kinetics. Using high framerate, high resolution fluorescent microscopy techniques combined with advanced image analysis tools, and atomic force microscopy, we will observe and quantify fibrin polymerization. In so doing, we will measure rates of fiber growth, changes in fiber stiffness, and branch point formation for the first time. Our experimental results will be tested against mathematical models to differentiate competing hypotheses about these processes.

Specific Aim 2: Validate fibrin structural properties with multiple techniques and correlate the final structural properties of fibrin gels with the early stages of fibrin formation. Using two or more different techniques, we will measure fibrin structural properties including fibrin fiber length and diameter as well as network pore size and branch point density. These network structural properties will be correlated with polymerization processes to identify which aspects of polymerization determine fibrin structures.

Specific Aim 3: Determine how early clot structures affect lysis. We will measure the fibrinolysis rates of whole networks and individual fibers within the network using novel fluorescence-based microscopy approaches. We will correlate lysis rates with polymerization processes such as fiber growth rates, to test our hypothesis that the entire hemostatic process is determined by the early stages of polymerization.

Project Narrative

Blood clots form at the site of injuries, preventing the loss of blood during wound healing. Fibrin fibers hold blood clots together until they are broken down by enzymes after the wound healing process. In this project, we seek to study the polymerization of fibrin fibers to determine the mechanisms of fiber growth and branching and to understand how these processes affect the final clot structure and its enzymatic breakdown.