Experimental measurement of spectral transmission of platelet thrombus in comparison to whole blood

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Introduction: Thrombosis is the pathological formation of platelet aggregates, or thrombi, which occlude blood flow causing strokes and heart attacks–leading causes of death in developed nations. While current commercial and research methods are able to evaluate thrombosis through the formation of small, concentrated platelet thrombi, analysis of such thrombi is limited by the need for sample preparation, fluorophores, microscopy, and/or image post-processing [1]. Thus the field would benefit from the development of a label-free and microscope-free method for thrombus detection in whole blood. Previous work has shown the detectable differences in light transmission through *dilute* platelet solutions vs. whole blood are greatest at 500-600 nm [2]. In this work, we experimentally verified these transmission-based differences through *concentrated* platelets thrombi formed inside a microdevice vs. whole blood using spectrometry and two quasi-monochromatic optical systems.

Materials and Methods: Platelet thrombi were formed within PDMS microfluidic devices from whole porcine blood as described in prior work [3]. Relative differences in light transmission (ΔT) through platelet thrombi vs. through whole blood (n=18 samples) were measured by over the visible spectrum (See Figure 1). Measurements of ΔT were taken as $\Delta T=[(I_t-I_b)/I_b)\ 1\ \square]$ where I_t is the intensity of light transmitted through platelet thrombi, I_b is the intensity of light transmitted through whole blood, and λ indicates wavelength. From these spectrometry experiments, we further verified the optimal wavelength by with quasi-monochromatic sources and photodiodes, one near the maximum ΔT at 590 nm and the other at 671 nm which we had used in previous work [3]. Measurements of light transmission were calculated using the signal-to-noise ratio, defined as $SNR=[(\mu_t - \mu_b)/ 1\ \square_b)]$, where σ_b and μ_b are the standard deviation and mean of the transmitted intensity through whole blood, respectively, and μ_t is the mean transmitted intensity through the fully formed occlusive platelet thrombus.

Results and Discussion: Results from spectrometry showed the maximum difference in ΔT to be near 590 nm (13.2± 4.04, n=18), with a lower magnitude ΔT at 671 nm at (5.06±2.02, n=18) (See Figure 1). Consistent with spectrometer predictions, the SNR of the monochromatic system at 590 nm (672.72, n=1) were much larger than those measured at 671 nm (87.4±146, n=68). Large standard deviations are attributable to natural variations in size and density of thrombi detected.

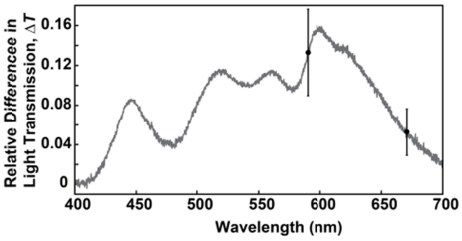

Figure 1. Relative difference in transmission (ΔT) between platelet thrombi and whole blood was measured by spectrometry over the visible spectrum (400-700 nm). Optimal ΔT occurs at wavelengths near 590 nm in contrast to those at wavelength of 671 nm. Results shown are averaged readings for n=18 occlusive thrombi samples vs. whole blood, with error bars indicating standard deviations.

Conclusions: Signal-to-noise ratio of thrombus detection by light transmission in the 400-700 nm range was optimal at 590 nm from experiments using broadband spectrometry and two quasi-monochromatic optical systems. The optical detection described is applicable to current commercial and research methods.

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