Pressure Generation as a Metric to Evaluate Vascular Graft Thrombogenicity *In Vitro*

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Introduction: Small-diameter (<6 mm) vascular grafts fail in the short term primarily due to thrombus formation that prevents blood flow[1]. Many strategies effectively assess the thrombogenicity of graft *materials*, but few probe this attribute by assessing graft function as it relates to patency. Furthermore, most systems suffer from a low signal-to-noise (SNR) ratio stemming from nontrivial activation of blood from contact with non-graft material[2].

Coagulation within a vessel results in both increased blood viscosity and decreased cross-sectional area of the conduit[1]. Both of these phenomena create a greater resistance to flow that can be quantified by measuring the increase in pressure proximal to the coagulating region. This metric is physiologically relevant, as it accounts for vessel-specific geometry and directly assesses vessel patency. Here, we present a system possessing a high SNR that is designed to monitor the pressure generated in response to purging grafts containing a fixed volume of fluid.

To model coagulation within the mock grafts, pressure was monitored while the viscosity of the solution and the fractional occlusion of the grafts were varied. In addition, stages of thrombosis were mimicked by purging fibrin glue at various degrees of polymerization from mock grafts. A system sensitive to these changes in the physical properties of blood would be able to monitor the progress of coagulation with high fidelity, permitting comparison of the thrombogenicities of vascular grafts.

Methods: *System Development*: Minimal lengths of fluorinated Tygon® tubing (3 mm ID) and 3-way connectors were used to connect a 5 mL syringe to two pressure transducers (MPX5100GP (less sensitive) and MPXC2011DT1 (more sensitive), FreeScale Semiconductor) and a mock vascular graft (Figure 1).

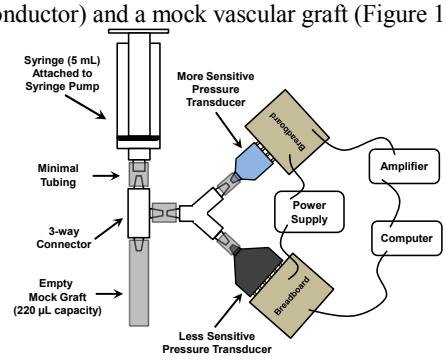

Figure 1: Overview of the devised system.

Pressure transducers were driven by a power supply supplying 5 V and output signals were recorded in LabView software. The signal from the more sensitive transducer was filtered and amplified by hardware prior to acquisition. Mock grafts were segments of the same Tygon® tubing cut to possess capacities of 220 µL.

Mimicking Thrombosis in Grafts: Increased viscosity during coagulation was mimicked by generating a series of glycerin solutions (0-100% in H2O with 10% increments). Fractional occlusion of grafts was simulated by reducing the cross-sectional area of mock graft outlets to 18, 9, 2 and 0% of its original value. Coagulation of blood was imitated using fibrin glue that was formulated to clot after reacting for 120 sec (0.25 U/mL bov. thrombin, 6 mg/mL fibrinogen in PBS). *Making Measurements*: A syringe pump was used to draw 200 µL of a glycerin or fibrin glue solution into a mock graft. Following a "dwell time" that depended upon conditions, data acquisition began and solutions were purged from grafts at 2.4 mL/min. Raw voltage signals were then processed and converted to pressures.

Results: Increasing viscosity and greater fractional occlusion were observed to generate significantly higher pressures while purging mock grafts, but delineation of conditions that produced either low (<5 mmHg) or high (>65 mmHg) pressures was impossible without acquiring data from both types of transducers simultaneously (Figure 2). When only the less sensitive transducer was monitored,

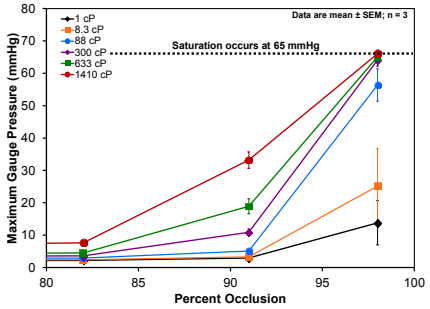

Figure 2: Max pressures while purging glycerin solutions from mock grafts of various fractional occlusions. Shown data acquired only from one (less sensitive) pressure transducer.

>80% fractional occlusion was required to generate significantly higher pressures except at extreme (>633 cP) viscosities.

The degree of fibrin glue polymerization strongly influenced the pressure generated when purging grafts. Prior to reaching the gel point, fibrin glue behaved similarly to water. Beyond the gel point, maximum theoretical pressures persisted until the clot was expelled or structurally compromised. Fibrin clots of greater strength (higher degree of crosslinking) resisted clot disruption for longer times under the same static pressure.

Conclusions: Quantifying differences in flow-induced pressure generation may be a valid and functionally relevant technique to monitor thrombosis in vascular grafts. Simultaneous use of pressure transducers with different sensitivities provided the necessary dynamic range to observe both low and high pressure regimes that exist during coagulation. Having observed coagulation-induced physical changes, future work will include observing the relative onset of these changes in blood-containing ePTFE vascular grafts to compare their relative thrombogenicities.

References:

1. Kannan, R.Y. JBMR. 2005 (74(1):570-581).
2. Roohk, HV. Trans. Am. Soc. Artif. Intern. Organs. 1977. (23(1):152-160).