Supplemental Material

Blood Sampling and Processing

Subjects for the study were healthy volunteers (ages between 18 and 60) not taking any medications known to alter hemostatic function and with no history of thrombotic or hemorrhagic disorders. Blood samples were obtained from a peripheral vein of the arm into evacuated tubes containing 3.2% sodium citrate anticoagulant. Per standard phlebotomy procedures, the first tube was always discarded. The remaining tubes were placed on a rocker and gently agitated for a minimum of 30 minutes at room temperature before further processing. Sample testing was performed by transferring the blood sample from a tube into a standard 3 ml syringe (Becton Dickinson, Franklin Lakes, NJ), which connects directly to a luer-lock fitting integrated on the Quantra cartridge.

Samples with modified levels of fibrinogen were prepared by mixing pooled normal plasma with an assigned fibrinogen level (George King Biomedical, Overland Park, KS) with fibrinogen-depleted plasma (Affinity Biologicals, Ancaster, ON) to obtain final fibrinogen levels of 75, 100, 150, 200, 250, and 286 mg/dL. This modified plasma was then added to the buffy coat and erythrocytes obtained by centrifugation (2000 RPM for 20 minutes at 4°C) of citrated whole blood.

To prepare samples with modified plasma factor levels, citrated whole blood samples were spun at 2000 RPM for 20 minutes to isolate the erythrocytes and platelets from plasma. The cells were then washed 4 times in phosphate buffer saline (PBS) by centrifuging for 5 minutes at 500 RPM before reconstitution with plasma deficient in human Factor VIII, X or XII (George King Biomedical).
Reagents

Kaolin powder (Avantor Performance Materials, Center Valley, PA) was suspended in a HEPES solution with stabilizers and used at a final concentration of 0.1 mg/ml in whole blood. Pacific Hemostasis Thromboplastin DS was obtained from Thermo Scientific (Waltham, MA) and used at a 1:50 dilution. The anticoagulant effect of sodium citrate was reversed with either 0.2 M calcium chloride or 0.2 M calcium acetate hydrate (Sigma Aldrich, St. Louis, MO). For every experiment presented here, reagents were loaded into each channel of the Quantra consumable cartridge and sealed with a layer of Polysil™ silicone transfer tape before test initiation.

Low molecular weight heparin (LMWH) enoxaparin sodium (Winthrop U.S., Bridgewater, NJ) was used at final concentrations of 0, 1, 2 and 4 U/ml in whole blood. Unfractionated heparin (Hospira Inc., Lake Forest, IL) was used at a concentration of 6.0 IU/ml to mimic a cardiac surgery patient on bypass. Heparin reversal was attained with 0.1-0.3 IU/ml of Heparinase I (Ibex Technologies, Mont-Royal, QC) or with polybrene (Hexadimethrine bromide, Sigma Aldrich). The monoclonal antibody abciximab (ReoPro®, Eli Lilly and Company, Indianapolis, IN) was used at final concentrations of 0, 2, 4, and 6 µg/ml.

A plasma-based control material was prepared to test the reproducibility of the technology and the overall performance of SEER Sonorheometry.

Comparative Studies

For comparison studies, a TEG 5000 analyzer was used. The TEG determines changes in sample viscoelastic properties by measuring the rotation of a cup around a pin suspended in the blood sample\textsuperscript{13}. The R parameter, measured in minutes, corresponds to the lag time before clot initiation, and MA, measured in units of millimeters, corresponds to the maximum stiffness of the
clot. The MA parameter was converted in units of Pascals (G parameter) using the transformation \( G = \frac{(500 \times MA)}{(100 - MA)} \), as previously shown in the TEG 5000 operator’s manual as well as by Lang et al. and Solomon et al.\textsuperscript{26,27}.

Additionally, a STart4 Hemostasis analyzer (Stago, Asnières-sur-Seine, France) was used with the STA Fibrinogen 5 kit and Owren-Koller buffer for measurements of fibrinogen concentration. Both the TEG 5000 and the STart4 systems were operated according to the manufacturers recommended guidelines.