Supplemental Material

Contact activation of blood coagulation on a defined kaolin/collagen surface in a microfluidic assay

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Supplemental Discussion

The interaction between kaolin and collagen was not significantly affected by the surface modification in this case. We prepared collagen/unmodified kaolin surface (medium surface concentration) and took picture of the surface under bright field after repeated wash with 0.1% BSA buffer. Surface coverage was found to be ~ 8% with the threshold tool in ImageJ (Supplemental Fig. 7), which is very consistent with the calculated surface coverage of collagen/modified kaolin (medium surface concentration, Figure 2B).

The 8-channal device has previously been characterized by Maloney et al (1). Flow rates in eight channels were confirmed to be equal by spiking a unique bead tracer in each inlet reservoir and quantifying the tracer bead concentration in the combined outlet of the device. The hemodynamics and transport conditions in each reactive interaction zone were shown to be statistically indistinguishable in 15 different experiments using 5 different devices.

Colace et al. (2) demonstrated a dramatic increase in shear rate under constant flow mode only occurs when full channel occlusion is approached. The embedded table in Figure 4 shows the occlusion times under constant flow mode are very close with little variation. The alteration in shear condition should be similar in all eight channels since all channels were approaching occlusion at roughly the same time.

Fibrin(ogen) signal was abolished by PPACK or high dose of CTI (Supplemental Fig. 6). There was no visible fibrin formation in the PPACK channel (Supplemental Fig. 8) indicating the fibrin(ogen) fluorescence is fibrin dependent.
Supplemental References

Supplemental Fig. 1. Fluorescent intensity vs. surface concentration (pg/μm²) for pure fluorescent kaolin surface. A linear dependency of fluorescent intensity of kaolin particles on its surface concentration was observed.

\[ y = 68.052x \]
\[ R^2 = 0.9581 \]
Supplemental Fig. 2. PPP was prepared from whole blood treated with both citrate (1:9 WB) and high CTI (40 µg/mL). A thrombin specific fluogenic substrate Boc-Asp(OBzl)-Pro-Arg-AMC was added into re-calcified (10 mM) PPP to detect thrombin generation in. A blend of lipids shortened the initiation time of thrombin generation (5% substrate converted) in PPP by about 10 min. The prothrombotic effects of kaolin was enhanced with the presence of lipids. However, lipids failed to further advance the initiation time when kaolin concentration increased from 3 to 30 µg/mL.
Supplemental Fig. 3. Citrate (1:9 WB) and CTI (4 µg/mL) treated whole blood (1:4 HBS) was recalcified (10 mM) right before measurements. Kaolin was added to trigger contact pathway. A thrombin specific fluorogenic substrate Boc-Asp(OBzl)-Pro-Arg-AMC was used to monitor the thrombin generation. Kaolin sped up thrombin formation in a dose dependent manner. Fastest thrombin generation was observed at 10 min with 300 µg/mL kaolin, which is a comparable concentration with kaolin suspension used for kaolin/collagen surface preparation.
Supplemental Fig. 4. Platelet (red) aggregation on kaolin/collagen surface at 5 representative time points under four flow conditions. Arrow indicates flow direction. Platelets tend to form plugs at front side of kaolin/collagen surface at low shear rate. High shear rate forced platelet mass downstream forming more elongated thrombi along the flow direction. Embolization or partial dislocation of thrombi was rarely seen under pressure relief mode since excessive pressure was released through the adjacent empty channels.
Supplemental Fig. 5. Citrate (1:9 WB) and CTI (4 µg/mL) treated whole blood (1:4 HBS) was recalcified (10 mM) right before measurements. Kaolin or diluted recombinant tissue factor was added to trigger either extrinsic or contact pathway. A thrombin specific fluorogenic substrate Boc-Asp(OBzl)-Pro-Arg-AMC was used to monitor the thrombin generation. Tissue factor induced faster thrombin generation.
Supplemental Fig. 6. Platelet deposition and fibrin generation on kaolin/collagen surface with blood treated with CTI (4 or 40 µg/mL) or PPACK (100 µM). The kinetic data was obtained at venous shear rate (100 s⁻¹) under constant flow mode.
Supplemental Fig. 7. Unmodified kaolin/collagen surface under bright field before (right) and after (left) thresholding.
Supplemental Fig. 8. Platelet aggregation (red) and fibrin formation (green) on kaolin/collagen surface (100 s⁻¹, constant flow) with low CTI (4 μg/mL) or PPACK (100 µM) treated blood. White dashed line outlines flow channels and arrow indicates flow direction. There is no fibrin formation in the PPACK channel, which indicates the fibrinogen fluorescence is fibrin dependent.