# Using Microfluidics to Assess Thrombus Formation Caused by Abuse-Deterrent Opioid Tablet Formulations Administered via a Non-Intended Intravenous Route

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## Synopsis

Altered abuse-deterrent opioid tablets may cause thrombotic microangiopathy (TMA) when injected by abusers, rather than taken orally as intended. An *in vitro* microfluidic platform has been developed to better understand the mechanism and likelihood of blood clot formation in opioid abusers.

## Introduction

**Background:** The abuse of prescribed opioids is a major public health issue. Abuse-deterrent formulations (ADFs), such as tablets containing high molecular weight (HMW) polyethylene oxide (PEO), were developed for opioids to alleviate the problem. However, case reports continue to reveal safety issues when abusers intravenously inject opioids with PEO excipient. Complications include microangiopathic hemolytic anemia, thrombocytopenia, and renal failure with thrombosis observed in the kidneys, although the mechanism by which PEO causes aberrant thrombosis remains largely unknown. The development of microfluidic models to simulate blood flow in capillaries can help us to understand and prevent thrombotic events, which are thought to be associated with adulterated opioid intravenous injection.

**Purpose:** This cross-cutting, collaborative effort is intended to elucidate the relationship between tablet excipients, manufacturing and manipulation methods, and toxicological outcomes associated with ADF opioid abuse by developing simple *in vitro* tools to evaluate non-intended uses of ADFs.

## Material and Methods

In total, we developed two *in vitro* test methods for this project. Previously, a needle model test system was designed and evaluated for feasibility, sensitivity, and reproducibility to use as a potential regulatory tool for assessing ADFs. Here, we introduce a microfluidic platform with dimensions similar to the kidney arterioles to observe the interactions between PEO and blood components under high shear conditions (Table 1).

**Table 1.** Benefits of the microfluidic model compared to the previous 30 gauge needle model.

| | 30G Needle model | Microfluidic model |
|---|---|---|
| Advantages | - Reproducibility<br>- Simplicity<br>- Low cost<br>- Axisymmetric | - Mechanistic study<br>- Cell and blood clot visualization<br>- Low blood volume<br>- Easily adjustable geometry |
| Potential Endpoints | - Plasma free hemoglobin<br>- Blood cell counts<br>- Viscosity | - Plasma free hemoglobin<br>- Pressure differential<br>- Cell free plasma layer thickness<br>- Clot formation<br>- Blood cell deformation |

To prepare the test solutions, HMW (7MDa) PEO powder was dissolved into phosphate buffered saline to make different concentrations of PEO solutions: 1, 2, 4, and 8 mg/mL. The PEO solution was gently mixed with ACDA-anticoagulated porcine blood for 5 min. The PEO-blood mixture was then transferred to 10 mL syringes for the perfusion studies. and a static background sample was collected. Testing was performed at flow rates up to 1.0 mL/min to generate a high shear environment.

## Material and Methods (cont.)

Microfluidic channels with narrowed flow (stenotic) regions were designed to generate isolated high shear blood flow for comparison to the previously developed needle model. Different PEO formulations were introduced into blood. The PEO-blood mixture and a control containing only blood flowed through the 100 µm x 100 µm channels of varying length. The blood flow rate was adjusted from 0.5 to 1.0 mL/min to visualize and quantify hemolysis and thrombus formation. Free plasma hemoglobin concentration was measured via the Cripps' method using a spectrophotometer to assess damage to red blood cells.

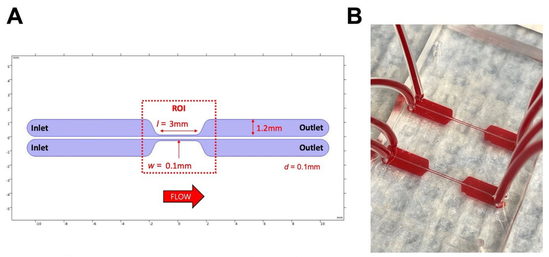


**Figure 1.** Dimensions and geometry of the microfluidic model.

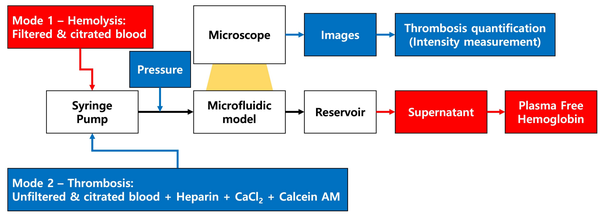


**Figure 2.** A schematic of the process for using microfluidics to assess blood damage in terms of both hemolysis and thrombosis.

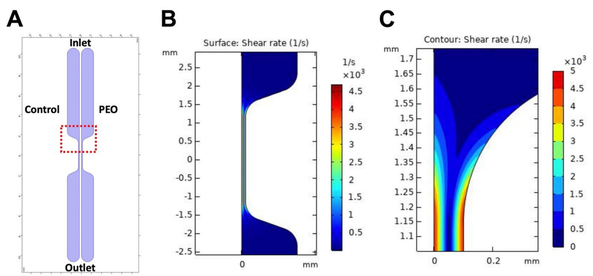


**Figure 3.** The region of interest for thrombus formation (throat of the stenosis) had elevated shear rates and fluid acceleration. Computational fluid dynamics simulations were performed to predict the flow characteristics of the stenotic region within the microfluidic model.

## Results

The *in vitro* microfluidic model generated measurable levels of blood damage similar to the levels observed in the corresponding small animal studies. These results indicate that HMW PEO in opioid tablets (7 MDa) is more likely to cause hematotoxicity, thrombosis, and kidney injury when administered via acute intravenous injection than lower molecular weight excipients. The microfluidic system developed in this study enabled us to visualize and quantify the interactions between PEO and blood elements, such as microthrombus formation, clot size (< 100 µm), and pressure changes (>100 mmHg), in real-time over a 30-minute test duration. The plasma free hemoglobin value of the static background generated by the blood alone was subtracted from the perfused sample value.

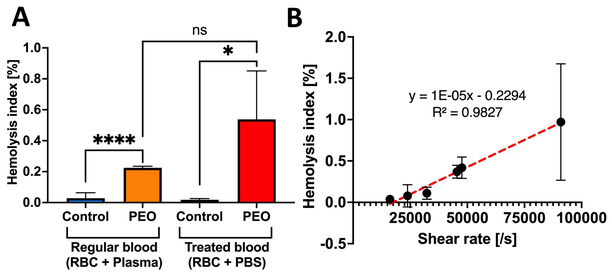


**Figure 4.** Hemolysis levels (plasma free hemoglobin / total hemoglobin x 100%) produced by the microfluidic model for both whole blood and concentrated (washed) RBC solutions using a concentration of 40 µg/mL, 7MDa PEO in blood flowing at 1 mL/min. B) Blood flow rates were varied.

After compiling all the hemolysis data from the microfluidic model and the needle model, the resulting dose response curve spans a large range of shear rates and exposure times. The effects of these flow characteristics on hemolysis are shown for both models in Fig. 5.

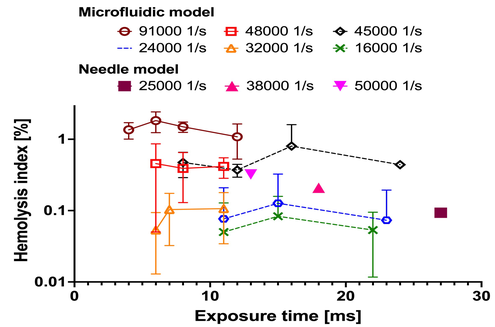


**Figure 5.** Dose response of PEO hematotoxicity in both the microfluidic and needle models is shown on a logarithmic scale for a range of shear rates and exposure times, by varying the flow rate and channel length.

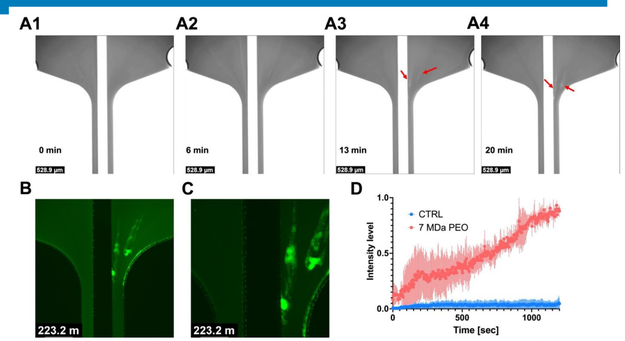


**Figure 6.** Visualization of thrombus deposition and growth over time near the entrance of the stenosis in the microfluidic model. The control (leftmost channel in each image) is without PEO and shows no thrombus. An intensity level of 1.0 is the maximum area of fluorescence detected during the test.

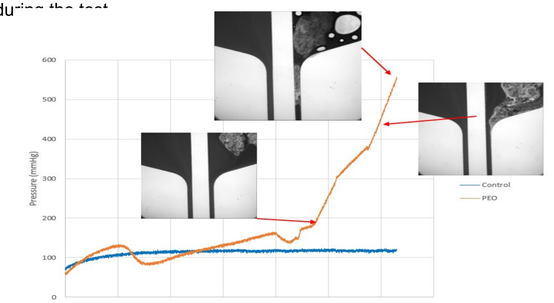


**Figure 7.** Feasibility test showing that the inlet pressure increases over time in the channel containing the PEO-blood mixture (right in each image), which correlates with visual thrombus formation.

## Conclusions

The proposed *in vitro* microfluidic tool allows us to observe interactions between PEO and blood in a dynamic environment using different flow parameters and ADFs. The results from the microfluidic model validate previous findings from the needle model.

- ❖ Increased hematotoxicity, in terms of hemolysis and thrombosis, was observed with higher PEO concentrations (i.e., 7 MDa) and higher shear rates in both the microfluidic and needle models.
- ❖ Feasibility testing was conducted to demonstrate that visible thrombus deposition corresponded to increases in inlet pressure.

The results from the *in vitro* models will be correlated to *in vivo* small animal studies conducted at the Univ. of Maryland. The main goal of this project is to develop regulatory tools that can be used to assess the safety of different abuse-deterrent formulations in a least burdensome manner.

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