Development of *In Vitro* Predictive Models for Abuse-Deterrent Opioid Safety Assessment

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Synopsis

Manipulated abuse-deterrent opioids can cause thrombotic micro angiopathy (TMA) when injected rather than taken as intended, orally. This project aims to develop *in vitro* models for assessing the safety of abuse-deterrent formulations and determining the root cause of TMA in opioid abusers.

Introduction

Background: The abuse of prescribed opioids is a major public health issue. Abuse-deterrent formulations (ADF) for opioids, which include high-molecular weight (HMW) polyethylene oxide (PEO), were developed to combat this issue. HMW PEO has shown to be effective for deterring abuse through snorting, but not necessarily via intravenous injection. Many case reports have revealed complications when patients intravenously inject extended-release opioids with PEO excipient. Opioid abusers have presented with microangiopathic hemolytic anemia, thrombocytopenia, and renal failure with TMA observed in the kidneys. It is not clear how PEO is causing TMA-associated complications. Currently, there are no recommendations for determining the safety of PEO excipients when opioids are manipulated and taken through the non-intended route of injection.

Purpose: CDER, CBER, and CDRH are collaborating to elucidate the relationship between excipients, manufacturing and manipulation methods, and toxicological outcomes associated with ADF opioid abuse, and to develop simple, reproducible *in vitro* tools for evaluating ADF safety.

Materials and Methods

We developed two *in vitro* test methods with different objectives. A needle model test system similar to one proposed by Persich et al, 2020, is being evaluated for feasibility, sensitivity, and reproducibility to use as a potential regulatory tool. Concurrently, a microfluidic platform with dimensions similar to the kidney arterioles is being used to observe the interactions between PEO and blood components under high shear conditions (Table 1).

Table 1. Benefits of each *in vitro* model.

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

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

Materials and Methods

Two replicate samples were collected in 1.5 mL centrifuge tubes for each test. Free plasma hemoglobin concentration, the primary metric used to indicate the hematotoxicity of PEO, was measured via the Cripps' method using a spectrophotometer to assess damage to red blood cells. The key test parameters for this preliminary study are summarized in Table 2.

Table 2. Preliminary test parameters of two *in vitro* models.

	Needle model	Microfluidic model
Cross-sectional dimensions	27G (ID = 200 µm) 30G (ID = 150 µm)	100 µm x 200 µm
Channel length	12.7 mm	18 mm
PEO molecular weight [MDa]	7	
PEO concentration in blood [µg/mL]	5 - 40	
Flow rate [mL/min]	0.5 - 1	

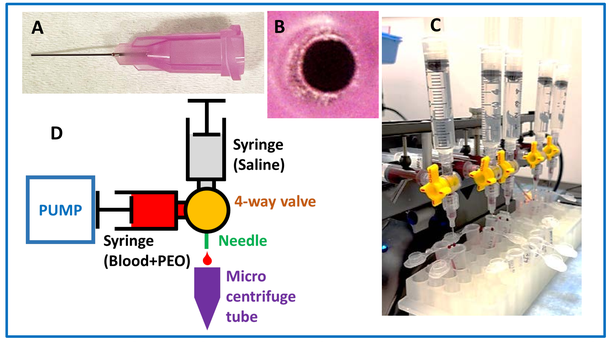

Figure 1. Needle model. A) 30G needle. B) Image of inside of the needle with minimal flashing. C) Experimental set-up with 4 test needle models and 1 control. D) Schematic of the needle model.

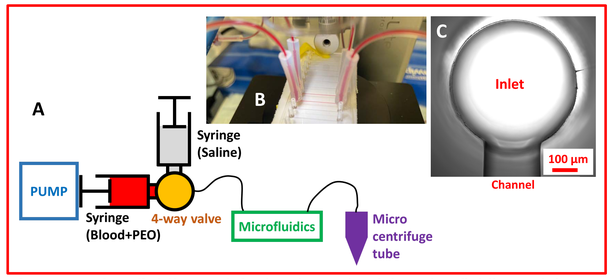

Figure 2. Microfluidic model. A) Schematic of the microfluidic model. B) Image of the preliminary experimental set-up with 1 test microfluidic channel and 1 control. C) Inlet to the microfluidic channel does not contain a sharp edge.

Results and Discussion

The results shown below are for PEO in saline because PEO in saline produced less hemolysis and less variability in the test results compared to the PEO in water. To evaluate the test sensitivity and reproducibility of the needle (27G and 30G) and microfluidic models, the blood-PEO mixture was perfused through each system at a constant flow rate of 1 mL/min with two PEO concentrations (5 and 40 µg/mL PEO in blood). The free plasma hemoglobin value of the static background control generated by the blood alone was subtracted from the perfused sample value. We found an increase in hemolysis level with a higher PEO concentration (40 µg/mL), smaller needle diameter (30G), and in the microfluidic channel (Fig. 3). All of the initial test conditions studied were reproducible, with a coefficient of variation less than 15%.

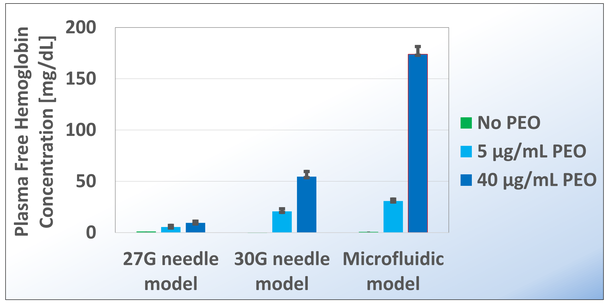

Figure 3. Plasma free hemoglobin levels produced by the 27G and 30G Needle model, and the preliminary Microfluidic model, for both low (5 µg/mL) and high (40 µg/mL) PEO concentrations at 1 mL/min.

Additional PEO concentrations (10 and 20 µg/mL) were evaluated using the needle model to generate a dose response curve (Fig. 4). Hemolysis levels increased as the PEO concentration in blood increased. The effect of shear rate on hemolysis is also shown for both the needle and microfluidic models in Fig. 5.

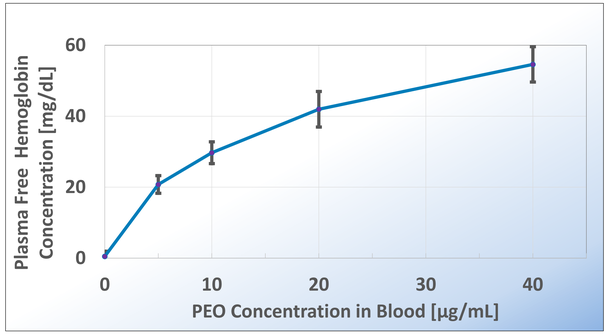

Figure 4. Dose response of PEO hematotoxicity in the 30G Needle model. Four PEO concentrations (5, 10, 20, and 40 µg/mL) and a dynamic control (without PEO) were perfused at 1 mL/min.

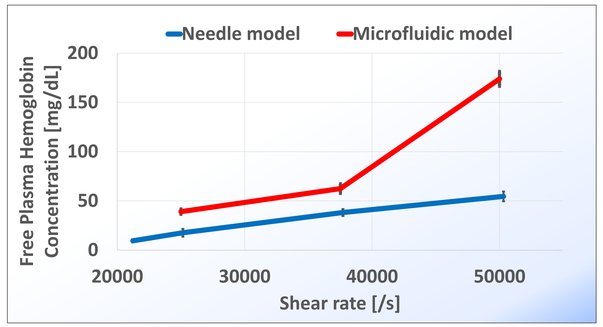

Figure 5. Shear rate dependency of PEO hematotoxicity in Needle model and Microfluidic model. Blood containing 40 µg/mL PEO was perfused through the needle and microfluidic models and the results are plotted with respect to shear rate.

Conclusions & Future Work

In order to develop an excipient safety evaluation tool and fully understand the mechanisms driving HMW PEO-induced TMA, two sensitive and reproducible *in vitro* platforms are being developed concurrently by FDA.

- The simple needle model generates reproducible hemolysis levels (coefficient of variation less than 15%).
- Increased hematotoxicity was observed with higher PEO concentrations and higher shear rates. In preliminary testing, the microfluidic model has generated comparable levels of hematotoxicity relative to the needle model.
- We have begun to establish a dose response curve and shear dependent hematotoxicity when PEO solution is perfused through the needle model.

In future testing, we will continue to develop and optimize the microfluidic model that will allow us to visualize and better study the interaction between blood and PEO in a dynamic environment. We also plan to fully characterize both models to study the effects of the following on clinical outcomes:

- Excipient molecular weight
- Dosage
- Physical manipulation of excipient
- PEO injection method
- Blood properties
- Hemodynamics

The results from the *in vitro* models will be correlated to the ongoing *in vivo* guinea pig studies conducted in CBER. The main goal of this project is to develop a regulatory tool that can be used to assess the safety of different abuse-deterrent formulations in a least burdensome manner.

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