

# Microplastic Effects on Thrombin-Fibrinogen Clotting Dynamics Measured via Turbidity

**Nathan Stelflug**<sup>1†</sup>, Daniela Q. Tran<sup>2,3†</sup>, Abigail Hall<sup>3</sup>, Tanmaye Nallan Chakravarthula<sup>3,4</sup>, Nathan J. Alves<sup>3,4</sup>

1. Indiana University School of Medicine, Indiana University, Indianapolis, IN 46202, USA
2. Fischell School of Bioengineering, University of Maryland, College Park, MD 20742, USA
3. Department of Emergency Medicine, Indiana University School of Medicine, Indiana University, Indianapolis, IN 46202, USA
4. Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA

## Background/Hypothesis:

Widespread use of plastic has created a world where exposure to microplastics is inevitable leading to their presence in our circulatory system. This raises questions about microplastics impact on thrombosis. Aminated polystyrene (aPS) plastics have been shown to increase platelet aggregation and thrombus formation in animal models. Given this, we hypothesized that aPS administration will increase the rate of fibrin clot formation in a simple thrombin-fibrinogen clot model.

## Project Methods:

We evaluated how concentrations of 25-200  $\mu$ g/mL of 100 nm aPS particles affect fibrin clot formation using turbidity assays. To determine the effect of surface charge, experiments were also performed with non-modified polystyrene (nPS) particles. Microplastics were pre-incubated either with physiological concentrations of fibrinogen or thrombin and the clot formation was measured using turbidity at 405 nm every 10 seconds over 45-minutes. Clotting parameters such as maximum turbidity (Turb<sup>Max</sup>), time to 90% maximum turbidity (Turb<sup>Time</sup>), and clot formation rate ( $V_{max}$ ) were determined and compared to controls without microplastics.

## Results:

When increasing concentrations of aPS were preincubated with thrombin or fibrinogen, there was less than a 2-fold change in  $V_{max}$ , Turb<sup>Max</sup>, and Turb<sup>Time</sup>. When increasing concentrations of nPS were preincubated with thrombin, there was up to a 27-fold decrease in  $V_{max}$ , 2.4-fold decrease in Turb<sup>Max</sup>, and 4.36-fold increase in Turb<sup>Time</sup> compared to the control. Whereas preincubation of nPS with fibrinogen resulted in 1.86-fold decrease in  $V_{max}$ , 1.63-fold decrease in Turb<sup>Max</sup>, and 2.30-fold increase in Turb<sup>Time</sup>.

## Potential Impact:

In this simplified clotting model, it was surprising to find inhibitory effects on clot formation and that they were more pronounced with nPS than with aPS. However, these results align with increase prothrombin time observed in literature in presence of aPS. Therefore, future studies with more complex clotting models need to be performed before claims can be made on the impact of microplastics on thrombosis.