# Investigating Organic Redox-active Crystals for Thrombotic Response

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**Statement of Purpose**: A material to combine biofilm prevention and real-time bacteria monitoring is being developed. The material is composed of redox-active RZx crystals deposited upon graphitic electrode sheets that detect bacterial viability by recognizing associated pH changes as well as direct electron exchange with bacterial cells.[1] One application of this material would be in the fabrication of blood contacting catheters where the RZx-carbon electrode would have vascular contact. This study investigates two RZx-carbon electrode fabrication protocols with respect to their behaviors in blood to ensure that these materials are not activators of thrombosis and determine which configuration is better suited for use in a device.

**Materials and Methods:** The two materials investigated both contain redox-active RZx crystals created from an organic solution with essential components including KCl, resazurin, and tryptone, and both materials are integrated with a nafion polymer. For the RZx/nafion coated carbon electrode material, RZx crystals were deposited upon pyrolytic graphite sheet (PGS) and covered in a protective layer of nafion. For the RZx/nafion embedded carbon electrode material, nafion was deposited directly upon the PGS electrode and RZx crystals were grown within the nafion layer using a pulsed electrodeposition technique. Blood compatibility testing included plasma coagulation assays and platelet adhesion testing. Scanning electron microscope (SEM) and atomic force microscopy (AFM) was used to analyze the topography of the materials. X-ray photoelectron spectroscopy (XPS) analysis was performed as well for comparison of surface elemental compositions in order to understand the effects of different materials chemistries for the two different electrode configurations.

**Results:** Testing suggested that the RZx/nafion coated carbon electrode material had RZx crystals protruding through the nafion layer, while the RZx/nafion embedded carbon electrode appeared to lack the nafion coating. This was confirmed via topographical analysis showing large numbers of visual crystals and XPS analysis revealing nitrogen (from resazurin) at the coated material's surface. XPS also showed a lack of fluorine from the nafion in the embedded samples. Despite these topographical and compositional differences, plasma coagulation testing of the two materials showed no statistically significant difference in the activation of plasma clotting (Figure 1). The embedded material does show a significantly higher clotting time (less activating) than polyurethane control ($p<0.05$), but all polymers tested show a significant decrease in clotting time (more activating) compared to polystyrene vials lacking any material activator ($p<0.05$). Platelet adhesion testing displays an approximately 10x greater platelet count on the embedded material than on

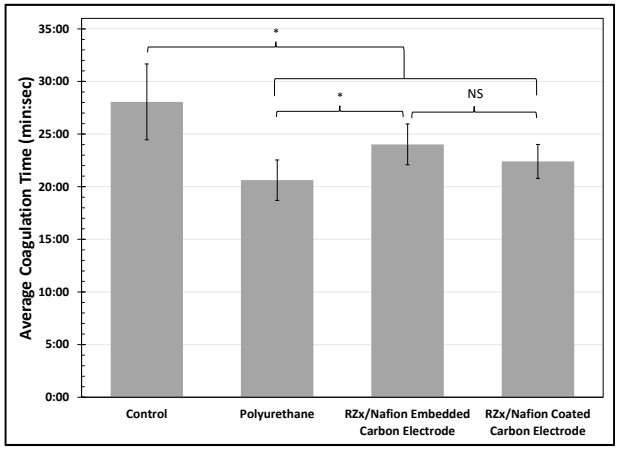


**Figure 1:** Coagulation data from multiple experiments (n=4) showing clotting time for 4 activators (*$p<0.05$, NS=not significant).

polyurethane but yields inconclusive results for the coated material. The fluorescent label utilized for platelet visualization may have interacted with the protruding RZx crystals of the coated material, making the platelets indistinguishable under microscopy.

**Conclusions:** XPS analysis reveals an absence of nafion on the surface of the RZx/nafion embedded material, suggesting the surface is likely composed primarily of RZx crystals. RZx crystal protrusion through the nafion layer of the coated material indicates that the nafion is not uniformly deposited across the surface or that thicker layers of nafion are needed in fabrication. It is expected that the inconsistency of the nafion layer resulted in some leakage and dissolving of the crystals for the coated material. This may have unexpected effects in the context of blood contacting devices. Further platelet adhesion testing should be completed for more comprehensive conclusions to be drawn. A different method will need to be utilized to accurately assess the coated material's platelet adhesion values in future experiments.

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**References:** [1] Bolotsky A (2021). *Biosensors and Bioelectronics.* 172:112615.