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# Giving Biofilm the Slip

## An *in vitro* evaluation of a new catheter material for the reduction of thrombus and bacterial adhesion

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## Purpose

An *in vitro* study designed to simulate catheter-related bloodstream infection evaluated resistance to bacterial (*staphylococcus aureus*) and thrombus adhesion on a novel hydrophilic biomaterial (HBM) used for vascular catheters. For comparison, catheters made from thermoplastic polyurethane (TPU) and fluorinated thermoplastic polyurethane (Fluoro-TPU) were also evaluated. A secondary pilot study using the same *in vitro* model compared resistance to yeast, *Candida albicans, and* adhesion in a catheter constructed of HBM versus a chlorohexidine-coated antimicrobial device.

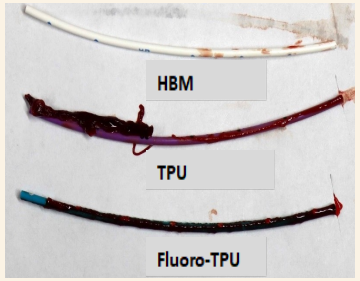
## Methods

***Study design.*** Two control catheters (TPU, Fluoro-TPU) and one test (HBM) were placed in a bovine, whole blood loop with radiolabeled platelets and $10^5$ colony-forming units per milliliter (CFU/ mL) of *S. aureus* at 37°C. Catheter segments of 10 cm were rinsed with sterile 1x PBS solution at 37°C for 2 hours before placement in the flow loop. The blood was circulated to mimic the shear rate in the superior vena cava. Twelve samples (n=12) were analyzed in each group, where duplicates were placed in 6 parallel flow loops, connected to a central reservoir. Exposing the catheter for 2 hours of physiologically representative fluid, phosphate buffered saline, allows the materials to equilibrate to temperature and water content.

Similarly, *C. albicans* was tested in triplicate where the HBM was compared with a chlorhexidine-coated TPU catheter (CHX) and an uncoated TPU catheter.

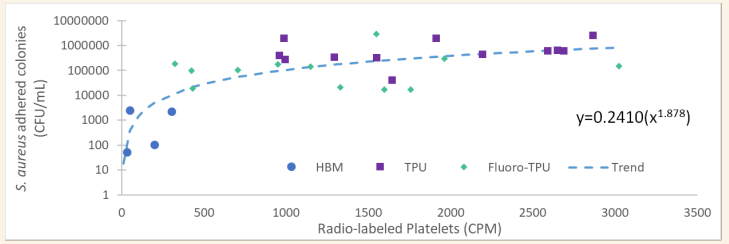
***Data analysis.*** Thrombus was quantified with a Geiger counter in counts per min (CPM) from the adhered platelets, while the bacteria were sonicated, rinsed, plated, and incubated for 24 hours at 37°C and serially diluted to count colony-forming units. Bacterial data were $\log_{10}$ transformed and analyzed using a paired student t-test, where a p-value < 0.05 was considered statistically significant. Thrombus data were not transformed but analyzed the same. Data is presented in its linear format.

***Figure 1.*** Catheter segments removed from a *S. aureus*-laden blood flow loop.




## Results

***Figure 2***. Bacterial adhesion vs. platelet adhesion in an *in vitro* blood loop model.




***Tables 1 & 2.*** Relative $\log_{10}$ reductions in microbial and thrombus adhesion in an *in vitro* blood loop model.

| $\%RED = 1 - \frac{Test}{Control}$ | *S. aureus (n=12)* | |
|---|---|---|
| | Relative Bacterial Reduction | Relative Thrombus Reduction |
| $1 - \frac{HBM}{TPU}$ | 99.998% ± 0.07%<br>P = .002 | 96.3% ± 1.7%<br>P < .001 |
| $1 - \frac{HBM}{FluoroTPU}$ | 99.989% ± 1%<br>P = .01 | 94.9% ± 2.7%<br>P < .001 |
| $1 - \frac{FluoroTPU}{TPU}$ | 81.36% ± 12%<br>P < .001 | 28% ± 13%<br>P = .02 |

| $\%RED = 1 - \frac{Test}{Control}$ | *C. albicans (n=3)* | |
|---|---|---|
| | Relative Bacterial Reduction | Relative Thrombus Reduction |
| $1 - \frac{HBM}{TPU}$ | 99.999% ± 0.005%<br>P = .03 | 99.94% ± 0.01%<br>P = .009 |
| $1 - \frac{HBM}{CHX}$ | 99.99% ± 0.02%<br>P = .008 | 99.4% ± 0.2%<br>P =.02 |
| $1 - \frac{CHX}{TPU}$ | 87.6% ± 4.2%<br>P = .02 | 89.8% ± 0.52%<br>P= .008 |

**HBM catheters had significantly less microbial and thrombus adhesion than the control groups.** HBM reduced *S. aureus* adhesion by 99.998%, compared to the TPU and Fluoro-TPU groups *(Table 1, top)*. The Fluoro-TPU also showed a more modest, but significant improvement in this test method compared to the HBM catheter. Visible evidence of the bacteria and thrombus adhesion of one set of samples is found in Figure 1. All p-values were less than 0.02 indicating statistical significance.

Data from the *C. albicans* pilot study demonstrated that HBM catheters had improved resistance to adhesion *(Table 2, bottom)*. Residual chlorhexidine may have reduced *C. albicans* concentrations across all devices as it leached into the blood flow loop; however, the magnitude of bacterial adhesion reduction was comparable to the *S. aureus* study performed without an antimicrobial agent. All p-values were less than 0.03 indicating statistical significance.

**A parabolic relationship between bacterial and thrombus adhesion was found in the larger *S. aureus* group** (*Fig. 2*). This trend suggests that thrombotic material can accelerate bacterial colonization on the surface of the catheter. Samples with no detectable thrombus or bacteria were omitted from the trend. In the *S. aureus* model, 7 of the 12 HBM samples had no detectable bacteria and 5 of the 12 HBM samples had no detectable thrombus adhered to the catheter surface.

## Conclusions

HBM significantly reduces *S. aureus* and *C. albicans* adhesion and thrombotic adhesion in a simulated bloodstream infection model. This *in vitro* model is a repeatable quantitative method used to determine how different biologic materials may interact with catheter materials in a simulated bloodstream infection environment.

*Note:* No correlation between in vitro and in vivo testing methods and clinical outcomes have currently been ascertained.

## Future Directions

A spectrum of clinically relevant organisms will continue to be explored.

## Disclosures

We acknowledge the help of Derek Solt and Nancy Moureau for their assistance in this work.

Daniel Donahue, Kristen LeRoy, and Eddie Korycka are employees of Access Vascular, Inc