Combined Antimicrobial and Antithrombogenic Properties of Betaine Polymers on Carbothane® Substrates

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Purpose:

Significant proof-of-concept data has been generated to-date on betaine materials demonstrating the potential to reduce both bacterial biofilm formation and protein and cell adhesion. Early work was performed primarily on gold and glass substrates using Atom Transfer Radical Polymerization (ATRP).[1-3] This work demonstrated the potential of betaine surface modifications to simultaneously reduce infection and thrombosis related to medical devices with the chemical stability required to provide protracted long-term activity. The goal of this research was to translate these proof-of-concept studies to more practical, manufacturable formulations on relevant biomaterial substrates.

Methods:

Betaine polymers are highly water-coordinating materials that may prevent the first stages of thrombosis and biofilm formation: protein and cell attachment. Further, polymers of betaines promise increased stability *in vivo* as compared to phosphorylcholine (PC) or poly(ethylene glycol) (PEG) based materials.

Recently these chemistries were adapted to medical-grade polyurethane (Carbothane® with 20% $BaSO_4$) using two in-house surface modification methodologies. Samples have been assessed for both antimicrobial and anti-thrombogenic activity using the methods described below:

A 24 hour colonization assay was used to assess antimicrobial performance after serum exposure. Briefly, test articles were incubated with 50% fetal bovine serum for 24 hours at 37 ˚C with agitation, rinsed, and transferred to individual conical tubes containing a starting inoculum of 10e5 CFU/ml bacteria suspended in 1% TSB:PBS. Test articles were agitated for 24 hours at 37 ˚C to promote contact of bacteria to the test article and control surfaces. Following the 24 hr period, test articles were rinsed and sonicated, and aliquots were plated to enumerate adherent bacteria.

Additionally, samples were tested in a widely utilized flow loop model of thrombosis at the Medical Device Evaluation Center. Treated and control rods were subjected to heparinized fresh bovine blood for two hours, and thrombus was measured both visually and quantitatively using radio-labeled platelet counts.

Results:

As shown in Figure 1, both betaine surface modifications yielded ~ 2 log (99%) reductions in bacterial colonization following serum exposure. These results were statistically significant ($p<0.001$).

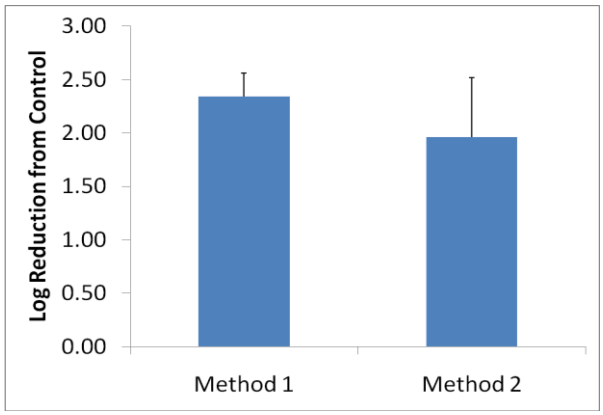
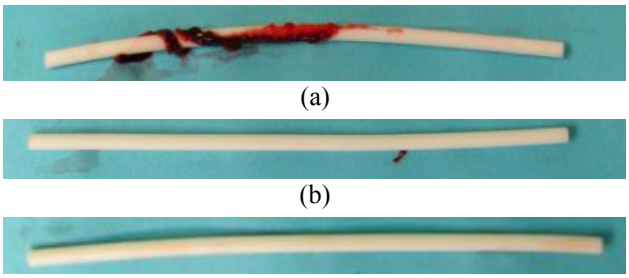


Figure 1: Log reduction in bacterial colonization following serum exposure vs. Carbothane® control.

As shown in Figure 2, both betaine surface modifications also demonstrated marked reductions in thrombus formation relative to control. These results are representative of six duplicate experiments which showed similar activity.



(a)

(b)

(c)

Figure 2: Thrombus formation on (a) Carbothane® control (b) Betaine-coated Carbothane (Method 1) (c) Betaine-coated Carbothane (Method 2)

Conclusions:

These results demonstrate the potential for betaine surface modifications to simultaneously reduce bacterial colonization and thrombus formation. This dual-functional technology represents a significant advancement in the field of surface modification and highly biocompatible biomaterials. Future studies will examine longer-term performance, and if successful this technology platform may find application for a range of medical devices where chronic biofilm and thrombus formation significantly complicate treatment.

References:

1. Cheng, et al. Biomaterials, 28 : 4192-4199 (2007)
2. Zhang, et al. Langmuir, 22: 10072-10077 (2006)
3. Zhang, et al. Biomacromolecules, 7 (12): 3311-3315 (2006)