

## Bacterial Colonization of Suture Biomaterials with Varied Substrate Architecture and Chemistry

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**Statement of Purpose:** Braided multifilament sutures have been reported to potentiate wound infection by providing microbial pathogens with a safe harbor that is impenetrable by larger immunocompetent cells<sup>1</sup>. It has been proposed that microbial colonization may be reduced in part by the use of monofilament materials in place of high surface area multifilaments with complex braid architectures. Further, the clinical utility of multifilaments comprising broad spectrum synthetic biocides is relatively unknown. In this study, representative multi and monofilament biodegradable lactone-based sutures (some containing the biocidal agent triclosan) were challenged *in vitro* with *Staphylococcus aureus* and *Escherichia coli* isolates for up to six days. Sutures were evaluated for zone of inhibition, quantified for suture-adherent organisms following serial debridement, and visually inspected using scanning electron and confocal microscopy.

**Methods:** All suture biomaterials were sterile, size 2-0, and within expiration date. Degradable lactone-based polyesters used for preparing the multi and monofilament substrates were synthesized from high purity L-lactide, glycolide, caprolactone, and dioxanone via anionic ring opening melt polymerization. All materials (from monomer to packaged sterile suture) were prepared using standard operating procedures (Syneture, US Surgical, North Haven, CT).

Isolates of *Staphylococcus aureus* and *Escherichia coli* were maintained on tryptic soy agar (TSA). Zone of Inhibition (ZOI) studies were conducted with an inoculum ( $10^5$  CFU/ml) prepared in molten TSA containing 5cm test articles. Following 24hrs, ZOI size was reported in millimeters. For direct challenge in high density bacterial broths, individual suture strands were aseptically placed into sterile test tubes inoculated with a bioburden of  $10^3$  CFUs/ml of an organism monoculture. Tubes were briefly vortexed to homogenously suspend the cells, and were incubated in a rocking water bath (60 cycles per minute) at 37°C for a predetermined 24hr increment of time (up to 144hr). Following the challenge period, strands were removed and processed via serial debridement to remove suture-adherent cells. This process consisted of repetitive mechanical (vortex) and energetic (sonication) debridement steps in sterile medium. Aliquots of the terminal debridement media were

plated on blood agar using sterile spreaders, incubated overnight at 37°C for 24 – 48hr, and enumerated for CFUs using a darkfield colony counter.

Scanning Electron Microscopy (SEM) was conducted on fixed, dried, and gold-palladium sputter coated samples using low voltage techniques (<5keV). Confocal imaging was conducted using a Zeiss META 510 confocal microscope (Carl Zeiss, Germany). Suture images were obtained using the 488 channel Argon2 laser for the Sytox-labeled bacterial signal. The Cy3 channel (Neon-Helium/1 laser) was used to obtain background auto-fluorescence in order to delineate the suture surface and braid architecture. These conditions were tailored such that no bleed-through between red (Cy3) and green (Sytox) signals were observed. For each sample, five random suture stacks were obtained using the 20x objective, taking 43 x 1µm slices.

**Results/Discussion:** Despite ZOIs observed for triclosan impregnated lactone sutures, no differences in bacterial colonization were observed for any multifilament braid specimens when challenged by direct immersion in high density bacterial broths for 1, 2, or 6 days and quantified for colony forming units following serial debridement and plating. Monofilament suture biomaterials remained at  $10^1$  CFU/ml for the duration of testing (6 days). Sessile counts for multifilaments were significantly greater than comparative monofilaments ( $10^3$ – $10^4$  CFU/ml). SEM and confocal microscopy with Sytox Green nuclear staining confirmed these findings and suggest that multifilaments, even those comprising a biocidal agent (triclosan), are vulnerable to biofilm formation and significant bacterial colonization.

**Conclusions:** Monofilament suture biomaterials may provide a means of reducing suture colonization when compared to high surface area braid architectures, including those containing a synthetic biocide (triclosan). These results in concert with the recent reports of emerging triclosan resistance raise concerns of both clinical utility and potential detriment associated with triclosan impregnated devices<sup>2</sup>.

References available on file<sup>1,2</sup>.