Effect of topical negative pressure and foam type on *Pseudomonas aeruginosa* biofilm utilizing an *in vitro* model.

FINAL REPORT

PERFORMING LABORATORY:

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Background

Prosthetic implant infection (PII) is an increasing issue in both plastic surgery and the wider surgical community (Shirtliff 2009). This is due to the increasing use of a variety of prosthetic materials fuelled by the demand for prosthetic replacement from the aging of the Baby Boomer generation. The principle mode of infection is in the establishment of a bacterial biofilm on the surface of the prosthetic, most likely due to contamination at the time of implantation from commensal skin flora around the surgical site (Costerton 2009). The conditions of a surgical wound, including the presence of clotted blood, compromised soft tissues and a prosthetic surface provide an ideal environment for the formation and establishment of a biofilm.

Bacteria exist in two phases: free floating (or planktonic) and sessile. In the presence of a suitable biological or artificial surface, a series of genetic changes occur to allow rapid primary attachment of bacteria. The initial electrostatic and potentially reversible attachment is followed by secretion of an extracellular polymeric sticky glycoprotein matrix. This matrix irreversibly bonds to the surface and provides protection to the bacteria, deeply embedded within the construct. The construct of bacteria and self-secreted matrix has been termed biofilm (see figure below). It has been shown that biofilm associated infections are more resistant to antimicrobial agents as compared with planktonic organisms (Anwar 1992)(see figure below). A prosthetic device contaminated with biofilm will eventually fail as the bacterial load increases and the interface between the implant and the tissues is subjected to chronic inflammation.

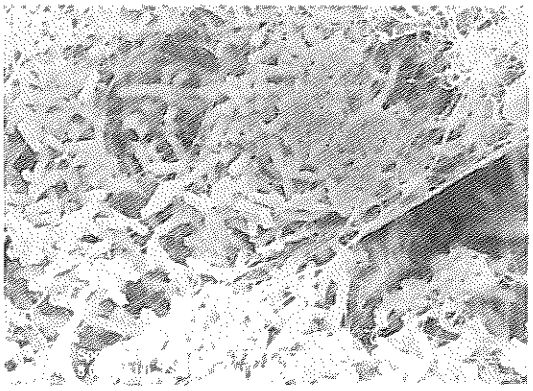


Figure 1: Scanning electron micrograph of a biofilm attached to prosthetic surface.

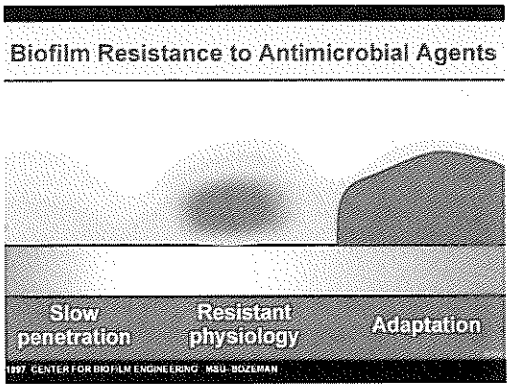


Figure 2**:** Some possible mechanisms of biofilm resistance to antimicrobial agents.

A growing list of PII has now been described in surgery. These include indwelling urinary catheters, peritoneal dialysis catheters, vascular grafts, orthopedic joints, pacemakers, kirschner wires, intravenous catheters, contact lenses and mammary implants (Tamboto 2009). Clinical presentations of biofilm disease include chronic inflammation, capsular contracture and implant loosening (Shirtliff 2009).

As the true impact of chronic biofilm infection is further delineated, strategies for both prevention and treatment are required. We have sought to investigate the role of combined physical and chemical assault of biofilms *in vitro.*

Topical negative pressure therapy has been previously described as a means of reducing bacterial load in infected wounds (Sadat 2008). Silver impregnated dressings and topical silver have also shown to have intrinsic antibacterial activity (Strohal 2005). We sought to test a combination of TNP and silver impregnation on a surface *Pseudomonas aerugenosa* biofilm using an *in vitro* model. Direct testing of these strategies on biofilm activity has not been previously investigated.

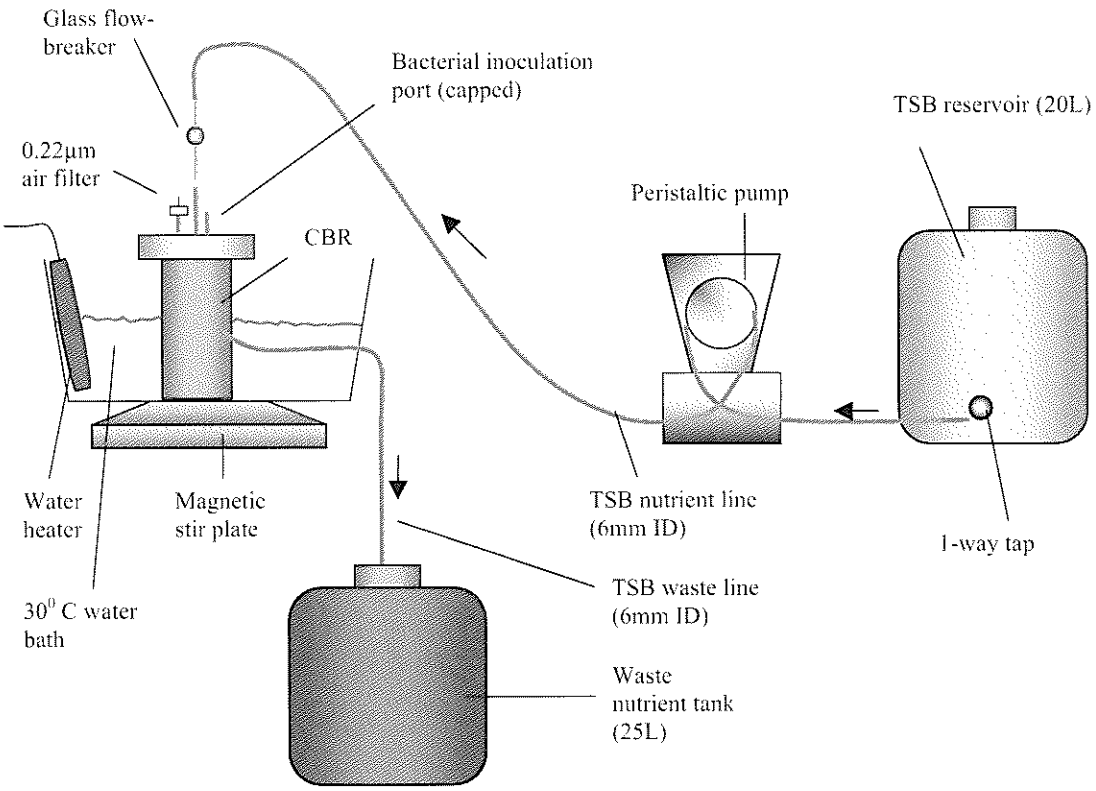
Materials and Methods

Preparation of biofilm covered coupons

Pseudomonas aeroginosa (ATCC 25619) biofilm was grown under shear stress (125rpm) on 24 borosilicate coupons in a CDC bioreactor (BioSurface Technologies Corp, Bozemman USA)

in 400mL of 300mg/L Trypton Soy Broth (TSB Sigma Aldrich, USA) at 30 °C in batch phase for 24 hours. Fresh media (TSB 100mg/L) was then allowed to flow into the top of the chamber at 11.7 ml/minute and out of the chamber side for a further 24hrs (Figure 1). Filtered air (3L/min) was passed across the CDC bioreactor media surface.

Figure 1 CDC Bioreactor



At the end of 48 hrs growth coupons were washed twice in phosphate buffered saline (PBS pH 7) to remove loosely attached planktonic bacteria and the biofilm from one surface of the coupon was removed by a combination of scrapping and killing with bleach. Biofilm on one surface of the coupon was protected with 3% bovine serum albumen. Coupons were washed another three times in PBS to remove any residual biocide.

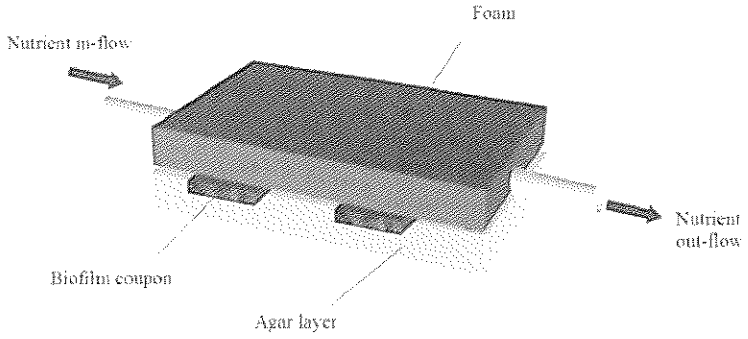
In vitro model

An *in vitro* model was designed such that it created an artificial surface, which incorporated the biofilm, covered coupons and could sustain biofilm viability while at the same time allowing application of therapy. In the physiological environment, a bacterial biofilm would be in constant contact with moisture, and nutrients. The biofilm covered coupons were

implanted onto an artificial surface and was nourished with a constant supply of moisture and nutrients to maintain bacterial viability. Overlaying sterile dressings prevented desiccation and contamination of the model.

All components for the model were either obtained sterile from the manufacturer or were autoclaved prior to use. The model was assembled in a Class II Biological Safety Cabinet using aseptic technique. Bacteriological agar base was used as a substitute low-nutrient and moist surface conducive to biofilm development. Various agar concentrations were tested and 3% was found to be resilient enough to withstand the 125mmHg negative pressure exerted by the TNP therapy device. The glass coupons were placed biofilm upwards into preformed depressions in the agar base (Oxoid, Hampshire, England), covered with foam dressing which was overlaid with an impervious sterile adhesive drape (V.A.C. dressing kit, KCI medical San Antonio TX, USA). Each chamber held 6 coupons. To simulate *in vivo* conditions, the model was supplied at one end with 40ml/hr of 1% TSB, excess fluid was drained from the opposite end via a drainage tube for control chambers (not subjected to TNP) or via a T.R.A.C. pad for chambers subjected to TNP (Figure 2). TNP was supplied by a V.A.C Instill machine (KCI medical San Antonio TX, USA). The model is a closed system with flow rates regulated by the instillation device. There was no variation in flow or oxygenation between control and experimental groups.

Figure 3: In vitro model for examination of biofilm



Coupons remained in situ for the designated time. Upon removal they were individually placed in 5 mls of PBS and sonicated in an ultrasonic bath (Soniclean, JMR Australia) with a sweeping frequency of 42-47 kHz for 5 minutes. Residual bacterial number (colony forming units, CFU) for each coupon was determined by standard serial dilution and plate counting.

Foam types

V.A.C. ® GranuFoam® Dressing (PU). Black polyurethane foam
V.A.C. ® GranuFoam Silver ®Dressing (SPU). Micro-bonded metallic silver is uniformly distributed throughout the polyurethane foam dressing.

Temporal viability of *Ps aeruginosa*

Biofilm covered coupons (n=6) were placed into control models, covered with PU foam for 1, 3 or 7 days.

Effect of silver impregnated foam and TNP on *Ps aeruginosa* biofilm *in vitro*.

Chambers containing 6 biofilm covered coupons each, were overlaid with either PU foam or silver SPU foam and subjected to TNP at 125 mgHg continuous mode for 24 hours, 3 days or 7 days. Control chambers containing biofilm covered coupons and either PU or SPU foam were not subjected to TNP.

Statistical Analysis

Data was transformed into $\log_{10}$ values for analysis. To examine for differences between the number of CFU attached to coupons harvested from the four different chambers an Analysis of Variance (ANOVA) with the Holm-Sidak method of multiple comparisons and if normality or variance failed the Kruskal-Wallis One-way analysis on Ranks using Tukey Test method of multiple comparisons were used. All statistical analysis was conducted using the SigmaPlot 11 Statistical Program.

Results

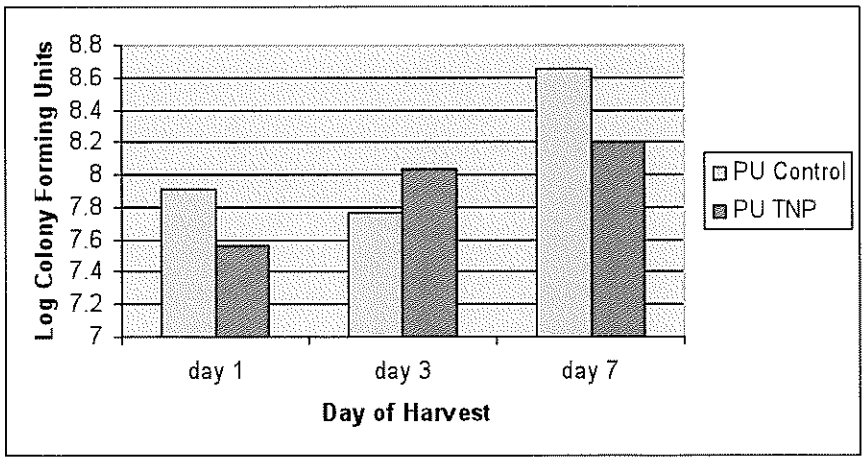
1. Temporal viability of *Ps aeruginosa* biofilm

There was no loss of *Ps aeruginosa* biofilm viability whilst being in the control model (PU foam) for up to 7 days. Mean ± standard deviation values for bacterial viability counts of coupons harvested on days 1, 3 and 7 were $8.1 \times 10^7 \pm 3.3 \times 10^7$, $5.76 \times 10^7 \pm 2.7 \times 10^7$, $4.5 \times 10^8 \pm 3.1 \times 10^8$ CFU per coupon respectively.)(Figure 3)

2. Effect of TNP on *Ps aeruginosa* biofilm using black polyurethane (PU) foam (V.A.C. ® GranuFoam® Dressing).

Application of TNP had a slight (approximately 0.5 $\log_{10}$) and variable effect on bacteria numbers on coupons ($P<0.05$)(Figure 3)

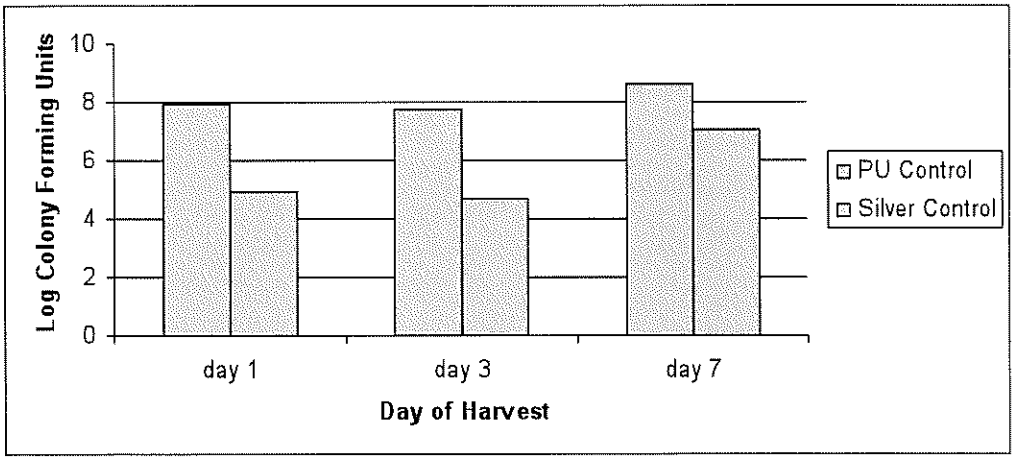
Figure 3. Temporal viability of *Ps aeruginosa* biofilm when covered with V.A.C. ® GranuFoam® Dressing. Coupons harvested on days 1, 3 and 7.



3. Effect of Silver impregnation of PU foam (V.A.C. ® GranuFoam Silver ®Dressing) on biofilm viability.

Overlaying the biofilm covered coupons with GranuFoam Silver foam significantly decreased bacterial numbers when compared to those overlaid with PU foam throughout the experimental period ($P<0.001$). During the first three days this decrease was a thousand fold with CFU/coupon decreasing by approximately 3 $\log_{10}$. Exact bacterial counts for day 3 were not obtained for GranuFoam Silver due to technical difficulties. However by day 7 the effect of silver impregnation was decreasing and bacterial numbers increased significantly ($p=0.003$) but were still 1.5 $\log_{10}$ lower than counts obtained for the PU control ($P<0.001$) (Figure 4).

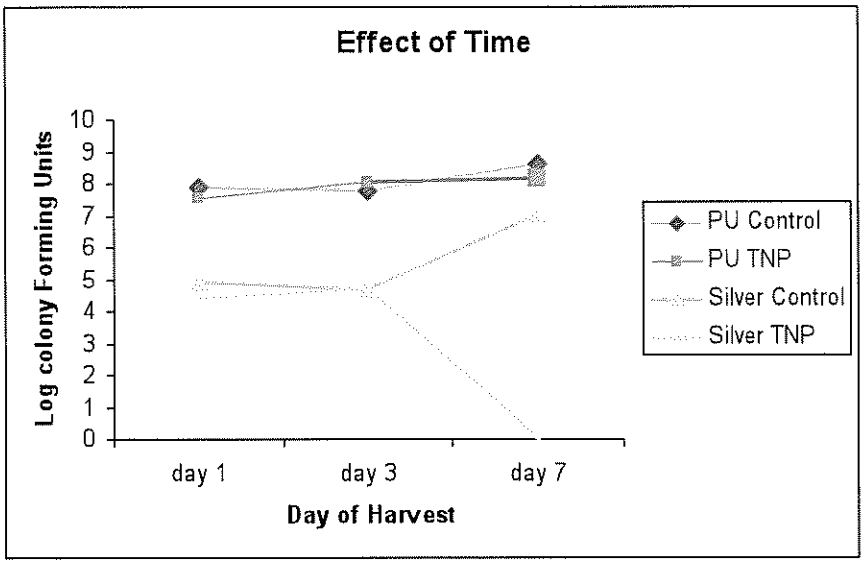
Figure 4. Comparison of V.A.C. ® GranuFoam Silver ®Dressing and V.A.C. ® GranuFoam Dressing on bacterial viability over time.



4. Effect of V.A.C. ® GranuFoam Silver ®Dressing combined with TNP on biofilm viability

Bacterial counts for GranuFoam Silver on day three represent the maximum possible, as exact numbers of CFU could not be determined due to technical difficulties. Application of TNP containing GranuFoam Silver foam did not decreased bacterial viability in the first 3 days. However, bacterial numbers were significantly decreased in the chamber subjected to TNP by day 7 (P=0.002) (figure 5).

Figure 5. Comparison of GranuFoam (PU) and GranuFoam Silver foam with and without TNP applied over 7 days.



Discussion

In this study the efficacy of a mechanical insult (TNP) and a chemical insult (micro-bonded metallic silver) and their combined efficacy against biofilm growing *Ps aeruginosa* was measured using an *in vitro* model. GranuFoam Dressings have no anti-bacterial properties and this was demonstrated by the maintenance and even increase in bacterial numbers in the models when the biofilm covered coupons were overlaid with GranuFoam. This is consistent with our

previous results showing maintenance of bacterial viability in a wound model for up to 14 days (Ngo, 2009).

GranuFoam Silver has antibacterial properties due to the addition of micro-bonded metallic silver and this was demonstrated by the 3 $\log_{10}$ decrease in bacterial numbers evident at day 1 and 3. In the control chambers, the antibacterial effect of silver decreased at the longer time period of 7 days suggesting that the silver ions had leached from the foam. However, this lack of antibacterial activity of the foam was more than compensated for by the addition of TNP. After 7 days of combined TNP and Granufoam Silver no viable bacteria were detected, representing over an 8 log decrease from the GranuFoam chambers.

We have shown that the combination of physical and chemical assault has the potential to disrupt surface *Pseudomonas aerugenosa* biofilm in an *in vitro* model. These findings could be translated to the clinical setting where there has been surface contamination of prosthetic devices. Treatment of PII could potentially salvage a failing prosthesis, thereby reducing the morbidity and significant cost of revision surgery. Future investigations utilizing a variety of other antibacterials and bacterial species are planned.

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Grant: Song Nipple Reconstruction

Evaluator—Mustoe
Grant fulfills the mission of ASERF
Study is well designed. I think it is worth funding. No other expenses are included except for the Radiesse. The only issue is whether this should be industry funded, but I am inclined to suggest funding.

Grade 4

Grant: Holzman, pressors effect on flap survival in rat
Evaluator Mustoe
Grant fulfills mission of ASERF
Study desing is fine. However, the problem studied is of minor clinical importance. Systemic pressors rarely necessary. Major issues whether levels of pressors in rat will translate into human relevance.
Grade 2