The Effect of Chlorhexidine Catheter Coating Compared to a Biomimetic Catheter on the Reduction of Fibrin Sheath Formation in the presence of *Staphylococcus aureus* colonization in an *in vivo* Clinically Simulated Ovine Model

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INTRODUCTION

Intravascular catheters are the most frequently used medical devices in healthcare. They are also associated with serious and life-threatening complications. A catheter in the bloodstream crosses from non-sterile to sterile tissue spaces and interferes with the circulatory flow. Both the cutaneous insertion site and the internal lumen of the catheter serve as entry points for microbial contamination[1]. Over 150 years ago, Virchow observed that reduced blood flow, vessel wall injury, and alterations in coagulation precipitated the formation and propagation of thrombus. In addition to vessel trauma and venous stasis, the catheter itself becomes a thrombogenic surface when exposed to blood. Plasma proteins instantaneously adhere to the catheter on blood contact. This proteinaceous "conditioning layer" in turn modulates platelet and leukocyte activation, inducing the release of inflammatory mediators into the bloodstream[2]. The release of the inflammatory mediators then in turn induces thrombogenesis. The adherent plasma proteins present attachment sites for adhesion of microorganisms to the catheter surface supporting biofilm formation[3-5]. Bacterial endotoxin release and leukocyte activation initiate procoagulant pathways.

The correlation of catheter-related infection and thrombosis has been observed clinically[6-9]. The "bundle" approach of implementing a set of interventions to reduce the risk of catheter-related bloodstream infection (CRBSI) is believed to be instrumental in reducing the incidence of CRBSI in critical care units. These interventions are directed primarily to the prevention of microbial contamination during the insertion procedure and have no relationship with post-insertion colonization and thrombus prevention. Pathogenesis-based strategies including catheter surface modification technology could lead to improved strategies to prevent CRBSI and thrombotic events.

The efficacy of a chlorhexidine (CH) coated catheter was previously studied in an *in vivo* ovine model and was found to be superior to an uncoated catheter in the reduction of extra- and intraluminal catheter colonization[10-11]. During the sample analysis it was observed that the CH catheter had reduced fibrin sheath formation on the extraluminal surface. The absence of fibrotic material on the catheter surface may influence the potential for catheter colonization. The use of a catheter that is protective as both antiinfective and antithrombogenic would be of significant clinical benefit.

PURPOSE

The purpose of this study was to compare antithrombogenic properties of a chlorhexidine coated catheter (CH) to an uncoated catheter (C1) and an antithrombogenic (biomimetic) surface treated catheter (C2) in the presence of *Staphylococcus aureus* colonization in a clinically simulated ovine model.

METHODS

Catheterization

This study was approved by an institutional IACUC for animal subjects. Ten adult male or female sheep weighing between 40-65 kg were randomly assigned to receive either a Test catheter: (5.5 Fr Teleflex (CH) chlorhexidine coated double lumen (DL) PICC, Teleflex Medical, Research Park Triangle, NC) or one of 2 control catheters; Control 1 (C1): 5 Fr. DL PICC, Teleflex Medical, Research Park Triangle, NC or Control 2 (C2): 5 Fr. biomimetic coated DL PICC, r4 Vascular, Maple Grove, MN) in the right or left jugular vein. Sheep were anesthetized and the neck was clipped and scrubbed with soap and water followed by application of 70% IPA. A 2 cm^2 area of skin over the insertion site was marked with a sterile surgical marking pen and inoculated with a 10µL inoculum of *Staphylococcus aureus* 10^6 CFU. Catheters were inserted using a modified Seldinger technique per manufacturer's instruction through the inoculated skin. The catheter tip was positioned in the distal superior vena cava/right atrial junction and confirmed by fluoroscopy. Catheters were flushed with heparin and a neutral needleless connector closed the catheter for the duration of the study. Catheters were secured in place with prolene suture and covered with sterile gauze and elastic vet wrap. Daily observations of body temperature, food intake, attitude, posture and behavior were performed. Insertion site care was done twice per week with sterile saline cleansing and sterile gauze. If the sheep displayed clinical signs of infection such as decreased food intake, depression, elevated body temp (≥ 105˚ F x 2 days), increased WBC count, they were euthanized within 24-48 hours. Blood sampling included CBC, chem panel, pro-thrombin time, and blood culture. Insertion site skin cultures were taken weekly and prior to euthanasia.

Fibrin sheath, catheter and vessel sample analysis

After euthanasia the jugular vein was dissected and removed en bloc. The vein was aseptically longitudinally excised and fibrin sheath length and width were measured. The fibrin sheath was stripped from the catheter and weighed. The jugular vein was fixed in 10% NBF, embedded in paraffin, cut at 3-5 µm and stained with H&E for histological evaluation by a veterinay pathologist. The catheter and fibrin sheaths were shipped in sterile packaging to the Center for Biofilm Engineering for analysis. All samples were treated with DE broth for chlorhexidine neutralization before analysis. Representative 5 cm sections of the catheter (vein insertion, middle, and tip) were vortexed, sonicated, serial diluted, plated and incubated overnight. Colony counts are expressed in CFU/cm^2. The fibrin sheath was homogenized, vortexed, serial diluted, plated and incubated overnight. Colony count results are expressed as CFU/gram.

Statistical analysis

Statistical analysis was performed separately on each of the length and weight of the fibrin sheath, and on the bacterial log densities using a one-way ANOVA, with catheter-type (CH, C1 and C2) as a factor. The ANOVA was used to compare the CH catheter to the C1 and C2 catheters. Tukey tests and confidence intervals for all pairwise comparisons were generated from the ANOVA models. All analyses were performed using the statistical software package Minitab v.16.

RESULTS

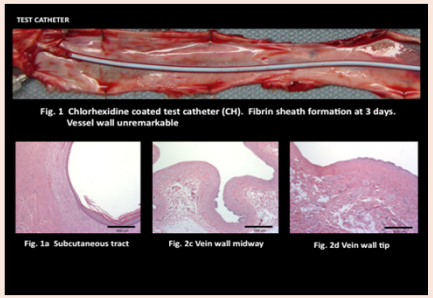

Fig. 1a. Subcutaneous catheter tract just proximal to the vein insertion site lined by keratinized stratified squamous epithelium consistent with in-growth from the skin insertion site surrounded by connective tissue.
Fig 1b. Transverse section of vein, adjacent connective and adipose tissue. Up to 25% luminal surface lined by multifocal hyperplastic intima consisting of endothelium and connective tissue (up to 110µm thick).
Fig 1c. Vein wall at approximate level of catheter tip. One section with single layer endothelium another with intimal hyperplasia (10% luminal surface, up to 25µm thick).

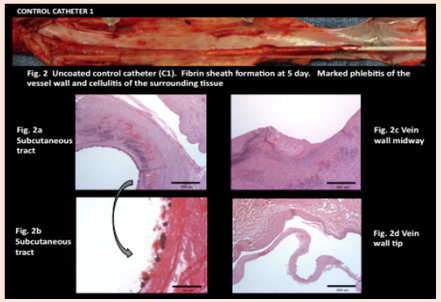

Fig. 2a Catheter tract (bottom left) adjacent to vein (upper right) just before insertion. Luminal surface (catheter space) is lined with multifocal organized basophilic coccoid biofilm colonies, The tract wall is composed of loose dense fibrin with large amount of necrotic inflammatory cell debris (up to 1µm thick). Transmural necrosis of vein wall.
Fig. 2b Gram Stain of coccoid biofilm colonies catheter tract surface (1-3µm) in Fig. 2a
Fig. 2c Vein wall section with fibrin lining the luminal surface (center) and transmural necrosis (left). Surrounding connective tissue thickened by fibrin, proteinaceous edema, RBCs, neutrophils, lymphocytes, and necrotic inflammatory cells entrapping adipose tissue lobules.
Fig. 2d Transverse section of vein wall with valve leaflet, connective and adipose tissue. Approx. 25-50% luminal surface lined by mild, intimal hyperplasia consisting of endothelium and connective tissue (up to 25µm thick). Other areas of intimal hyperplasia have fibrin and RBCs attached. Valve leaflet has similar changes.

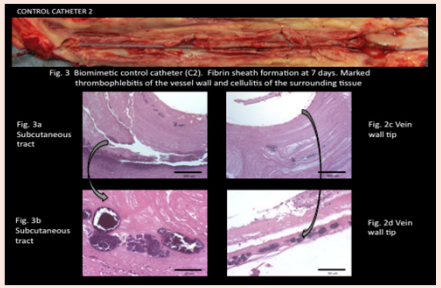

Fig. 3a Transverse section of vein at level of catheter insertion with catheter tract, adjacent connective and adipose tissue. Catheter tract composed of large amounts of fibrin and protein in concentric layers. The fibrin and luminal surface contains RBCs, necrotic inflammatory cells and multifocal biofilm colonies.
Fig. 3b Higher magnification of Fig 3a with biofilm colonies within the fibrin.
Fig. 3c Transverse section of vein, connective and adipose tissue at level of catheter tip. The vein wall has diffuse transmural necrosis. Luminal surface lined by large amounts loose fibrin with multifocal biofilm colonies.
Fig. 3d Gram Stain of luminal wall and associated fibrin of Fig. 3c. Luminal surface fibrin with coccoid biofilm colonies.

STATISTICAL ANALYSIS

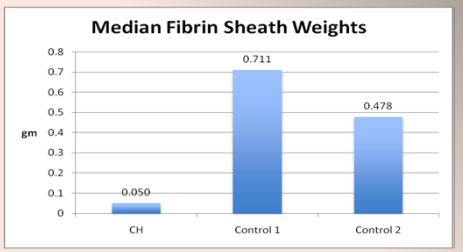

Figure 4 shows the estimated median values of the fibrin sheath lengths for each of the catheter groups. Note that the CH catheter was statistically significantly different from C1 (p-value = 0.0012) and the CH catheter was statistically significantly different from C2 (p-value = 0.0032). The two control catheters were not statistically significantly different from each other (p-value=.684).

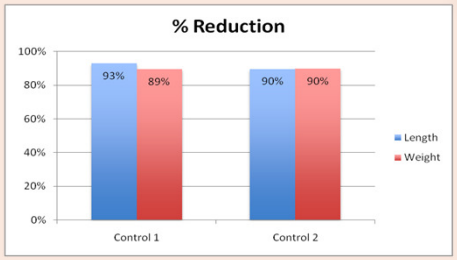

Figure 5 shows the % Reduction in relation to either the length or weight of the fibrin sheath achieved by CH when compared to either C1 or C2 catheters.

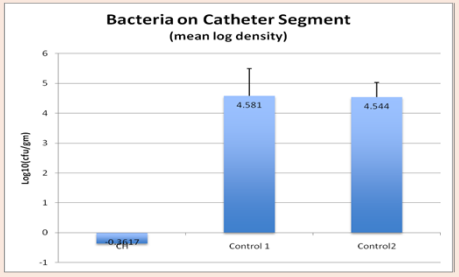

Figure 6 shows the mean log density and SD of bacteria on the catheter segment. The CH catheter was statistically significantly different from both the C1 (p-value = 0.00005) and the C2 catheters (p-value = 0.00005). The two control catheters were not statistically significantly different from each other (p-value = 0.325).

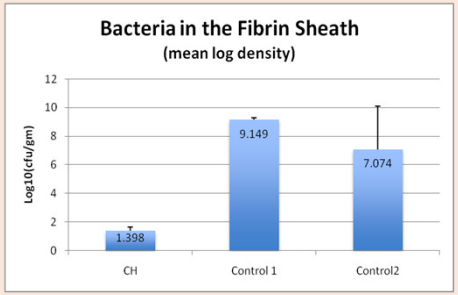

Figure 7 shows the mean log density and SD of the bacteria in the fibrin sheath. Note that the CH catheter was statistically significantly different from both the C1 (p-value = 0.0011 and the C2 catheters (p-value = 0.0066). The two control catheters were not statistically significantly different from each other (p-value = 0.325).

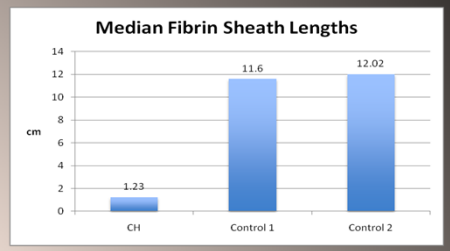

Figure 8 shows the estimated median values of the fibrin sheath lengths for each of the catheter groups. Note that the CH catheter is statistically significantly different from C1 (p-value = 0.01150) and the CH catheter was statistically significantly different from C2 (p-value = 0.0106). The two control catheters were not statistically significantly different from each other (p-value=.9980).

CONCLUSIONS

- The Chlorhexidine coated catheter showed a statistically significantly smaller fibrin sheath than either of the control catheters.
- The Chlorhexidine coated catheter showed a clinically significant reduction in fibrin sheath formation than the control catheters
- The Chlorhexidine coated catheter showed a statistically significantly reduced bacterial burden within the fibrin sheath and the catheter itself.
- There was an impressive macroscopic and microscopic reduction in cellulitis, thrombophlebitis and necrosis of the tissues surrounding the catheter tract of the Chlorhexidine coated catheter compared to the control catheters.
- Based on histologic findings, there was less intimal hyperplasia in the veins of the catheters with no evidence of infection (all chlorhexidine catheters and one control 2 catheter).
- The Chlorhexidine coated catheter was protective for 31 days against thrombotic events and infection in the presence of colonization (except for one C2 catheter) while the control catheter cases exhibited septic thrombophlebitis at 5 and 7 days.

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