

Research Article

An *In Vivo* Rabbit Model for the Evaluation of Antimicrobial Peripherally Inserted Central Catheter to Reduce Microbial Migration and Colonization as Compared to an Uncoated PICC

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Infection is the leading complication associated with intravascular devices, and these infections develop when a catheter becomes colonized by microorganisms. To combat this issue, medical device manufacturers seek to provide healthcare facilities with antimicrobial medical devices to prevent or reduce the colonization. In order to adequately evaluate these devices, an *in vivo* model is required to accurately assess the performance of the antimicrobial devices in a clinical setting. The model presented herein was designed to provide a simulation of the subcutaneous tunnel environment to evaluate the ability of an antimicrobial peripherally inserted central catheter (PICC), coated with chlorhexidine based technology, to reduce microbial migration and colonization compared to an uncoated PICC. Three samples of control, uncoated PICCs and three samples of coated PICCs were surgically tunneled into the backs of female New Zealand White rabbits. The insertion sites were then challenged with *Staphylococcus aureus* at the time of implantation. Animals were evaluated out to thirty days and sacrificed. Complete *en bloc* dissection and evaluation of the catheter and surrounding tissue demonstrated that the chlorhexidine coated catheter was able to significantly reduce microbial colonization and prevent microbial migration as compared to the standard, un-treated catheter.

1. Introduction

Indwelling intravascular devices, which are becoming integral components of modern medical practice, are associated with the high incidence of nosocomial bloodstream infections especially in intensive care patients, those receiving chemotherapy and those dependent upon haemodialysis. Central venous catheters (CVCs) account for approximately 90% of all catheter-related bloodstream infections (CRBSIs) [1]. PICCs are associated with similar rates of CRBSI as CVCs, placed in internal jugular or subclavian veins (2 to 5 per 1,000 catheter days) [2]. It has been estimated that nearly 80,000 CRBSIs occur in intensive care units alone and result in costs approaching 2 billion dollars and 20,000 deaths annually [3–5]. Colonization of the catheter by microorganisms forming a biofilm is the first stage of

the infection process [6]. Sources for microbial colonization may be environmental contamination, skin organisms, post-placement subcutaneous tract infection, intraluminal contamination, or hematogenous seeding [7]. Whether derived from the patient's skin, hematogenously, or from a health care worker, microorganisms present in the catheter display the characteristic reduced susceptibility to antimicrobials, which has become a hallmark of biofilms [8]. Biofilms are a cohesive matrix of microorganisms, mucopolysaccharides (slime), and extracellular constituents that exist in virtually every natural environment [9]. They form on a solid surface (such as a catheter or other implanted medical device) in the presence of shear force or flow (as would be encountered in the environment surrounding an implant medical device), as a mechanism to avert being removed from that environment [10]. Biofilm formation is a developmental process moving

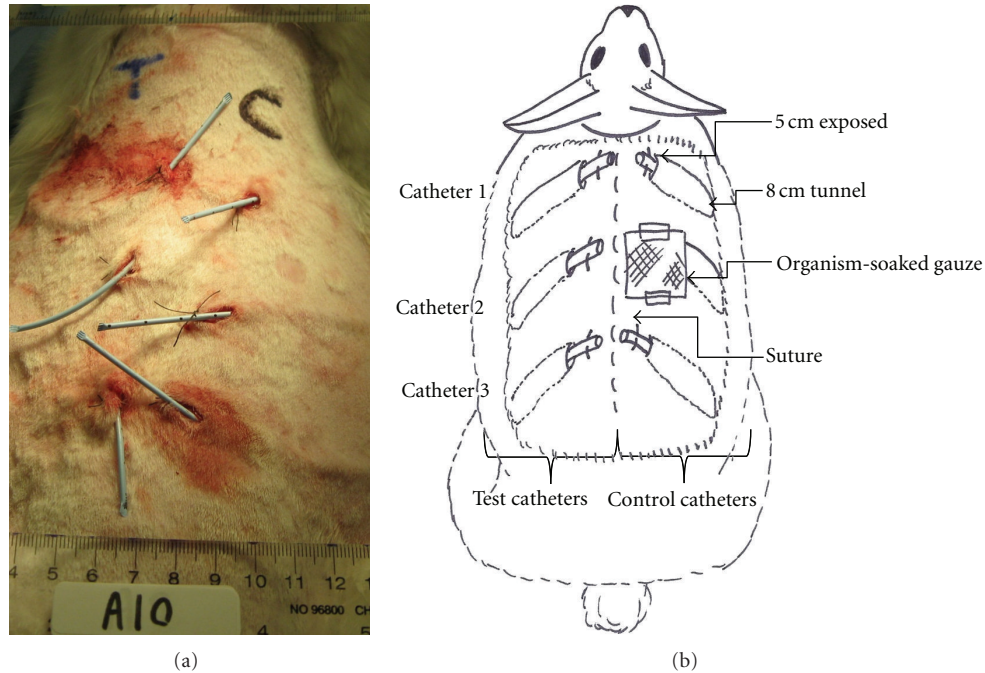


FIGURE 1: Left side image shows actual catheter placement (rabbit 10 shown) prior to challenge and bandaging. Right side is a cartoon diagram representing catheter placement and challenge procedures. The diagram displays only those portions of the device which are evaluated. Note that the test and control sides are randomized between animals and groups.

TABLE 1: A diagram and table describing the preparation of the explanted catheter. Note that 13 cm sections are implanted, but 3 cm which is heat sealed and remains outside of the animal is removed prior to evaluation.

(a) The bolded (underlined numbers) represent tissue segments and catheter material that were exposed to the subcutaneous tunnel environment

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
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(b) The table describes what the various segments were used for in the analysis of each segment

Segment no.	Microbiology	Histology	SEM
1	X		
2	X		
3	X		
4	X		
5		X	X
6	X		
7		X	X
8	X		
9		X	X
10	X		

from attachment to microcolony formation and then to mature biofilm development under the control of specific biofilm genes [11]. The production of a mucopolysaccharide (slime) on the surface, which further protects the biofilm, can often be seen with the naked eye [9]. Microbial

biofilms demonstrate recalcitrance towards a wide range of antimicrobial treatments and have been reported to be 100–1,000 times less susceptible than their planktonic counterparts [12]. This resistance is due to the presence of extracellular polysaccharide matrix, the physicochemical heterogeneity developed within such consortia, acquiring of multiantimicrobial resistance genes, and the presence of cells of highly recalcitrant physiology (persisters) [13]. Thus, once an implanted medical device (such as a PICC) is colonized by biofilm bacteria, the bacteria are protected from both immune response and antimicrobial therapy, and thus, such infections are rarely resolved [14–16]. This fact often leads to the failure of conventional antibiotic therapy and necessitates the removal of infected devices. Removal of the infected device is often not practical (as in the case in pace makers or artificial joints) or not at good strategy as this can lead to the production of detached, slime-enclosed, antibiotic-resistant aggregates that can initiate endocarditis or pneumonia by dissemination in the blood stream [17]. With this in mind, medical device manufacturers and researchers in biofilm microbiology have developed strategies and technologies to prevent the initial colonization and prevent the subsequent biofilm colonization of various implant medical devices. A good approach to the reduction of CRBSI includes surface modification of devices to reduce microbial attachment and biofilm development as well as the use of CVCs precoated with antimicrobials such as chlorhexidine and/or silver sulfadiazine and antibiotics such as minocycline and rifampin [18–22].

The proper development and evaluation of antibiofilm efficacy of antimicrobial coated or eluting medical devices

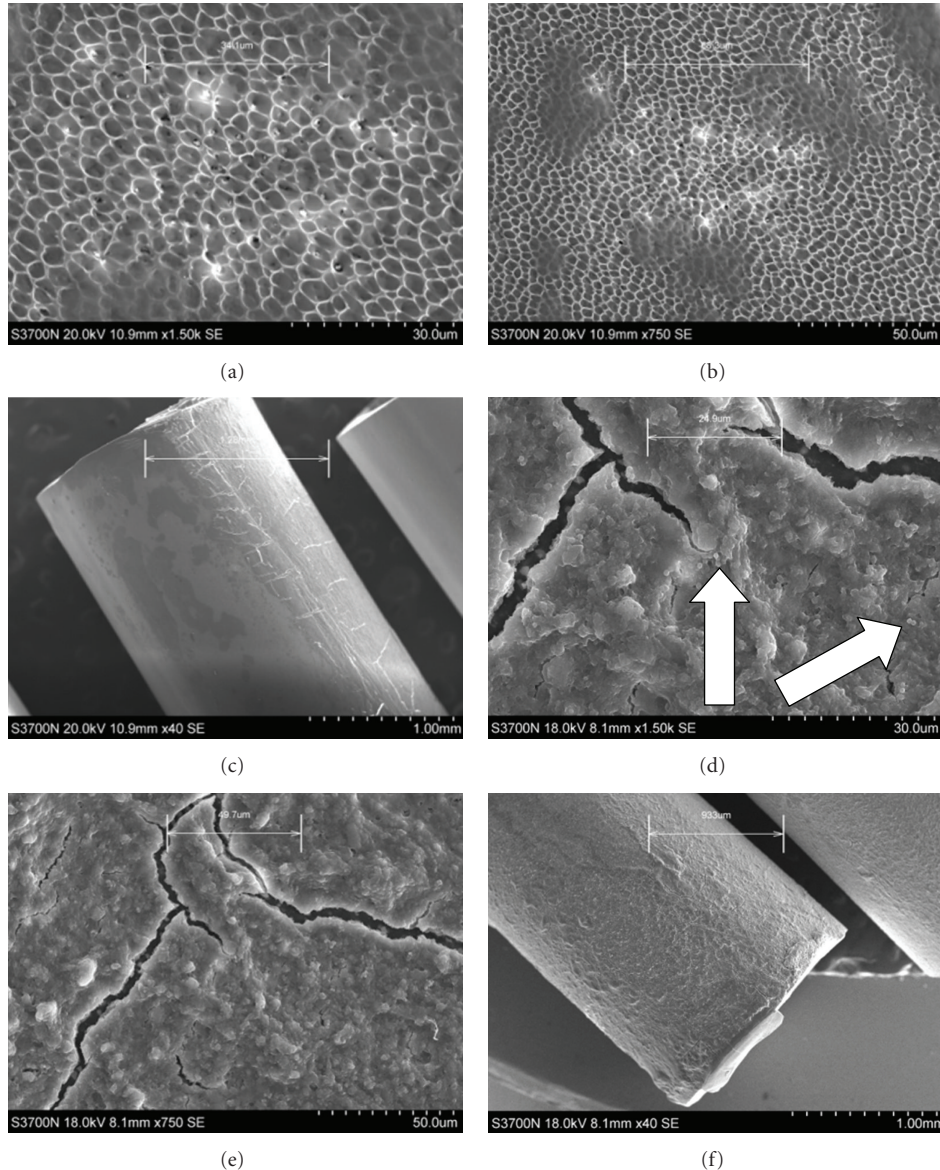


FIGURE 2: Representative SEM micrographs taken of test catheter 3 (segment 7) and control catheter 3 (segment 7) from animal 10 on day 21. (a) 1500x magnification of test catheter, (b) 750x magnification of test catheter, (c) 40x magnification of test catheter, (d) 1500x magnification of control catheter, (e) 750x magnification of control catheter, and (f) 40x magnification of control catheter. Note: the fissures seen in the biofilm in images D & E are artifacts from the SEM preparation process. Scale bars are presented on each image. Arrows point to isolated cocci.

has proven to be a daunting task due to the limitations of good *in vitro* evaluation tools. Rapid screening tools like the MBEC assay can be utilized to evaluate short-term exposures and isolated antimicrobials and potential antibiofilm agents [23–25] but do not evaluate the antimicrobial agent as it is presented on the actual medical device. Most commonly, the testing of potential implant materials or medical devices is often done by measuring planktonic growth in the presence of the respective material or by “zone of inhibition” determination [26, 27]. Placing selected bacterial suspensions and exposing them to short-term incubation (<24 hr) directly

on implant/coated materials and analyzing viable counts is also a standardized method to examine antibacterial activity (Japanese Industrial Standards, as described in [28]). Capillary flow cells that study different biofilm stages under constant nutrient, salt, and pH conditions [29] or disc/drip flow reactors that expose implant/coated materials to biofilm under shear [30] account for the dynamic environment the devices may be exposed to and the biofilm state of the organisms colonizing the device but are limited in that only partial devices or components thereof can be assessed. The so-called “roll plate,” method first described by Maki et al.

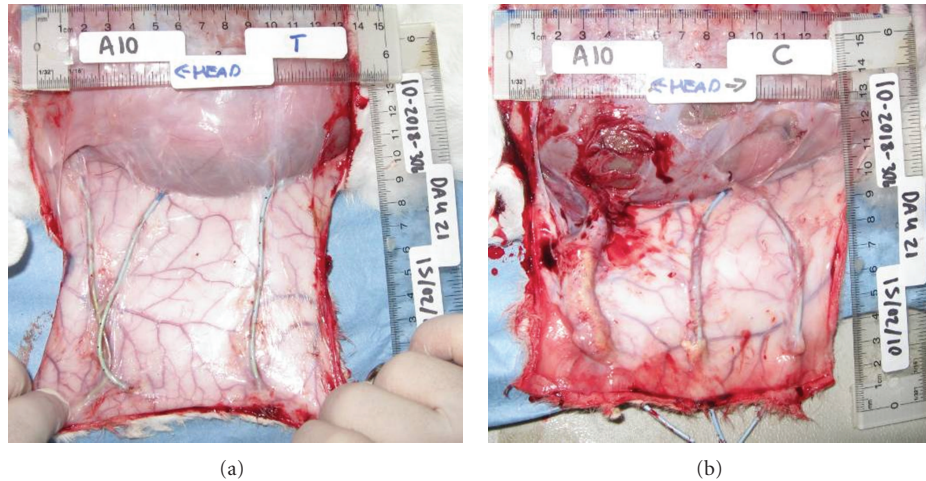


FIGURE 3: Representative digital micrographs taken during catheter explant and necropsy on rabbit 10. (a) Test article side: note that the fibrous capsule is minimal, and there is no evidence of pus or irritation. (b) Control article side: the digital image demonstrates indicators of infection uniformly across the three control replicates. Extensive pus, hyperemia, and fibrosis can be seen associated with the tissue around the control catheters.

[31], can evaluate the entire surface of the catheter but is also limited in its practicality and potential operator errors associated with the manipulations of the catheters.

When evaluating and developing implant medical devices with surface modifications, the study of the antibacterial/antibiofilm effects must take into account the actual device itself and be tested while exposed to an appropriate *in vitro* test system that mimics the environment in which it is expected to perform. In addition, demands of regulatory agencies such as the American Food and Drug Administration (FDA) [32] require the utilization of effective *in vitro* (preferably supported by *in vivo*) data to support any label claims. This evaluation needs to simulate human clinical situation which includes (1) using the actual device (preferably sterile finished goods), (2) preconditioning or exposure of the device to body fluid, (3) using a dynamic (rather than static) environment, (4) evaluating the actual contact time with the body, (5) at body temperature, and (6) assuring that the organisms used are relevant to the label claim [32, 33].

To address the needs and testing gaps described above, we developed an *in vivo* test method to provide a high-throughput, repeatable, and cost-effective simulation of the subcutaneous tunnel environment with multiple end points to evaluate the ability of an antimicrobial PICC to reduce microbial migration and colonization as compared to an uncoated PICC.

2. Methods and Materials

2.1. Animals. A total of 10 healthy, ~15-week-old, ~3 Kg, New Zealand white female rabbits (Charles River Laboratories, Canada) were used in this study. Five rabbits were allocated to each sample time (21 and 30 days, resp.). All animals received a thorough physical examination before entry into the study. Any animal that did not meet the health

and weight criteria was excluded from the study. Animals with any infection, inflammation, dermatitis, muscular disease, or apparent abnormalities of the urinary tract as well as any animal deemed unsuitable by the study director were also excluded from this study. The study was conducted in compliance with the guidelines of the Canadian Council on Animal Care after the appropriate review by the Institutional Animal Care and Use Committee.

2.2. Catheters. All catheter materials were custom manufactured to 13 cm lengths from the tip and heat sealed at ends. The catheters were individually packaged and sterilized by Arrow International, Inc. (Reading, PA). The test article consisted of 5.5 French (Fr) double lumen Arrow Antimicrobial PICC with Chlorag + ard Technology (Teleflex Medical Inc, Reading, PA). The control/predicate material consisted of uncoated 5.5 Fr double lumen PICC material (Teleflex Medical Inc, Reading, PA).

2.3. Experimental Design. Animals were allocated to test groups using a random block procedure and randomly assigned to groups 1 (21-day catheterization) and 2 (30-day catheterization). As each animal served as its own control and treatment, control/treatment sides were allocated to each treatment group using a random block procedure and randomly assigned to each rabbit. Test and control sides were randomized between animals and groups (see Figure 1).

2.4. Preparation of Inoculum. *Staphylococcus aureus* ATCC 29213 (American Type Culture Collection, Manassas, VA) was selected due to its role in CRBSI [33]. This strain was selected because it has been used successfully based on our experience to cause infection in NZW rabbits, and this strain was used in an *in vitro* challenge experiment using artificially aged PICC material and demonstrated as significant ($> \text{Log}_{10} 4$ reduction compared to controls). Using

TABLE 2: Tissue (CFU/g) Log reduction summary tables for day 21. Log reduction values higher than 4 are bold and underlined.

(a) For pairwise comparison, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit per segment

Day	Sample no. Rabbit no.	Sample location				
		3	4	6	8	10
21	1	0.81	4.28	6.03	289	2.44
	<i>P</i>	0.50	0.05	0.00	0.31	0.83
21	4	-2.46	-1.54	-0.44	1.93	1.18
	<i>P</i>	0.18	0.35	0.86	0.37	0.59
21	7	2.90	3.08	1.87	1.70	3.22
	<i>P</i>	0.13	0.14	0.37	0.50	0.12
21	9	1.23	1.10	1.48	0.00	1.59
	<i>P</i>	0.37	0.37	0.37	N/A	0.37
21	10	5.30	3.68	3.73	5.02	4.48
	<i>P</i>	0.00	0.13	0.14	0.01	0.01

(b) For test article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

Day	Sample no. Rabbit no.	Sample location				
		3	4	6	8	10
21	1	2.62	5.67	6.86	5.55	5.72
	<i>P</i>	0.50	0.05	0.00	0.31	0.83
21	4	1.49	2.53	4.41	6.86	5.38
	<i>P</i>	0.18	0.35	0.86	0.37	0.59
21	7	6.86	6.86	6.86	5.29	6.86
	<i>P</i>	0.13	0.14	0.37	0.50	0.12
21	9	6.86	6.86	6.86	6.86	6.86
	<i>P</i>	0.37	0.37	0.37	N/A	0.37
21	10	6.86	6.86	6.86	6.86	6.86
	<i>P</i>	0.00	0.13	0.14	0.01	0.01

(c) For control article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

Day	Sample no. Rabbit no.	Sample location				
		3	4	6	8	10
21	1	1.81	1.39	0.82	265	3.28
	<i>P</i>	0.50	0.05	0.00	0.31	0.83
21	4	3.95	4.07	4.85	4.93	4.20
	<i>P</i>	0.18	0.35	0.86	0.37	0.59
21	7	3.96	3.77	4.99	3.59	3.64
	<i>P</i>	0.13	0.14	0.37	0.50	0.12
21	9	5.63	5.75	5.38	6.86	5.26
	<i>P</i>	0.37	0.37	0.37	N/A	0.37
21	10	1.56	3.18	3.13	1.84	2.38
	<i>P</i>	0.00	0.13	0.14	0.01	0.01

TABLE 3: Catheter (CFU/catheter segment) Log reduction summary tables for day 21. Log reduction values higher than 4 are bold and underlined.

(a) For pairwise comparison, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit per segment

Day	Sample no. Rabbit no.	Sample location						
		1	2	3	4	6	8	10
21	1	-0.47	-0.07	1.58	2.44	0.49	0.00	0.87
	<i>P</i>	0.78	0.94	0.37	0.15	0.78	1.00	0.37
21	4*	-1.99	0.71	0.17	-0.87	1.45	1.95	0.00
	<i>P</i>	0.23	0.76	0.93	0.50	0.27	0.27	1.00
21	7	1.84	2.38	1.31	0.97	1.03	1.03	0.00
	<i>P</i>	0.12	0.12	0.37	0.37	0.37	0.37	1.00
21	9	-0.67	0.43	1.15	1.28	0.00	0.00	0.00
	<i>P</i>	0.65	0.06	0.37	0.37	1.00	1.00	1.00
21	10	2.08	0.33	1.48	3.18	2.92	2.60	1.50
	<i>P</i>	0.08	0.85	0.37	0.16	0.13	0.14	0.37

(b) For test article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

Day	Sample no. Rabbit no.	Sample location						
		1	2	3	4	6	8	10
21	1	1.33	2.49	6.86	6.86	5.99	6.86	6.86
	<i>P</i>	0.78	0.94	0.37	0.15	0.78	1.00	0.37
21	4*	4.86	5.62	5.73	5.99	6.86	6.86	6.86
	<i>P</i>	0.23	0.76	0.93	0.50	0.27	0.27	1.00
21	7	6.86	6.86	6.86	6.86	6.86	6.86	6.86
	<i>P</i>	0.12	0.12	0.37	0.37	0.37	0.37	1.00
21	9	5.12	3.34	6.86	6.86	6.86	6.86	6.86
	<i>P</i>	0.65	0.06	0.37	0.37	1.00	1.00	1.00
21	10	5.12	5.89	6.86	6.86	6.86	6.86	6.86
	<i>P</i>	0.08	0.85	0.37	0.16	0.13	0.14	0.37

(c) For control article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

Day	Sample no. Rabbit no.	Sample location						
		1	2	3	4	6	8	10
21	1	1.80	2.56	5.27	4.42	5.50	6.86	5.99
	<i>P</i>	0.78	0.94	0.37	0.15	0.78	1.00	0.37
21	4*	6.86	4.90	5.55	6.86	5.40	4.90	6.86
	<i>P</i>	0.23	0.76	0.93	0.50	0.27	0.27	1.00
21	7	5.02	4.47	5.54	5.89	5.83	5.83	6.86
	<i>P</i>	0.12	0.12	0.37	0.37	0.37	0.37	1.00
21	9	5.79	2.90	5.71	5.58	6.86	6.86	6.86
	<i>P</i>	0.65	0.06	0.37	0.37	1.00	1.00	1.00

(c) Continued.

		Sample location						
Sample no.		1	2	3	4	6	8	10
Day Rabbit no.		Control article LogR (based on initial inoculum)						
21	10	3.04	5.56	5.37	3.68	3.94	4.25	5.35
	P	0.08	0.85	0.37	0.16	0.13	0.14	0.37

* indicates that control catheter 3 on rabbit 4 was not available for analysis.

a cryogenic stock (at -70°C), a first subculture of the bacterial organisms listed above was streaked out on tryptic soy agar (TSA) (Becton Dickinson, Sparks, MD). The plate was incubated at $35 \pm 2^{\circ}\text{C}$ for 24 hours and stored wrapped in parafilm at $4 \pm 2^{\circ}\text{C}$. From the first sub-culture, a second sub-culture was streaked out on TSA and incubated at $35 \pm 2^{\circ}\text{C}$ for 24 hours. The second sub-culture was used within 24 hours starting from the time it was first removed from incubation. From a fresh streak plate, each study organism was inoculated in 200 mL of sterile Trypticase Soy Broth (TSB) (Becton Dickinson, Sparks, MD). Organisms were grown in TSB at $37 \pm 2^{\circ}\text{C}$ on a rotary shaker (at approximately 150 rpm) for 12–18 hours. This achieved an inoculum density of approximately 10^9 colony-forming units (CFU)/mL. The inoculum was adjusted to an approximate cell density of 10^6 CFU/mL by diluting *Staphylococcus aureus* in sterile 0.9% saline (Baxter, Canada). The cell density was confirmed by serially diluting and spot plating triplicate samples of the inoculum.

2.5. Surgical Procedures and Catheterization. Rabbits were fasted approximately 12 hours prior to surgery. Anesthesia (Isoflurane) (Benson Medical, Markham, ON, Canada) was administered by the qualified veterinary staff per test facility standard operating procedure. Selection of the dose and agents followed the standard operating procedures of the test facility. The hair over the dorsal thorax and abdomen was clipped with a number 40 Osler clipper blade and scrubbed with a soap that did not contain any disinfectant or antibiotic. Three incisions were made on either side of the dorsal midline for catheter insertion. The catheters (13 cm long) were inserted subcutaneously in each rabbit so ~ 8 cm tunneled under the skin and 6 cm was on the skin surface (see Figure 1). The catheters were anchored to the skin with adhesive tape and sutures. Animals were observed for any adverse reactions. The catheters were challenged by inoculating the insertion site with ~ 1 mL of inoculum. To supplement this significant challenge, a gauze sponge with 5 mL of the prepared bacterial suspension was also placed over each insertion site. Opsite (Smith and Nephew, Hull, England) was placed over all catheters and inoculum-soaked sponges. Tensoplast (BSN Medical, Pinetown, South Africa), an occlusive bandage, was then placed over all catheters to prevent self-mutilation and removal of the catheters. All animals were observed for morbidity and mortality, overt signs of toxicity (including abstinence of water), and any signs of distress throughout the study per test facility procedures.

TABLE 4: Tissue (CFU/g) Log reduction summary tables for day 30. Log reduction values higher than 4 are bold and underlined.

(a) For pairwise comparison, the mean Log_{10} CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log_{10} CFU/g recovered from the three replicates of control articles/rabbit per segment

		Sample location				
Day	Sample no. Rabbit no.	3	4	6	8	10
30	2	0.98	0.00	0.00	0.00	-0.74
	P	0.37	0.00	0.00	0.00	0.76
30	3	-1.05	2.43	1.16	2.48	4.52
	P	0.37	0.13	0.37	0.12	0.00
30	5	-2.57	2.18	2.41	1.06	1.04
	P	0.12	0.12	0.12	0.37	0.37
30	6**	3.91	0.00	0.00	0.00	0.00
	P	N/A	N/A	N/A	N/A	N/A
30	8	4.90	2.24	3.70	3.68	5.18
	P	0.01	0.37	0.14	0.13	0.00

(b) For test article log reduction, the mean Log_{10} CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log_{10} CFU from the initial inoculum counts that were exposed to the catheters at implantation

		Sample location				
Day	Sample no. Rabbit no.	3	4	6	8	10
30	2	6.86	6.86	6.86	6.86	5.02
	P	0.37	0.00	0.00	0.00	0.76
30	3	5.81	6.86	6.86	6.86	6.86
	P	0.37	0.13	0.37	0.12	0.00
30	5	4.28	6.86	6.86	6.86	6.86
	P	0.12	0.12	0.12	0.37	0.37
30	6**	6.86	6.86	6.86	6.86	6.86
	P	N/A	N/A	N/A	N/A	N/A
30	8	6.86	6.86	6.86	6.86	6.86
	P	0.01	0.37	0.14	0.13	0.00

(c) For control article log reduction, the mean Log_{10} CFU/g recovered from the three replicates of control articles/rabbit was subtracted from the mean Log_{10} CFU from the initial inoculum counts that were exposed to the catheters at implantation

		Sample location				
Day	Sample no. Rabbit no.	3	4	6	8	10
30	2	5.87	6.86	6.86	6.86	5.76
	P	0.37	0.00	0.00	0.00	0.76
30	3	6.86	4.42	5.70	4.37	2.34
	P	0.37	0.13	0.37	0.12	0.00
30	5	6.86	4.67	4.44	5.80	5.81
	P	0.12	0.12	0.12	0.37	0.37
30	6**	2.95	6.86	6.86	6.86	6.86
	P	N/A	N/A	N/A	N/A	N/A
30	8	1.95	4.62	3.15	3.18	1.67
	P	0.01	0.37	0.14	0.13	0.00

** indicates that control tunnels 1 and 3 on rabbit 6 were not available for analysis.

TABLE 5: Catheter (CFU/catheter segment) Log reduction summary tables for day 30. Log reduction values higher than 4 are bold and underlined.

(a) For pairwise comparison, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit per segment

		Sample location						
Sample no.		1	2	3	4	6	8	10
Day	Rabbit no.	LogR (pairwise comparison)						
30	2*	0.30	1.80	0.00	0.00	1.75	1.60	0.00
	<i>P</i>	0.88	0.27	0.00	0.00	0.27	0.27	0.00
30	3	-1.72	2.98	3.43	3.49	0.87	2.71	1.19
	<i>P</i>	0.34	0.10	0.00	0.00	0.37	0.12	0.37
30	5*	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	<i>P</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	6**	0.00	0.00	2.60	0.00	0.00	0.00	0.00
	<i>P</i>	N/A	N/A	N/A	N/A	N/A	N/A	N/A
30	8	2.49	2.56	1.97	2.40	2.38	2.40	1.13
	<i>P</i>	0.12	0.12	0.12	0.12	0.12	0.12	0.37

(b) For test article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of test articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

		Sample location						
Sample no.		1	2	3	4	6	8	10
Day	Rabbit no.	Test article LogR (based on initial inoculum)						
30	2*	<u>5.71</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	0.88	0.27	0.00	0.00	0.27	0.27	0.00
30	3	<u>4.27</u>	<u>5.50</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	0.34	0.10	0.00	0.00	0.37	0.12	0.37
30	5*	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	6**	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	N/A	N/A	N/A	N/A	N/A	N/A	N/A
30	8	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	0.12	0.12	0.12	0.12	0.12	0.12	0.37

(c) For control article log reduction, the mean Log₁₀ CFU/g recovered from the three replicates of control articles/rabbit was subtracted from the mean Log₁₀ CFU from the initial inoculum counts that were exposed to the catheters at implantation

		Sample location						
Sample no.		1	2	3	4	6	8	10
Day	Rabbit no.	Control article LogR (based on initial inoculum)						
30	2*	<u>5.40</u>	<u>5.06</u>	<u>6.86</u>	<u>6.86</u>	<u>5.10</u>	<u>5.25</u>	<u>6.86</u>
	<i>P</i>	0.88	0.27	0.00	0.00	0.27	0.27	0.00
30	3	<u>5.99</u>	2.52	3.42	3.36	<u>5.99</u>	<u>4.15</u>	<u>5.67</u>
	<i>P</i>	0.34	0.10	0.00	0.00	0.37	0.12	0.37
30	5*	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	6**	<u>6.86</u>	<u>6.86</u>	<u>4.25</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>	<u>6.86</u>
	<i>P</i>	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(c) Continued.

		Sample location						
Sample no.		1	2	3	4	6	8	10
Day	Rabbit no.	Control article LogR (based on initial inoculum)						
30	8	<u>4.36</u>	<u>4.30</u>	<u>4.89</u>	<u>4.46</u>	<u>4.48</u>	<u>4.46</u>	<u>5.73</u>
	<i>P</i>	0.12	0.12	0.12	0.12	0.12	0.12	0.37

* indicates that control catheter 1 on rabbits 2 and 5 was not available for analysis.
 ** indicates that control catheters 1 and 3 on rabbit 6 were not available for analysis.

TABLE 6: Infection prevention in the tissue samples: the total number of samples where significant (≥ 3.0 Log) bacterial load was recovered (from sample locations 4, 6, 8, and 10) is divided by the total number of catheters/tracts in each group. This is separated by sample type (tissue and catheter).

Sample day	Test catheter tunnel	Control catheter tunnel	<i>P</i> *
21	40% (6 of 15 tracts)	80% (12 of 15 tracts)	0.030216 (S)
30	6% (1 of 15 tracts)	76% (10 of 13 tracts)	0.000203 (S)

* Fisher exact test for statistical significance (for statistical significance (S), $P \leq 0.05$).

TABLE 7: Infection prevention on the catheter segments: the total number of samples where significant (≥ 3.0 Log) bacterial load was recovered (from sample locations 4, 6, 8, and 10) is divided by the total number of catheters/tracts in each group. This is separated by sample type (tissue and catheter).

Sample day	Test catheter	Control catheter	<i>P</i> *
21	0% (0 of 15 catheter segments)	43% (6 of 14 catheter segments)	0.006322 (S)
30	0% (0 of 15 catheter segments)	54% (6 of 11 catheter segments)	0.002007 (S)

* Fisher exact test for statistical significance (for statistical significance (S), $P \leq 0.05$).

2.6. *Euthanasia and Harvesting of Samples.* Animals were euthanized with an overdose of intracardiac-injected sodium pentobarbital at 21 (group 1) and 30 (group 2) days after catheterization, based on Alberta Veterinary Medical Association (AVMA) procedures and guidelines. Animals were immediately prepared for aseptic collection of tissue samples. A complete *en bloc* dissection was made excising the skin and catheter at the insertion site and the tissue underlying and surrounding the catheter. The distal and proximal ends of the tissue were surgically stapled to the PICC line to secure the location. A photo was taken to record complete length of the catheter positioned in the tissue bloc (*in situ*). The catheter (and surrounding tunnel) was segmented into 10 × 1 cm segments for analysis as described in Table 1. The procedure was repeated for each catheter.

2.7. *Microbiology Procedures*

Catheter Segments. 1 cm segments were aseptically separated from the surrounding tissue. Each catheter segment was

inserted into a sterile 6-well plate (Becton Dickinson, Franklin Lakes, NJ) containing 4 mL of D/E neutralizing broth (Becton Dickinson, Sparks, MD) in each well. The entire 6-well plate assembled above was sonicated in a bath sonicator (VWR 550T, VWR, Canada) for 30 minutes. Following sonication, 100 μ L from each well of the six-well plates was placed into the first 12 empty wells of the first row of a 96-well microtiter plate (Nunc, Roskilde, Denmark). 180 μ L of 0.9% sterile saline was placed in the remaining rows. A serial dilution (10^0 – 10^{-7}) was prepared by moving 20 μ L down each of the 8 rows. 10 μ L was removed from each well and spot plated on a prepared TSA plate. Plates were incubated at $37 \pm 2^\circ\text{C}$ and counted after approximately 24 hours of incubation. Data was evaluated as CFU/catheter segment.

Tissue Segments. Each 1 cm tissue segment was aseptically homogenized (Brinkman Polytron PT (VWR, Canada) in 2.5 sterile phosphate buffered saline (PBS: 2 g KCl, 2.4 g KH_2PO_4 , 80 g NaCl, and 14.4 g Na_2HPO_4 , to 1000 mL; pH 7.4)) in sterile preweighed 50 mL conical centrifuge tubes (Fisher Scientific, Mexico). Following homogenization, 100 μ L from each tissue homogenate specimen was placed into the first 12 empty wells of the first row of 96-well microtiter plates. The remaining wells all contained 180 μ L of 0.9% sterile saline. A serial dilution (10^0 – 10^{-7}) was prepared by moving 20 μ L down each of the 12 rows. From each well, 10 μ L was removed and spot plated on TSA plates. Culture plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 hours, after which the numbers of CFU were counted as CFU per gram of tissue.

2.8. Calculation of Log Reduction. Catheter efficacy is presented by Log_{10} reduction [23–25, 32]. Sample calculations to describe the data presented in Tables 2, 3, 4, and 5, are presented below.

Tissue Log Reduction. Total CFU recovered per gram of tissue = (raw plate count/0.01 mL {the volume plated}) * 2.5 mL {the total volume homogenized}/tissue sample weight. Log transformed value = $\text{Log}_{10}(\text{CFU}/\text{sample} + 1)$ [23].

Log reduction ($\text{Log}R$) = (mean Log_{10} control) – (mean Log_{10} test).

Catheter Log Reduction. Total CFU recovered per gram of tissue = (raw plate count/0.01 mL {the volume plated}) * 4.0 mL {the total sonication volume}. Log transformed value = $\text{Log}_{10}(\text{CFU}/\text{sample} + 1)$ [23].

Log reduction ($\text{Log}R$) = (mean Log_{10} control) – (mean Log_{10} test).

For statistical analysis, a nonpairwise, two-tailed Student's *t*-test was used. $P \leq 0.05$.

2.9. Scanning Electron Microscopy (SEM) Samples. One cm catheter segments were aseptically separated from the surrounding tissue. The samples were placed into empty receiver vials. In a fume hood, primary fixative (5% glutaraldehyde in 0.1 M Na cacodylate buffer pH = 7.5) was added to each

vial to completely cover all samples. The vials were capped and incubated at $4 \pm 2^\circ\text{C}$ for 16 to 24 hours. After-fixation, the samples caps were loosely removed. The loosely capped sample vials were placed into a fume hood, and the samples were allowed to air dry for 72 to 96 hours. Appropriate standard operating procedures (SOPs) for use of a Hitachi S3700N Scanning Electron Microscope were followed. SEM data was recorded as images and through notes describing each sample.

2.10. Histopathology. Tissue specimens were excised and fixed in 10% neutral buffered formalin. The samples were embedded in paraffin and sectioned, mounted, and stained with hematoxylin and eosin (H&E) per facility SOPs. Samples were reviewed by a blinded third-party board-certified pathologist for a scored report detailing findings.

3. Results

Animals 2, 5, and 6 removed some of the control catheters prior to the scheduled removal at day 30. This behavior is associated with infection and inflammation of the tissue around the catheters as animals cannot ethically be restrained to totally prevent them from accessing the catheters. At the time of sacrifice, the tissue tract associated with the catheter could be clearly identified allowing removal and analysis on animals 2 and 5. The loss of these samples is denoted by an asterisk (*) in the supporting data tables.

Animal models of infection of medical devices generally tend to produce variability. Infections may be associated with the test catheters due to the significant level of challenge. Also, infection may not be observed in all control devices due to the ability of the healthy (nonimmunocompromised) animal to resist or eliminate the infection. In this model, a significant and continual challenge was provided to both the control and the test catheters. In spite of the heavy challenge (10^6 CFU/mL), the test catheters consistently demonstrated prevention of catheter and associated tissue bacterial colonization and reduction in catheter and tissue colonization by the challenge bacteria (see Tables 2–5). *In vivo* efficacy was demonstrated by meeting the acceptance criteria (4 Log reduction of adhered biomass) [32] over a 30-day time period. This is further demonstrated by the observation that the test catheters significantly reduced tissue colonization (40% test article tissue colonization versus 80% control tissue colonization) on day 21 and (6% test article tissue colonization versus 76% control tissue colonization) on day 30 (see Table 6). More significantly, the test catheters significantly reduced catheter colonization (0% test article colonization versus 43% control article colonization) on day 21 and (0% test article colonization versus 54% control article colonization) on day 30 (see Table 7). The plate count data was further supported by the scanning electron microscopic (SEM) evaluation of the selected catheter segments analyzed. Generally, irrespective of sample location or time point, no major differences were seen between the various samples of the coated catheter material. Occasional patchy deposition of host proteins and occasional areas of

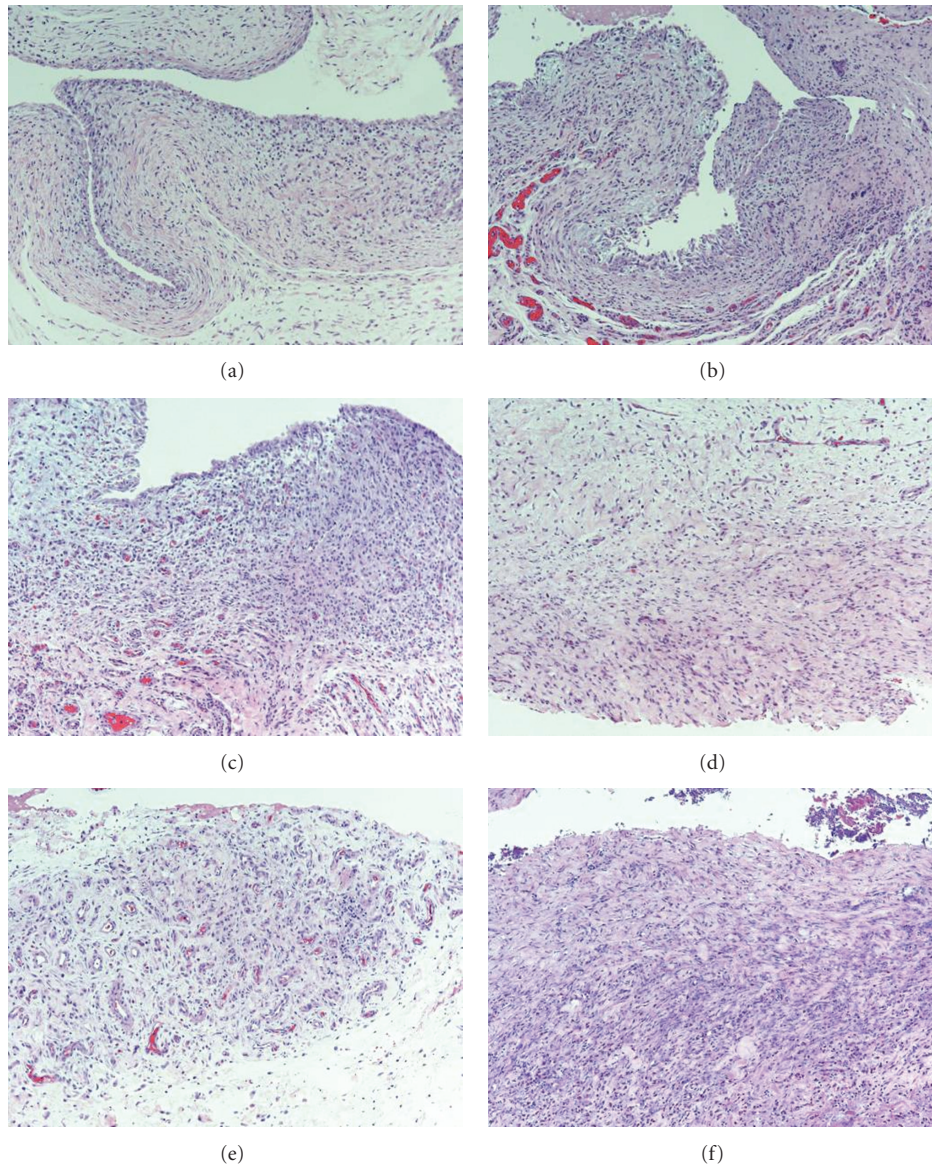


FIGURE 4: Representative digital micrographs (100x magnification) taken during the histological review of tissue segments from animal 10. (a) Test catheter 3, segment 5: neutrophils and/or macrophages are present, with inflammation in the lumen, (b) test catheter 3, segment 7: neutrophils and/or macrophages are present, with inflammation in the lumen. There is an inner layer which would have been adjacent to the catheter which is composed of well-vascularised immature connective tissue with a mild inflammatory reaction. There is also an outer layer of mature, well-organized, and uninflamed fibrous connective tissue. (c) Test catheter 3, segment 5: neutrophils and/or macrophages are present; inflammation extends to include the subcutis outside the connective tissue tunnel. (d) Control catheter 3, segment 5: neutrophils and/or macrophages are present, with inflammation in the lumen. (e) Control catheter 3, segment 7: neutrophils and/or macrophages are present; inflammation extends to include the subcutis outside the connective tissue tunnel. (f) Control catheter 3, segment 9: neutrophils, macrophages and lymphocytes are present; inflammation extends to include the subcutis outside the connective tissue tunnel. There is a thick necrotic mass of debris in lumen, with more general inflammation of wall.

aggregated host red blood cells (RBCs) were seen on the catheter surfaces. The catheter surface was largely visible on most samples. No significant amounts of bacteria were observed indicating that the log reduction data obtained during this study was due to the device preventing the adherence of the challenge microorganism, rather than killing or inactivating adhered cells (Figure 2). The control (or reference catheters) on the other hand, typically presented with heavy

deposition of host proteins, RBCs, and bacterial proteins and exopolysaccharide, was consistently observed across all samples. Heavy fibrin deposition was observed on several samples (data not shown). Most bacteria were embedded in the host/bacterial extracellular protein/polysaccharide matrix. Occasional healthy bacterial cocci were visible at the surface (Figure 2(d)). These observations are consistent with the viable cell count data obtained (Tables 2–5).

Clinical Efficacy. In addition to the plate count data, gross pathological findings clearly demonstrate the efficacy of the antimicrobial PICC as demonstrated by the digital images presented in Figure 3. The antimicrobial PICC presented with minimal pathological signs of infection (device associated swelling, capsule, pus), while the untreated control devices uniformly demonstrated clinical signs of infection (pus, swelling, extensive capsule surrounding the device, and necrotic tissue). A sample comparison set (animal 10) is presented to highlight this discussion point in Figure 3. The impact on the surrounding tunnel tissue was further examined by histological evaluation (Figure 4).

4. Discussion

This model was effective in comparing the patterns of microbial migration along the catheter segments proximal and distal to the entry site, comparing microbial colonization for antimicrobial PICC to that for untreated PICC and comparing the efficacy of the antimicrobial PICC for microbial colonization and inflammation mitigation up to 21 and 30 days after catheter insertion as compared to untreated catheters. In addition, the results showed that chlorhexidine-based technology was effective in reducing bacterial colonization on catheters and their surrounding tissue for up to 30 days. This model represents the subcutaneous space and not the vasculature; however, it is the subcutaneous space that is frequently infected, and tracking of the infection leads to vasculitis and cellulitis. In addition, the model does not simulate the use of the device in the clinical setting that includes repeated manipulations of the device under repeated challenge conditions and up to the projected life of the device. Rather, this model simulates the *in vivo* environment that the catheter is expected to be in contact with up to the projected life of the device. This feature of the model is important in that the risks of CRBSI increase as the duration of implantation increases [34, 35], and the replacement of PICCs is not a recommended strategy [17, 34] underlying the need for devices with long-acting antimicrobial coatings and models that can accurately evaluate these technologies.

The authors recognize that this model has several limitations: the first is that not all organisms will reproducibly colonize rabbit tissue, as such *S. aureus* was chosen for this model due to reproducibility in this model and in the literature and due to the fact that this species represents the causative agent of the majority of clinical CRBSIs [33]. Therefore, for product label claims that seek to cover the full spectrum of causative agents of CRBSI or catheter colonization, it is recommended that this model be used in conjunction with other recognized *in vitro* assays that can specifically test against this diverse group of organisms [33]. Secondly, these are healthy animals, and their immune systems can clear the infection over the duration of the study (see Tables 2–5). It can be hypothesized that the catheters may provide protection for only a few days, and then the animal's immune system clears the infection. For that reason, it is critical that control (nonantimicrobial) catheters be run concurrently

within the same animal to demonstrate that the antimicrobial effect is in fact due to the antimicrobial agent and not just the animal's immune response. This limitation was not valid and pertinent in this study because antimicrobial catheters did clearly demonstrate reduction in colonization on catheter surface, surrounding tissue, and clinical/localized infection symptoms. Lastly, to accurately predict and demonstrate duration of the efficacy, it is important to run a parallel *in vitro* study utilizing unchallenged 21- and 30-day explant materials or artificially aged materials followed by an *in vitro* challenge of the organism to test whether the materials can still resist biofilm colonization. Also, chronic irritation of a chronic wound will cause a rabbit to remove its bandage and its catheter. It is ethically not possible to provide such restraint to prevent removal of a severely irritated catheter. However, despite these limitations, this model does represent an effective *in vivo* assessment of antimicrobial implant medical device performance over an extended time period. We present this work to further expand the research tool box available to medical device manufacturers and regulatory agencies to develop, review, and assess new antimicrobial implant medical devices.

While many factors affect microbial colonization of the catheter, duration of implantation is a major complication leading to infection [33, 34]. Clearly preventing infections on the surface of indwelling medical devices over extended time frames is an important issue with the increased use of medical devices. To provide healthcare facilities with another tool to attain and sustain their goal of zero catheter-related infections, Teleflex has developed both a 4.5 Fr single lumen and a 5.5 Fr double lumen Arrow Antimicrobial PICC with Chlorag+ard Technology to reduce the potential risk for catheter colonization. Both the indwelling external surface and entire fluid pathway of the catheters are treated with chlorhexidine-based technology to address this issue. This model successfully demonstrated that this technology was effective in catheter colonization reduction up to 30 days and showed promise in reducing CRBSI.

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