# A study on the ability of quaternary ammonium groups attached to a polyurethane foam wound dressing, to inhibit bacterial attachment and biofilm formation.

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## Abstract

Bacterial infection of acute and chronic wounds impedes wound healing significantly. Part of this impediment is the ability of bacterial pathogens to grow in wound dressings. In this study, we examined the effectiveness of a polyurethane foam wound dressings coated with poly diallyl-dimethylammonium chloride (pDADMAC-PU) to inhibit the growth and biofilm development by three main wound pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, within the wound dressing. pDADMAC-PU inhibited the growth of all three pathogens. Time-kill curves were conducted both with and without serum to determine the killing kinetic of pDADMAC-PU. pDADMAC-PU killed *S. aureus, A. baumannii*, and *P. aeruginosa*. The effect of pDADMAC-PU on biofilm development was analyzed quantitatively and qualitatively. Quantitative analysis, (CFU) assay, revealed that pDADMAC-PU dressing produced more than 8 log reduction in biofilm formation by each pathogen. Visualization of the biofilms by either confocal laser scanning microscopy or scanning electron microscopy confirmed these findings. In addition, it was found that the pDADMAC-PU treated foam totally inhibited migration of bacteria through the foam for all three bacterial strains. These results suggest that pDADMAC-PU is an effective wound dressing that inhibits the growth of wound pathogens both within the wound and in the wound dressing.

## Introduction

Infected wounds are often difficult to heal. Bacteria infect both acute and chronic wounds including; burn wounds,[1] diabetic ulcers,[2] surgical wounds,[3, 4] and trauma wounds[5]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two of the most common bacterial pathogens that are detected in infected wounds.[1-5] The mortality rate from wound infections ranges from 26% to 55% [6, 7] for *P. aeruginosa* and from 19% to 38% for *S. aureus*.[8-10] Besides these two pathogens, *Acinetobacter baumannii,* which is considered among the most difficult antimicrobial-resistant Gram-negative bacilli to control and treat, is a serious wound pathogen.[11] The ability of *A. baumannii* to persist for extended periods of time on surfaces under a wide range of environmental conditions makes it a frequent cause of infections. [11] Due to the occurrence of these serious wound infections, it is critical to produce a wound dressing that efficiently inhibits bacterial growth within the wound and on the dressing itself. By blocking growth of biofilm formation on the dressing this would inhibit bacteria from reseeding the wound, which would allow the bacteria to overcome the natural host defense mechanisms.

Wound dressings generally fall into three main categories: 1) there are dressings that leach antimicrobial compounds into the wound; 2) dressings that are antimicrobial but do not leach into the wound and 3) those dressings which uses a combination of these two mechanisms.

Nanotechnology, which utilizes materials at their molecular dimension, is one of the potential approaches to eliminate microbial pathogen. A recent review by Seil and Webster[12] discussed in

details different antimicrobial nanoparticles including metal and metal oxides such as ZnO, Ag, Cu, $Fe_3O_4$, $Al_2O_3$, $TiO_3$ and $SiO_3$.[12] These metals and metal oxides efficiently inhibited the growth of different bacteria.[12] However, one of the main concerns in utilizing them is their toxicity to eukaryotic cells since they must leach off the material in order to be effective.[12]

The use of poly (quaternary ammonium) compounds covalently attached to materials, to form antimicrobial surfaces has been reported recently.[13, 14] These quaternary ammonium salts are considered to be the most commonly used cationic antimicrobials.[13] Among these compounds, the polymeric quaternary amines have shown the greatest promise in a role as a surface active agent.[13] The main other compound that has been attached to the surface of material to inhibit biofilm formation are organo-selenium compounds.[15] These organo-selenium compounds use the mechanism of catalyzing the formation of superoxide radicals in order to inhibit biofilms.

The current paper is a study of the antimicrobial properties of a polyurethane-foam wound dressing that has long-chain polymers (molecular weights of greater than 100,000 Daltons) with high densities of quaternary amines (pDADMAC) attached to its surface, so that it does not leach polymer from the dressing into the wound. This is the first report of this type of microbicidal cationic polymer applied to a very common type of dressing, a polyurethane foam. This pDADMAC-PU treated foam was evaluated for its potential as a wound dressing with respect to its antimicrobial efficacy, inhibition of biofilm formation and bacterial migration. In addition, kinetic studies were carried out to determine the rate at which the killing occurred. The results obtained were found to be in agreement with the proposed killing mechanism for quaternary ammonium compounds while still attached to the surface of polyurethane foam.

## Materials and Methods

**Foam Dressing**:

The wound dressing evaluated contained a high molecular weight (~250 k Daltons) polymer with a high density of quaternary amines, poly-diallyl-dimethylammonium chloride [p DADMAC, 0.3%] bonded onto a polyurethane foam. It is immobilized on the polyurethane dressing by a simple "dip" and "dry" process in which the polyurethane foam dressing is saturated with a dilute solution of the pDADMAC and dried with heating to evaporate all the water. The molecules of pDADMAC bind tightly to the polyurethane polymer fibers by a process that material engineers typically call "dehydration bonding". More specific details of the manufacturing process are provided in US Patents, US7045673B1 and US7709694B2. The pDADMAC-PU-containing foams were obtained from Viridis BioPharma Pvt. Ltd. Mumbai, India and Quick-Med Technologies, Inc., Gainesville, Florida, USA. The control (pDADMAC - free) foams were obtained from ABL Medical, USA. They were tested as 1-square inch segments. The polymer is attached onto the substrate by a polyelectrolyte complex, specifically by generating entanglement with an anionic polymeric species that is present at a low level. A long-chain cationic polymer (MW at $10^5$ daltons), is entangled by both physical and ionic interaction with an anionic polymer (MW level of $10^4$ daltons). This provides effective retention of the active agent on the target substrate. Retention of polymer is resistant to rinsing with about 35 ppm of active agent leaching during extraction testing. As per ISO 10993 procedures for biocompatibility testing, this is below threshold for antimicrobial activity.

**Bacterial Strains**:

All strains were grown in either Luria Bertani (LB) Broth, Mueller Hinton Broth, or on LB Agar plates at 37°C. The strains used were: 1) *Staphylococcus aureus* AH133 GFP which carries plasmid pCM11 which contains the gene that codes for the green fluorescent protein. To maintain the plasmid, erythromycin was incorporated in the growth medium at a concentration of 1 µg/ml;[16] 2) *Pseudomonas aeruginosa* strain PAO1/pMRP9-1 GFP. This strain carries plasmid pMRP9-1 which contains the gene that codes for the green fluorescent protein. To maintain the plasmid, the strain was grown in the presence of 300 µg/ml carbenicillin;[17] 3) *Acinobacter baumannii,* this strain was obtained from patients with infected wounds at Texas Tech University Medical Center. The strain was obtained through an approved Institutional Review Board protocol, Texas Tech University Medical center/Lubbock, Texas.

**Colony-forming unit assays**:

The biofilm assay was performed as previously described with some modifcations.[15] Bacteria were grown overnight, washed once with PBS (pH 7.4), re-suspended in PBS (pH 7.4) to an optical density ($OD_{600}$) of 0.5 ($10^8$ CFU/mL), and serially diluted [10-fold]. One hundred microliter aliquots containing $10^3$ colony-forming units were added to either an untreated (control) foam, or test foam coated with pDADMAC. After the bacterial inoculum was absorbed into the foams, they were placed on LB agar plates, and the plates were incubated at 37°C for 24 h. Biofilms were quantified by determining the CFU per square inch of foam. Following incubation, each foam piece was gently washed twice with sterile PBS to remove any planktonic bacteria. Excess PBS was drained from the foam by touching it to a sterile filter paper and the foam was then transferred to a sterile 15-ml conical tube containing 5 ml of PBS for enumeration

of bacteria. The tubes were placed in a water bath sonicator for 10 min to loosen the cells within the biofilm and then vigorously vortexed 3 times for 1 min to detach the cells. Suspended cells were serially diluted (10-fold) in PBS, and 10-μL aliquots of each dilution were spotted onto LB Agar plates. All experiments were done in triplicate, and all measurements were repeated at least three times.

**Analysis of the biofilms by confocal laser scanning microscopy:**

Pieces of foam were treated with bacteria as described above for the CFU assays. At 24 hour post-inoculation, three control and three pDADMAC-PU foam segments were then examined for the presence of biofilm by Confocal Laser Scanning Microscopy (CLSM). Visualization of the *S. aureus* and *P. aeruginosa* biofilms was accomplished with an Olympus IX71 Fluoview 300 Confocal Laser Scanning Microscope (Olympus America, Melville, NY, USA) with images acquired at 4 μm intervals through the biofilms. Three-dimensional biofilm image reconstructions were performed with NIS-Elements 2.2 software (Nikon) as previously described.[18] The biofilm structural features were analyzed with the COMSTAT program.[19] Several image stacks of each biofilm were examined by CLSM, and the images were analyzed as previously described.[18] Experiments were done in triplicate, and all measurements were repeated at least three times.

**Analysis of the biofilms by scanning electron microscopy**:

Since *A. baumannii* is a clinical isolate and does not contain the gene for GFP, biofilms formed by this strain were visualized by scanning electron microscopy. The biofilms were established as described above. The pieces of foam were prepared for Scanning Electron Microscopy (SEM)

by standard techniques as previously described.[15] After 24 h of incubation, each foam with the adherent bacteria was fixed with 2% (wt/vol) glutaraldehyde in filter-sterilized 0.05 M PBS (pH 7.4) at room temperature for 16 h and then rinsed three times for 15 min each in 0.05 M PBS. The fixed foams were then dehydrated in successive ethanol-water mixtures with increasing ethanol concentrations (20%, 40%, 60%, 80%, and 95% [vol/vol]) for 15 min each and then twice in absolute ethanol for 15 min. The samples were then placed in HMDS (hexamethyldisilazane) for 10 min. The HMDS was completely removed by a pipette. The samples were air dried in a hood at room temperature overnight. The dried samples were affixed to aluminum mounts with double-sided carbon adhesive tape and sputter coated with platinum and palladium to a thickness of 18 nm. Observations were performed at 1-3 kV accelerating voltage with a Scanning Electron Microscope (Hitachi S-3400N, Hitachi High Technologies America, Inc.). Five fields of view at magnifications of 500X were taken at randomly chosen areas from the optic surface of each sample. Each experiment was conducted in triplicate, and all measurements were repeated at least three times. A biofilm-positive field was defined as being occupied by biofilm over at least half of the visible area.

**Kinetic study of bacterial killing by the pDADMAC-PU foam**:

In order to assess the killing rate of bacteria within the pDADMAC-PU foam, a recovery assay was performed. Bacterial cultures were grown overnight in LB Broth supplemented with an appropriate antibiotic at 37°C, with shaking at a speed of 225 rpm. Overnight cultures were washed once with PBS (pH 7.4). Cell pellets were re-suspended in PBS (pH 7.4) to an optical density ($OD_{600}$) of 0.5 ($10^8$ to $10^9$ CFU/mL) and serially diluted [10-fold]. Three one-square inch foams were placed separately in 120 mm petri plates. 500-µl aliquots containing about $10^6$ to $10^7$

colony forming units were added to either untreated foams or foams coated with pDADMAC-PU. The foam squares were incubated at room temperature for different time periods (5 min, 1, 2, and 4 hrs). Following incubation, the foams were transferred to sterile 15-ml conical tubes containing 5 ml of PBS for enumeration of bacteria by the CFU assays as described above. All experiments were done in triplicate, and all measurements were repeated at least three times

**pDADMAC-PU activity in the presence of fetal bovine serum:**

The antimicrobial activity of pDADMAC-PU was tested using the same approach as described in the kinetic study of bacterial killing by the pDADMAC-PU foam. Overnight cultures were washed once with PBS (pH 7.4). Cell pellets were re-suspended in PBS (pH 7.4) containing 10% Standard Fetal Bovine Serum (#SH30088.03, HyClone, UT, Logan, Utah 84321) to an optical density ($OD_{600}$) of 0.5 ($10^8$ to $10^9$ CFU/mL) and serially diluted [10-fold]. 500-µl aliquots containing about $10^6$ to $10^7$ colony forming units were added to either untreated foams or foams coated with pDADMAC-PU. The foam squares were incubated at room temperature for 1 hour with *S. aureus* GFP AH133 and *A. baumannii* CI and 2 hours with *P. aeruginosa* PAO1 GFP. Following incubation, the foams were transferred to sterile 15-ml conical tubes containing 5 ml of PBS for enumeration of bacteria by the CFU assays as described above. All experiments were done in triplicate, and all measurements were repeated at least three times.

**Bacterial Migration Test:**

The migration test is an indicator of the ability of a pathogen to migrate from the wound bed to wound periphery and beyond. This test determines the ability of pDADMAC-PU foams to prevent the migration of microorganisms through the foams from a contaminated section to a

sterile section. A positive result suggests that it is possible that microorganisms could be transported laterally out of contaminated wound onto the surrounding skin or potentially move in the opposite direction from the intact skin into the wound itself. The experiment was performed as previously described[20] with some modifications. A 1 cm width ditch was created across a Nutrient Agar plate to make a trough by using a sterile scalpel. Overnight cultures were diluted until the turbidity matched the 0.5 McFarland standard using a spectrophotometer. A sterile cotton swab was dipped into the suspension, rotated several times, and gently pressed onto the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was then streaked over one half of the agar plate three times to ensure an even distribution of the inoculum. A final sweep of the swab was made around the agar rim. The foam was cut into 1-1.5 cm wide and 5-6 cm long strips. They were then placed at right angle across the trough and were gently pressed down against the Agar surface. The plates were then incubated at 37$^{o}$C for 48 hrs. After 48 hrs, the foam sections on the non-inoculated sides were cut and examined for the presence of bacterial growth by CFU assay.

**Statistical Analysis**:

Results of the CFU assays were analyzed with Prism® version 4.03 (GraphPad Software, San Diego, CA, USA) with 95% confidence intervals (CIs) of the difference. Comparisons of the in vitro biofilms formed on pDADMAC-PU-free and pDADMAC-PU foams were analyzed by a two-tailed unpaired t-test to determine significant differences. Each experiment had 3 samples, and each experiment was repeated at least three times. Values (**) represent significant differences.

## RESULTS

**pDADMAC-PU Foam Inhibits Biofilm Development by Three Main Wound Pathogens:**

We examined the effectiveness of pDADMAC-PU foam in inhibiting biofilm development by the wound pathogens *P. aeruginosa, S. aureus*, and *A. baumannii*. Either a control, or pDADMAC-PU foam was inoculated with the tested bacterial strain. The biofilms were developed for 24 hours and quantified as described in materials and methods. As shown in Figure 1, all three strains formed sufficient biofilms on the control foam (about 1x $10^8$ CFU/$cm^2$ of foam). However, no biofilm was recovered from pDADMAC-PU foam inoculated with either strain (Figure 1). Values are the means of 3 or more independent experiments ± SD and are CFU/1-square inch of foam.

**Visualization of the Biofilms:**

We confirmed the results of the quantitative analysis of the biofilms formed by *S. aureus* AH133 (Figure 1A,B) and *P. aeruginosa* PAO1/pRP9-1 (Figure 1C,D) using confocal laser scanning microscopy to view the green fluorescent protein in these two strains. We then utilized a program that generates a 3D structure, in order to examine the structure of each biofilm seen with the confocal laser scanning microscope (Figure 2). As can be seen, a mature biofilm formed on the control (Figure 2A,C) and no biofilm formed on the pDADMAC-PU (Figure 2B,D)

Analysis showed that on the control foam, the biofilm biomass was 311.0 and 373.9 ($\mu m^3/\mu m^2$) for AH133 and PAO/pRP9-1 respectively (Table 1). In addition, the average thickness of the

biofilms was 73.3 and 340.7 μm for *S. aureus* AH133 and *P. aeruginosa* PAO/pRP9-1 respectively (Table 1). However, the values for the biomass and average thickness of biofilms formed by either strain on pDADMAC-PU were zero (Table 1).

At this time, we do not have an *A. baumannii* strain that carries the gene that codes for the green fluorescent protein. Therefore, we visualized the biofilm formed by this strain using scanning electron microscopy. As shown in Figure 3A, *A. baumannii* formed an extensive well-developed biofilm that covered most of the control foam. However, we detected no bacteria on the surface of pDADMAC-PU (Figure 3B).

Taken together these results suggest that pDADMAC-PU strongly inhibits biofilm development by *S. aureus, P. aeruginosa*, and *A. baumannii*.

**Kinetic studies**:

In order to determine the rate at which bacterial killing occurred, kinetic studies were carried out. Untreated and pDADMAC-PU treated foam samples were allowed to react with the bacteria, while on top of a moist Agar plate, for various time periods and the number of surviving bacteria determined by CFU assay. The results of the CFU assay represented in Figure 4 A, B, and C show that complete killing was observed in less than 5 minutes for *S. aureus*; however complete killing took longer time for the Gram negative bacteria *P. aeruginosa* (< 2 hrs) and *A. baumannii* (< 1 hr.).

**Migration Studies**:

Foam samples were placed as bridges, on agar plates that had bacterial inoculum on half of the plate with a gap between the inoculated half and the bacteria free half as seen in Figure 6. The plates with the foam bridges over the gap were incubated for 48 hours. At the end of that time the plates were studied to see if bacteria could migrate through the foam with and without pDADMAC-PU. No bacteria were found to migrate across the pDADMAC-PU treated foam (Figure 5). However, in the case of the untreated foam, bacteria had migrated along the foam to the un-inoculated side of the agar (Figure 5).

**Growth of bacteria in PBS with 10% Fetal Bovine Serum:**

*S. aureus* GFP AH133 ($10^6$ CFU), *A. baumannii* CI ($10^7$ CFU) and *P. aeruginosa* ($10^7$ CFU) were inoculated and incubated for 1 hour and 2 hours respectively, on foam samples, in the presence of 10% fetal bovine serum (FBS). This was carried out to see if FBS would interfere with the action of the pDADMAC-PU. The results of the CFU assay represented in Figure 6 show that complete killing was observed for *S. aureus* (< 5 minutes) (A) and *P. aeruginosa* (< 2 hrs) (B), and a significant reduction in the number of surviving bacteria *A. baumannii* (< 1 hr.) (C) was recovered from the pDADMAC-PU treated foam as comparing to control foam, in 10% FBS. Studies were also carried out on *S. aureus* with 50% and 100% serum. These showed that killing did occur, however it was limited to a little over 3 logs in both cases (Figure 7).

## DISCUSSION

The first anti microbial polymeric amine that was used for wound dressings was probably a chitin derivative, chitosan, that interacts with bacterial cell membrane. It is made from chitin by

treatment with sodium hydroxide. This hydrogel polymer was first isolated over 200 years ago (1811), by Henri Bracannot,[21] a French chemist and pharmacist. Bracannot observed that a certain substance (chitin) found in mushrooms did not dissolve in sulfuric acid. Later in the 19th century, it was found to be present in outer skeletons of insects, crustaceans, and cell walls of mycelial fungi. Chitosan is a β-1,4-linked polymer of glucosamine (2-amino-2-deoxy-β-D-glucose) which contains lesser amounts of N-acetylglucosamine. The cationic polymer chitosan (poly-N-acetylglucosamine), has been used extensively as a wound dressing for decades due to its hemostatic, antimicrobial and wound healing properties.[22]

These antimicrobial properties of chitosan probably led to the early work on the action of quaternary amines on bacteria. Salton *et al*., (1951), showed by electron micrograph that *S. aureus* treated with cetyltrimethylammonium bromide (CTAB), resulted in dissolution of bacterial cell walls.[23] Dawson *et al*., (1953), showed CTAB caused cytoplasmic membrane disintegration and plasmolysis.[24] Babbs *et al*., (1956) along with Caldwell *et al*., (1961), studied a homologous series of the 1,1' polymethylene bis(4-aminoquinaldinium) salts, and 1-alkyl-4-aminoquinaldinium salts which showed antibacterial properties.[25, 26] Cox (1963, 1964) showed a relationship between chemical structure of these compounds and their antimicrobial activity, which appeared to indicate that the compounds interfered directly with cellular permeability.[27, 28] Cox (1965) also studied the site of action of these compounds in bacterial cells by morphological and biochemical studies.[29] More recent studies confirm this alteration of bacterial surface morphology and membrane permeability.[30] Most of these data support increased permeability or disruption of the cellular membranes.[30-33]

Attachment of quaternary ammonium groups to a solid surface (glass) has been studied and shown to result in approximately 2 logs of killing for Gram negative and Gram positive bacteria.[14] Studies with textiles also resulted in approximately the same amount of killing.[14] Studies by Murata *et al*.,[13] showed they could achieve higher killing percentages by increasing the charge density on the surface of a glass slide. At a high enough charge density, they could kill all the bacteria until the surface was completely free of bacteria. Thus, killing was dependent upon the surface area if there was a high enough charge density.

A recent study with polyurethane membranes as wound dressings, coated with quaternary ammonium compound (glycidyltriethylammonium chloride, GTEA) showed that fibroblasts could grow next to the membranes with no inhibition of growth at up to 30 mole % of GTEA in the polymer.[34] They showed bacterial killing at this concentration, however, no quantitative data was presented.

It is important to block bacterial biofilm formation in the bandage, since it has been shown in the past that wound dressings that are contaminated with bacteria can result in airborne dispersal of the bacteria.[35-38] Aerosolization of the bacteria on the bandage can play a role in contamination of wards, operating theaters and burn unit dressing stations.[37, 38] It was also found that dressings can shed bacteria into the wound and can play a role in cross contamination.[36] Thus it is important to find dressings that can prevent or minimize this possibility.

In this current study we present quantitative data on the killing of both Gram positive and Gram negative bacteria by a quaternary ammonium compound (pDADMAC-PU) attached to a

polyurethane foam for use as a wound dressing. The data shows over 8 logs of killing (100%) for *S. aureus, P. aeruginosa, and A. baumannii* (Figure 1). These biofilms were also quantitated using a Comstat program and the results are shown in Figures 2 and Table 1. In each case, the pDADMAC-PU treated polyurethane foam showed complete inhibition of the biofilm as compared with the control foam. Similar results were obtained for *A. baumannii* (Figure 3) using SEM. This is important in minimizing possible reseeding of the wound and cross contamination. It would also minimize the possibility of airborne dispersal of bacteria upon removal of the bandage.

Kinetic studies were performed to determine the killing rate of pDADMAC-PU treated foam. It was found that *S. aureus* was killed by the treated foam in less than 5 minutes (Figure 4A). However Gram negative bacteria such as *P. aeruginosa* and *A. baumannii* took more time (Figures 4B and 4C). It would appear that Gram positive bacteria are killed quickly on contact, but Gram negative bacteria required more time probably due to the presence of the lipopolysaccharide outer membrane. The mechanism proposed for killing by quaternary ammonium compounds is the disruption of the plasma membrane of the bacteria.[39, 40] This data would appear to indicate that the peptidoglycan layer outside the plasma membrane of Gram positive bacteria offers little or no resistance to the pDADMAC-PU compound, while it takes more time to penetrate the LPS outer membrane of Gram negative bacteria. However, total killing was achieved with all the three strains tested.

Studies were carried out to show inhibition of migration of bacteria through the pDADMAC-PU treated foam (Figure 5) while the control allowed migration of the bacteria through the foam.

This implies that pDADMAC-PU treated foams used as dressings for infected wounds will not allow migration of bacteria from the wound bed to the wound periphery. Also, it was found that serum that may be present in a wound had no effect on the action of the pDADMAC-PU foam against *S. aureus* and *P. aeruginosa* (Figure 6) however, 10% serum showed slight inhibition of pDADMAC-PU killing against *A. baumannii* (Figure 6), Experiments with *S. aureus* showed 100% killing in the presence of 10% FBS but with 50% and 100% FBS showed slightly over 3 logs of killing (Figure 7). These are important results since this should minimize both aerosolization of bacteria into the surroundings as well as to health care providers, when the bandages are changed.[37, 38] In addition, the finding that the bandages (with the exception of those in the presence of 50% and 100% serum), have no detectable bacteria growing on them, should minimize the chances of reseeding the wound bed with bacteria from a biofilm on the bandage.

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| | Average Biomass ($\mu m^3/\mu m^2$) | | Average Thickness (μm) | |
|---|---|---|---|---|
| | Control foam | pDADMAC foam | Control foam | pDADMAC foam |
| S. aureus | 311.0 | 0 | 73.3 | 0 |
| P. aaeruginosa | 373.9 | 0 | 340.7 | 0 |

Table 1. COMSTAT analysis of confocal data for the average biomass $\mu m^3/\mu m^2$ and average thickness of the biomass, using on the control and pDADMAC-PU foam, using *P. aeruginosa* PAO1 GFP and *S. aureus* AH133 GFP. Values represent means of 3 or more replicates.

Figure Legends:

Figure 1. Effects of pDADMAC-PU foam on biofilm development. Biofilms of either *S. aureus* AH133 GFP (A), *P. aeruginosa* PAO1GFP (B), or *A. baumannii* CI(C) were allowed to form for 24 h on pDADMAC-PU-free and pDADMAC-PU foams. Values represent the means of 3 or more independent experiments ± SD and represent CFU /1-square inch foam.

Figure 2. CLSM 3-D images of biofilm development of; (A, B) *S. aureus* AH133 GFP, (C, D) *P. aeruginosa* PAO1 GFP, Inhibition of biofilm was not seen on control foam (A,C) but was seen on pDADMAC-PU foam (B,D). Biofilm formation was examined in triplicate experiments. Three-dimensional analysis of the images: Bars, 100 pixels; box dimensions, width 800 pixels, height 600 pixels; depth 800 μm.

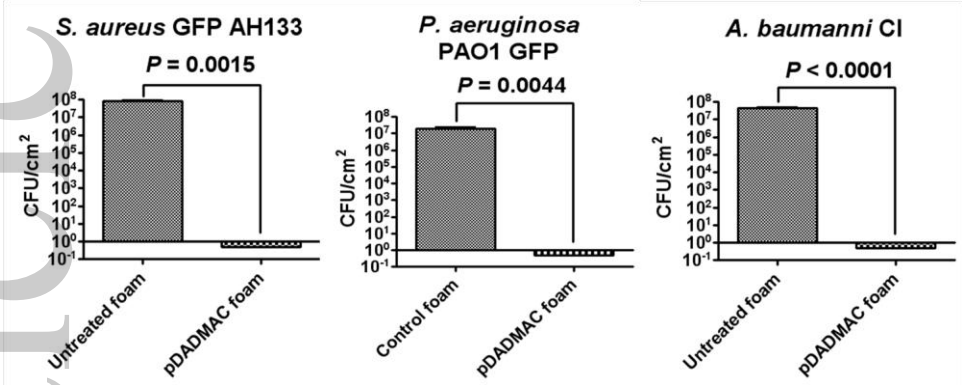
Figure 3. Scanning electron microscope images of control (A) and pDADMAC-PU (B) foam after treatment with *A. baumannii* CI for 24 hours.

Figure 4. Kinetic studies on the rate of killing of bacteria by control ( ) and pDADMAC-PU ( ) foam. (A) *S. aureus* AH133 GFP; (B) *P. aeruginosa* PAO1 GFP; (C) *A. baumannii* CI.

Figure 5. CFU analysis of the effects of pDADMAC-PU foam on *S. aureus* AH133 GFP (A), *P. aeruginosa* PAO1 GFP (B), and *A. baumannii* CI (C) migration from inoculated sides to sterile sides of control foams and pDADMAC-PU foams, after 24 and 48 hours.

Figure 6. Kinetics studies on the rate of killing of bacteria by control ( ) and pDADMAC-PU ( ) foam, in the presence of 10% fetal bovine serum. (A) *S. aureus* AH133 GFP; (B) *P. aeruginosa* PAO1 GFP; (C) *A. baumannii* CI.

Figure 7. CFU analysis of the effects of control foam and pDADMAC foam in the presence of : A) 10% Fetal Bovine Serum; B) 50% Fetal Bovine Serum; C) 100% Fetal Bovine Serum

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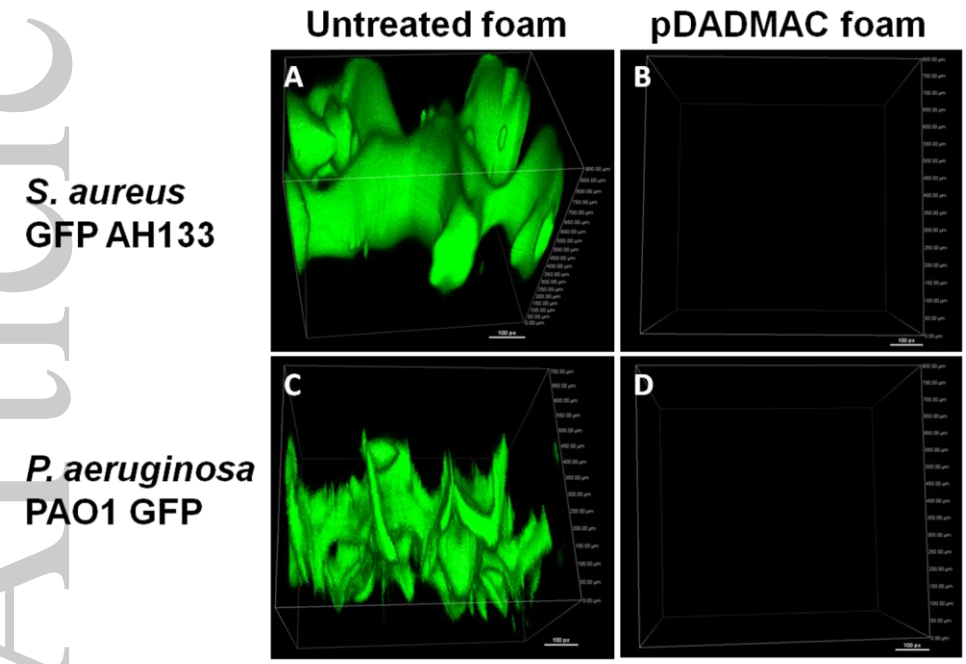


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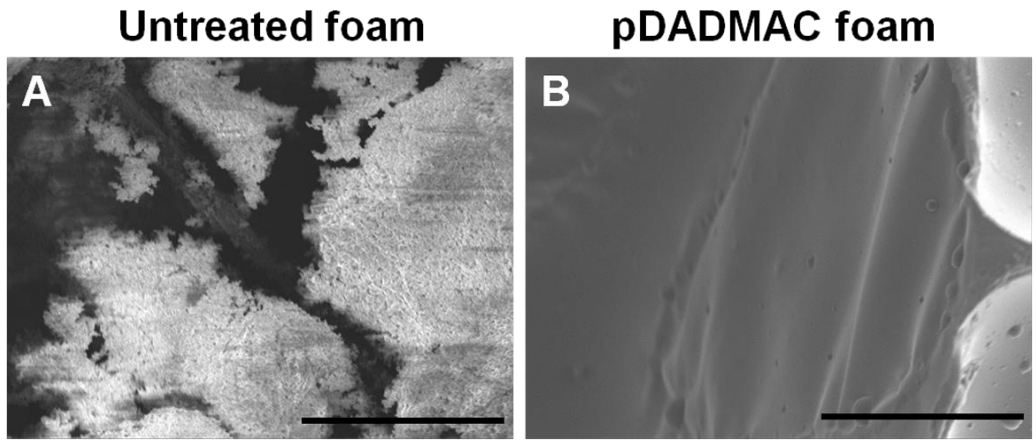


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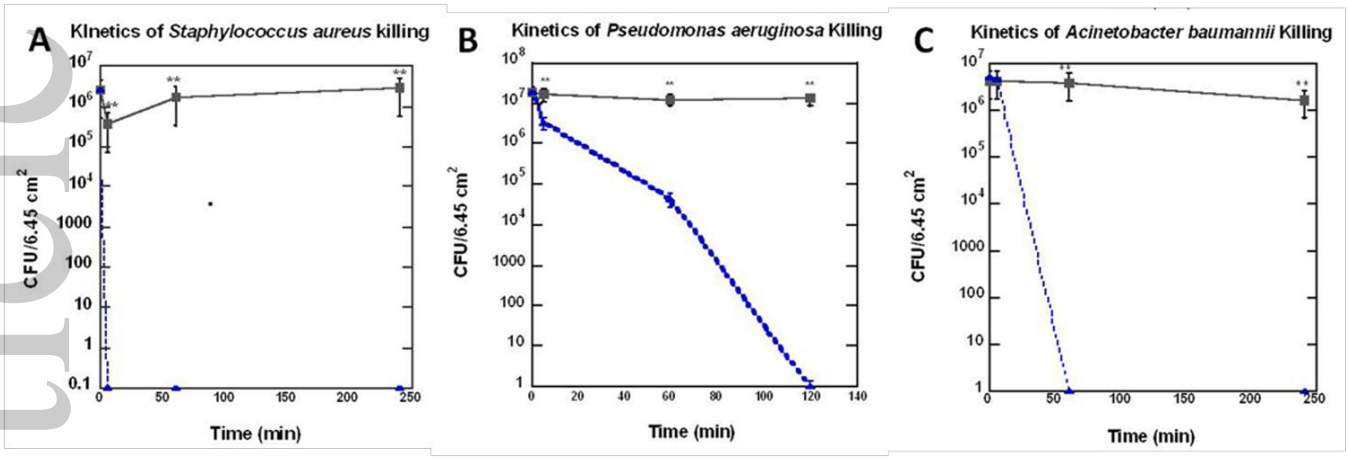


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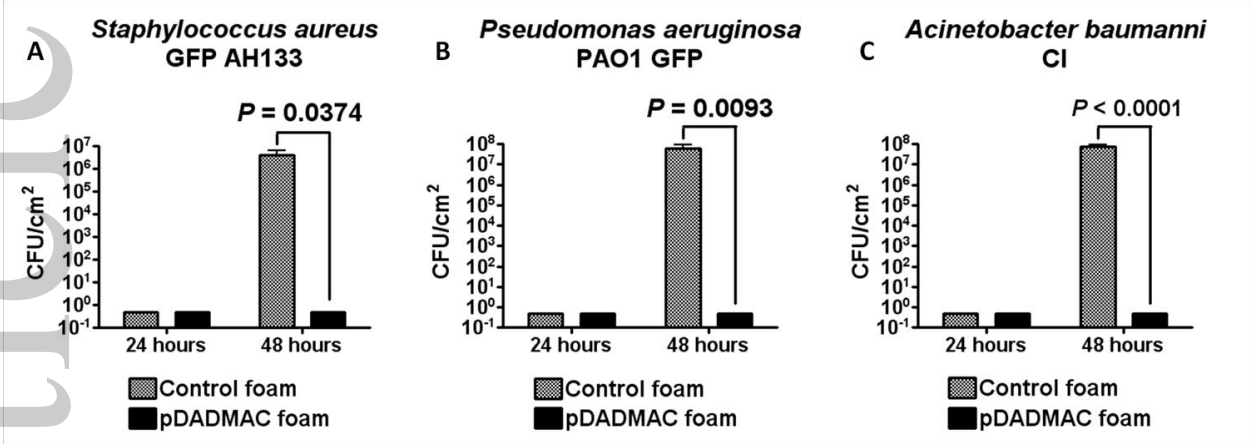


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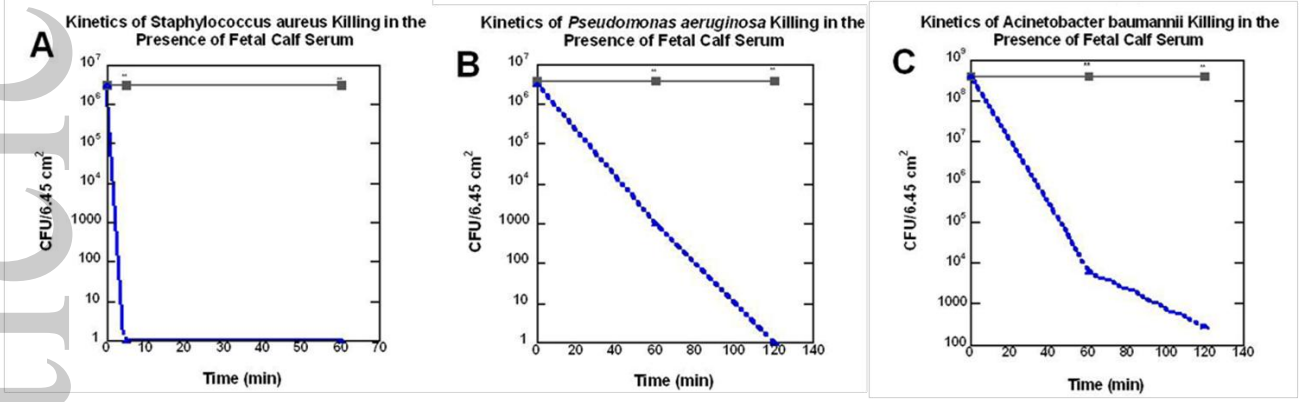


Figure 6.tif

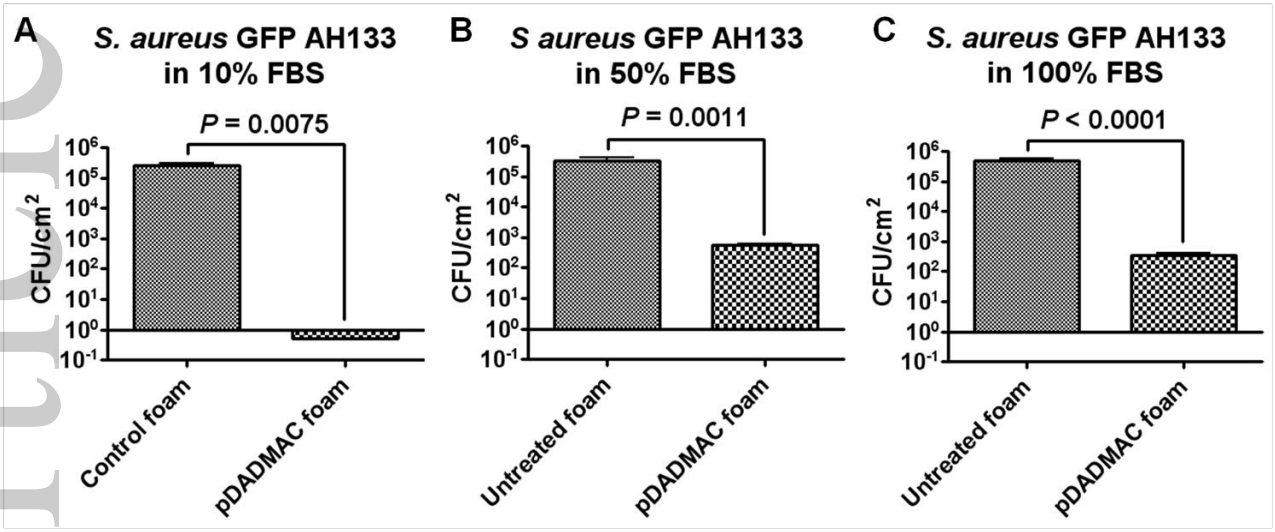


Figure 7.tif