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Development of Antimicrobial Peptide Conjugated PVC Catheters for Prevention of Catheter Associated Urinary Tract Infections

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***Abstract*— In postoperative care, generally, indwelling catheterization is used for patients who cannot empty their bladder without any help. Indwelling catheterization can be applied as long-term and long-term catheterization causes catheter-associated urinary tract infections (CAUTIs). CAUTIs remain an important concern in healthcare, with varying incidence rates globally. The most common microorganisms causing CAUTIs include *Escherichia coli, Klebsiella pneumonia*, and *Candida* species. Also, Methicillin-resistant *Staphylococcus aureus* poses challenges due to antibiotic resistance. Antimicrobial resistance (AMR) occurs when pathogens evolve to resist the presence of antimicrobial drugs through overuse and misuse. Resistant infections are challenging to treat and lead to prolonged hospital stays, increased medical costs, and higher mortality rates. To overcome AMR, multiple strategies have been explored and antimicrobial peptides are one of them (AMP). AMPs are found in various organisms and exhibit broad spectrum activity against bacteria, viruses, fungi, and tumor cells. AMPs are very promising in research because they are effective not only against multi-resistant bacteria but also because resistance is unlikely to develop. LFcinB(21-25)pal is an AMP with a sequence of RWQWRWQWR and has demonstrated very high activity against various pathogenic microorganisms. It shows antibacterial activity against *E. coli* and antifungal effects against several Candida species. In this study, we developed a urinary catheter with antimicrobial activity against Gram-negative and Gram-positive activity to prevent CAUTIs. For this purpose, LFcinB$_{(21-25)}$pal is conjugated to PVC urinary catheters first with cold atmospheric plasma application to create hydroxyl groups on the PVC surface. Then EDC/NHS chemistry is used for the covalent bonding of AMP. This developed catheter expressed bactericidal effect on both *E. coli* and MRSA strains, which proves the efficacy on gram-negative and gram-positive bacteria with resistant ones including. Future studies will focus on antibiofilm activity of the developed catheter and also the cytotoxicity tests will be conducted.**

Keywords—Urinary tract infections, urinary catheters, antimicrobial peptides, cold atmospheric plasma

I. Introduction

Urinary catheters are used when a person is unable to empty urine from the bladder automatically. There are different types of catheters including intermittent, indwelling and condom catheters [1]. Indwelling catheterization is commonly applied in postoperative care, however long-term indwelling catheterization is associated with bacterial colonization on the catheter surface and eventually clogging of the catheter and causing urinary catheter infections. Catheter-associated urinary tract infections (CAUTIs) account for more than 40% of hospital-related infections. CAUTIs cause increasing mortality, morbidity, hospital costs, and extended hospital stays [2]. To reduce CAUTIs main subject is to focus on minimizing catheter use if possible and to improve catheter care practices. Key strategies include implementing reminder systems to prompt timely catheter removal, which can reduce infection rates by up to 53% [3]. Daily multidisciplinary rounds to assess catheter necessity, nurse-driven removal protocols, and the use of catheter bundles have also been shown to be effective in reducing CAUTI rates [4]. The mentioned strategies are recommended for the prevention of CAUTIs, but may not be sufficient due to the high need for the use of urinary catheterization. Therefore, there is a search for developing a new strategy for preventing CAUTIs.

The most common uropathogenic is *Escherichia coli*, which is followed by several bacteria like *Candida* species, *Klebsiella*, and *Pseudomonas* [5, 6]. Furthermore, a substantial proportion of CAUTIs have been found to contain *Staphylococcus aureus*, particularly methicilin-resistant *S. aureus* (MRSA) [7]. Mostly antibiotics are used in the treatment of CAUTIs, however, the management of CAUTIs using antibiotics is facing an increasing challenge which is antimicrobial resistance (AMR). AMR occurs with the adaption of the bacteria and surviving in the antibiotic presence. This phenomenon keeps increasing due to the extensive use of antibiotics in agriculture, and animal and human health [8]. The frequency of antibiotic use is closely correlated with the occurrence of the resistance since bacteria evolve defense mechanisms against these medication [9]. A comprehensive strategy is required to address AMR such as reducing antibiotic use and applying a thorough set of controls to stop its spread.

Alternatives for antibiotics have been widely studied. Antibodies, bacteriophages, vaccines, and probiotics are some of the alternatives for antibiotics, however, antimicrobial peptides (AMPs) are one of the most frequently studied ones [10]. AMPs are short peptides composed of 10-50 aminoacids. They are naturally occuring molecules found in the immune defense system of many organisms and serves as a first defense

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line against pathogens [11]. AMPs can alter membrane potential and interfere with cellular functions by creating ion channels in bacterial membranes. Some AMPs can enter host cells, where they target intracellular bacteria and reduce the probability of antimicrobial resistance development [12]. The palindromic peptide LFcinB$_{(21-25)}$pal : RWQWRWQWR is derived from bovine lactoferricin and displays important antimicrobial and antitumor activities. This peptide exhibits antimicrobial activity against different strains, including *E. coli* and *S. aureus* [13]. Besides, it shows antifungal activity against several *Candida* species, including drug-resistant ones [14].

Here in this project, we aimed to develop a urinary catheter for the prevention of infections against gram-positive and gram-negative bacteria and even resistant ones. To achieve this PVC urinary catheters were functionalized with LFcinB$_{(21-25)}$pal antimicrobial peptide. Surface modification was conducted with cold atmospheric plasma to activate the surface with –OH groups. Then, LFcinB$_{(21-25)}$pal was covalently bonded to –OH groups using EDC/NHS chemistry. The efficacy of the developed catheters was examined on *E. coli* and MRSA strains.

II. Material And Methods

A. Solid Phase Peptide Synthesis

Peptide synthesis materials were acquired form AAPPTEC (Louisville, KY, USA). LfcinB was synthesized using the fluorenylmethoxycarbonyl (Fmoc) chemistry. 500mg 4- methylbenzhydrylamine (MBHA) resin (0.67 mmol/g loading capacity) was left in N, N-dimethylformamide (DMF) for 30 minutes. Coupling of aminoacids were conducted using amino acids with Fmoc protection (2 equivalent), N, Ndiisopropylethylamine (DIEA; 4 equivalent), and 2-(1Hbenzotriazol-1-yl)- 1,1,3,3- tetramethyluronium hexafluorophosphate (HBTU; 2 equivalent) in DMF. The amino acid solution was left in contact with resins for 4-6 hours. For the deprotection of Fmoc-protecting groups, resin was kept in 20% piperidine in DMF solution 30 minutes. After each aminoacid coupling and deprotection step, Kaiser (Ninhydrin) test was realized to detect the presence of unreacted amine groups. Until all aminoacids are added to the sequence, deprotection and coupling steps were repeated. After the sequence is completed, the resin was left to cleave in cleavage solution which contains trifuoroacetic acid (TFA) (95% (v/v)), triisopropylsilane (TIPS) (2.5% (v/v)), deionized water (2.5% (v/v)) for 2 hours. At the end of 2 hours the solution was transfered into cold ether and then the mixture was centrifuged for 10 minutes at 4500 rpm 3 times. The remaining ether in the precipitate was removed by vacuum evaporator and the solid part was freeze dryed.

B. Cold Atmospheric Plasma Application on Catheters

1 cm catheter sections were cut from 18 Ch PVC urinary catheters. Catheter diameters were measured in Charriere and 1 Charriere = 0.33 mm. The diameter of 18 Ch is equal to 5.94 mm. Samples were plasma treated with a microsecond pulsed high voltage source. A dielectric barrier discharge electrode of 50 mm x 75 mm was connected to this source and the power source was plasma treated for 5 minutes with 40kV voltage. 5 minutes of plasma application was decided with contact angle measurements (Figure 2).

C. Peptide Conjugation on Catheter Surface

LFcinB$_{(21-25)}$pal was prepared at 1mM concentrations by dissolving in sterile PBS (phosphate buffered saline). First, 1-ethyl (dimethylaminopropyl) carbodiimide (EDC) and Nhydroxy sulfosuccinimide (NHS) were used for amide bond formation between the catheter surface and peptide. For this purpose, catheters were incubated for 40 minutes in 2mM EDC and 5mM NHS added in 0.1 M 2-Morpholinoethanesulfonic acid (MES) solution at 37°C. After 40 minutes, samples were rinsed with PBS and peptide solution was added. Catheter samples were incubated at 4°C for 24 hours. Before using in the experiments the samples were washed with PBS three times.

D. FITC Labeling

Confirmation of covalent binding of the peptide to the catheter surface was performed by FITC labeling. The synthesized LFcinB$_{(21-25)}$pal peptide was labeled with the FITC molecule before removal from the resin. The labeling reaction was performed between the amine groups in the amino group of the peptide and the succinimidyl ester functional group in the FITC molecule. For the conjugaton of FITC labelled peptide to catheter surface the same protocol above was followed.

E. Colony Counting Assay

In colony counting assay the experimental groups were control and LFcinB$_{(21-25)}$pal with *Escherichia Coli* and MRSA. *E. coli* and MRSA strain cultures suspended at 10^8 CFU ml^{-1} were serially diluted to 10^5 CFU/ml^{-1} in PBS and 100 µl of these cultures were transferred onto catheter groups. Microorganisms were left in contact with catheters for 24 hours in room temperature. Then, serial dilution were conducted and the samples were spread on agar plates. Agar plates were incubated at 24 hours of incubation at 37°C. After 24 hours the colonies were counted.

F. Evaluation and Statistical Analysis of the Results

The Student-Newman-Keuls and Two-way ANOVA method were used as for all statistical analyses. Significant differences between groups were determined at p values of at least 0.05 (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

III. Results And Discussion

Kinetic growth analysis results of MRSA within the presence of different concentrations of LFcinB$_{(21-25)}$pal is given in Figure 1. With the increased bacterial viability, the absorbance value at 600nm is expected to increase. In Figure 1. it is seen that 25 µM, 50 µM, 75 µM, and 100 µM of LFcinB$_{(21-25)}$pal peptide provided a bacteriostatic effect, which means the antimicrobial peptide suppressed and delayed the bacterial growth. Peptide concentrations of more than 150 µM killed the MRSA completely and did not allow bacteria to

grow. That means a bactericidal effect can be achieved with peptide concentrations more than 150 µM.

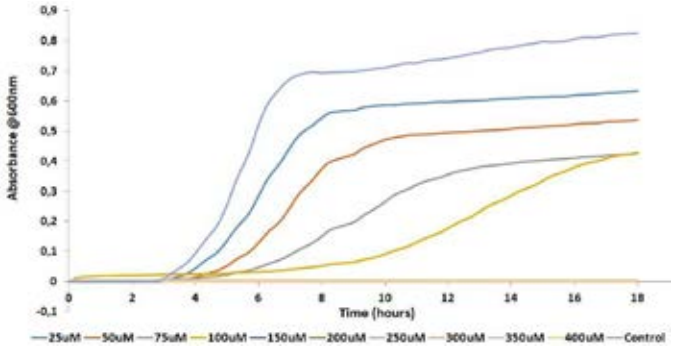


Figure 1. Kinetic growth results of MRSA at different, LFcinB$_{(21-25)}$pal concentrations.

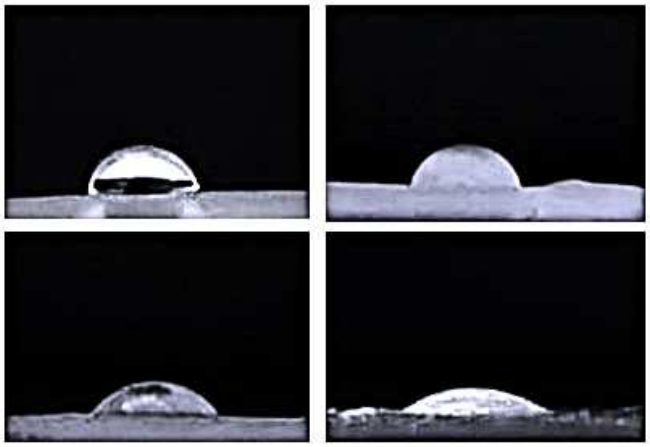


Figure 2. Contact angle images of A)0 sec. B)1 min. C)3 min. and D) 5min cold plasma applied PVC urinary catheters.

PVC is known to be a hydrophobic material. Interaction with water results in not even distribution of the water on the PVC surface [15]. Therefore, hydrophilicity is aimed to increase with cold atmospheric plasma application and the optimization of the plasma duration is conducted with contact angle measurements. Figure 2. shows the contact angle measurements of 0 min., 1 min., 3 mins., and 5, mins. The wettability of the PVC catheter surface has increased with the plasma exposure duration. Without plasma application, the contact angle of water droplet on PVC was 81.22°. 1 minute, 3 minutes, and 5 minutes of CAP application decreased the contact angle to 70.27°, 48.62°, and 30.20° respectively. This means plasma application increased the wettability with decreasing hydrophobicity of the PVC surface. The most effective duration was determined as 5 minutes. Therefore, the peptide conjugation for the experiments was conducted with 5 minutes of plasma exposure.

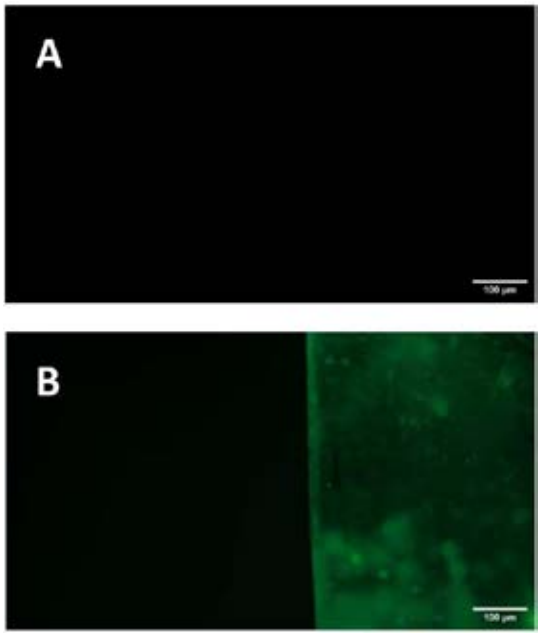


Figure 3. A) Pure PVC catheter, B) FITC labeled LFcinB(21-25)pal peptide conjugated PVC catheter images under fluorescence microscope (scale bar represents 100µm).

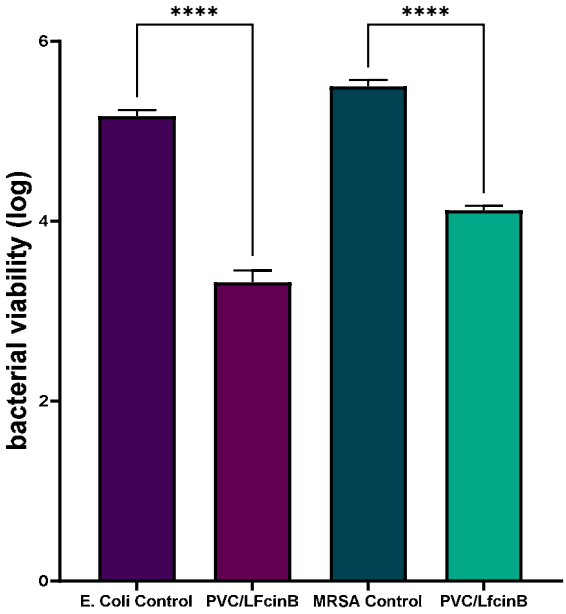


Figure 4. Colony counting results of 10^5 CFU/ml *E.coli* and MRSA after 24-hour incubation on PVC with PBS (Control), on plasma applied PVC (PVC/Plasma), and the conjugate of , LFcinB$_{(21-25)}$pal (PVC/LfcinB).

The confirmation of conjugated peptide on the PVC catheter surface was evaluated by observing the FITC intensity utilizing a fluorescent microscope. In Figure 3. FITC labeled LFcinB$_{(21-25)}$pal peptide conjugated PVC catheter image is showed. Figure 3.A. shows the image of PVC urinary catheter under fluorescence microscope. It is seen that there is no fluorescence illuminance exist in pure PVC. However, in FITC

labeled LFcinB$_{(21-25)}$pal peptide conjugated PVC catheter FITC molecules can be seen under fluorescence microscope. Using FITC labeling the peptide conjugation on PVC catheters was proven.

For colony counting assay, *E. coli* and MRSA strains were used. The *E. coli* viability on control PVC catheter was found 5.16 log and with LFcinB$_{(21-25)}$pal conjugated catheter this viability had decreased to 3.31 log (Figure 4). As in MRSA case, the viability of control group was 5.49 log and in PVC/ LFcinB$_{(21-25)}$pal group viability has decreased to 4.12 log. It is seen that MRSA was harder to kill compared to *E. coli* for LFcinB$_{(21-25)}$pal antimicrobial peptide. With the same concentration of peptide, more bactericidal effect has provided against *E. coli*. With an increase in the conjugated peptide molarity, higher bacterial death can be achieved in future studies.

IV. Conclusion

In this study, PVC urinary catheters were functionalized with antimicrobial peptide, LFcinB$_{(21-25)}$pal, and its effectiveness was investigated on MRSA and *E. coli* strains. First, the PVC surface was treated with cold atmospheric plasma to increase the hydrophilicity of the surface and optimal exposure duration was determined with contact angle measurements. For the conjugation of the peptide, EDC/NHS was used and the characterization was conducted with FITC labeling. Efficient conjugation of LFcinB$_{(21-25)}$pal achieved. The developed catheter provided bacterial death of both gram-positive MRSA and gram-negative *E.coli*. With this application, we provided an effective and alternative method to antibiotics for prevention and treatment of CAUTIs. We aimed to eliminate the possibilities of AMR occurrence and the problems that arise with AMR. In the future, the antibiofilm activities and the cytotoxicity of the developed catheter will be investigated.

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