Modification of a conventional polyurethane composition provides significant anti-biofilm activity against *Escherichia coli*

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Modification of a Conventional Polyurethane Composition Provides Significant Anti-biofilm Activity Against *Escherichia coli*

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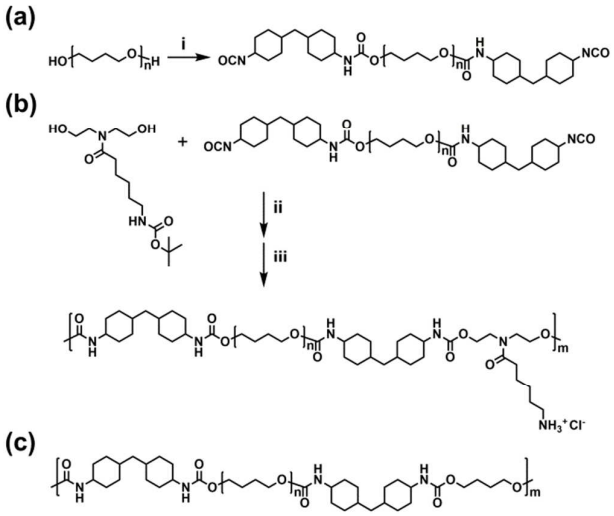
Catheter-associated urinary tract infections, caused by a build-up of biofilm on the catheter surface, are one of the most common nosocomial infections. For this work, we prepared a compositional variant of Tecoflex®, a commercial thermoplastic polyurethane (TPU), with peptide-like cationic pendant functional groups to study their effect on biofilm growth. Tecoflex® is used for fabrication of catheters and therefore a variant that shows decreased biofilm accumulation could have the potential to combat nosocomial infections. The cationic pendant groups were incorporated into the polyurethane composition similar to Tecoflex® by copolymerization of an amine functionalized N-substituted diol to give a cationic polyurethane (Tecoflex-NH_3^+). The antimicrobial properties of this cationic polyurethane were investigated using *Escherichia coli*, a Gram-negative bacterium and as confirmed by microscopy studies and bactericidal assays, the cationic polyurethane coating exhibited a contact-killing mechanism, and it significantly slowed down the build-up of biofilm compared to Tecoflex® coating. Moreover, the cationic polyurethane demonstrated minimal toxicity towards mammalian cells.

Catheter-associated urinary tract infections (CAUTI) are an emerging problem, causing approximately 40% of all hospital acquired infections.[1-5] While the catheter is in use, bacteria can attach to the catheter surface and initiate biofilm formation: a process in which bacteria replicate and secret extracellular polysaccharides that protect the growing bacterial community and impart resistance to host defense mechanisms.[6] The bacteria embedded inside the biofilm can detach and release, which may lead to an infection when the released bacteria overwhelms the host's defense mechanisms.[7] Several approaches to reduce the incidence of catheter infections have already been established; these include avoiding unnecessary catheterization, limiting the duration of catheter use, and utilizing alternative means of catheterization as well as different infection control strategies.[1,8-10] In addition to these routinely recommended methods, CAUTI can also be prevented by using innovative biomaterials that inhibit or slow down biofilm formation on the catheter surface.

One strategy to create antimicrobial surfaces involves the incorporation of antimicrobial agents, such as silver nanoparticles, antibiotics, or peptides, into the surfaces.[11-13] For example, Zhang *et al.* reported nano-silver functionalized Ti surfaces that released silver ions over a sustained period,



Scheme 1. Synthetic route for the preparation of (a) prepolymer and (b) amine functionalized polyurethane Tecoflex-NH_3^+. (c) Tecoflex® was synthesized by polymerizing the prepolymer with 1,4-butanediol. Reagents and conditions: (i) 4,4'-methylenebis(cyclohexyl isocyanate), CH_2Cl_2, DBTDL, 50 °C, 4 hours; (ii) CH_2Cl_2, DBTDL, room temperature, 24 hours; (iii) 4N HCl in 1,4-dioxane, CH_2Cl_2, 45 min.

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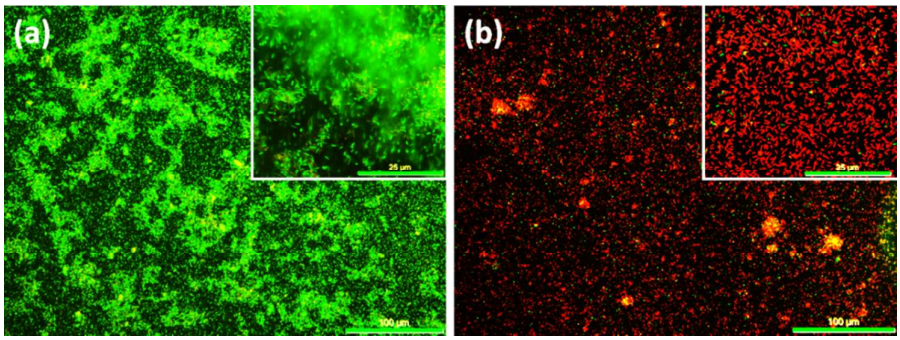

Figure 1. Live/dead staining of *E. coli* on Tecoflex® (a) and Tecoflex-NH_3^+ (b) after 24 h incubation

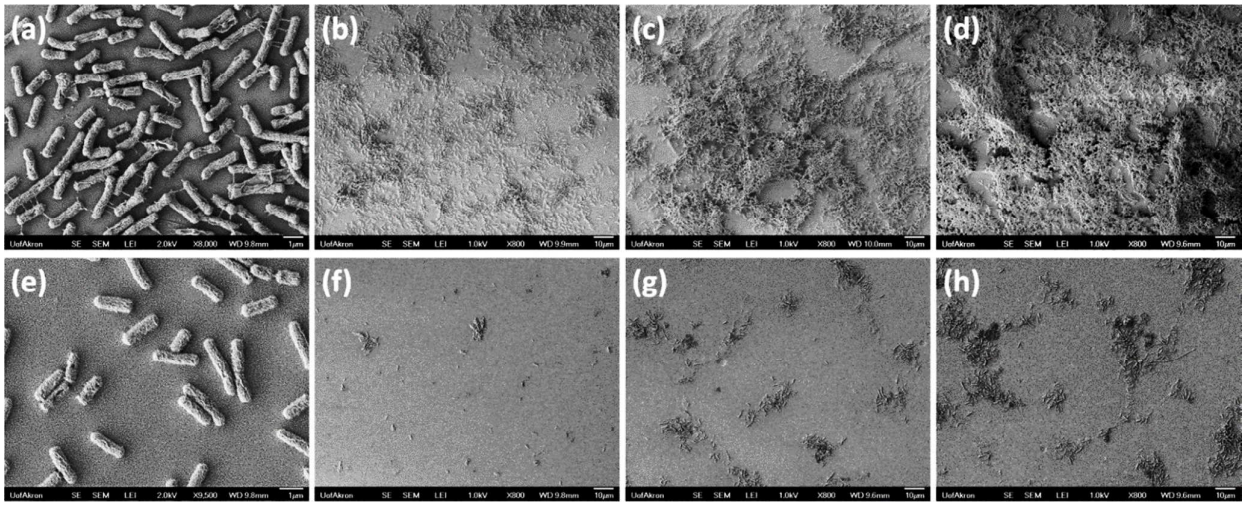

Figure 2. SEM images of Tecoflex® coating after incubation with *E. coli* suspension for 1 day (a, b), 3 days (c), and 5 days (d); SEM images of Tecoflex-NH_3^+ coating after incubation with *E. coli* suspension for 1 day (e, f), 3 days (g), and 5 days (h); the scale bars in a, e are 1 μm, and the scale bars in b, c, d, f, g, h are 10 μm

and showed excellent antimicrobial activity against both adherent and planktonic bacteria, such as *Staphylococcus epidermidis* and *Staphylococcus aureus*.[11] Another strategy is the use of contact-active antibacterial surfaces, wherein the antibacterial activity does not depend on release of biocidal substances, but on physical contact between bacteria and the surface.[13-17] Cationic functional groups, such as positively charged amine or quaternary ammonium compounds (QACs) are typically attached to the surface to interact with and disrupt the bacterial membrane when they come in contact with the bacteria. Joy *et al.* developed coumarin based polyesters with cationic amine pendant groups that are very effective against Gram-negative *Pseudomonas aeruginosa*.[16] Busscher *et al.* tethered quaternary-ammonium-compounds onto hyperbranched polyurea coatings, which exhibited efficient contact-killing of *Staphylococcus epidermidis*.[14]

Here, we describe the design and synthesis of a cationic antimicrobial polyurethane with anti-biofilm properties made by chemical modification of Tecoflex®, a commercial aliphatic polyether-based thermoplastic polyurethane (TPU). Tecoflex® was chosen as a model substrate in this work, since it is extensively used for biomedical applications and is a commonly used material for catheters. The peptidomimetic cationic functionalities were incorporated into the polyurethane through copolymerization using a pendant functionalized N-substituted diol monomer that was previously developed in our lab.[16,18-21] The antimicrobial polyurethane reported here has several advantages. First, the pendant functionalized N-substituted diol used here can also be incorporated into other step-growth polymerizations to confer antimicrobial properties to various polymers. Second, the antimicrobial polyurethane has a contact-killing mechanism which exhibits good anti-biofilm activities against *Escherichia coli* even after 5 days. Third, the cationic polyurethane showed low hemolytic activity and cytotoxicity, demonstrating good selectivity for bacteria over mammalian cells.

The amine functionalized polyurethane was synthesized *via* a three-step reaction (Scheme 1). First, a prepolymer was made by reacting 4,4'-methylenebis(cyclohexyl isocyanate) (MDI) with poly(tetramethylene oxide) (PTMO) in the presence of dibutyltin dilaurate (DBTDL) at 50 °C for 4 hours. The resulting prepolymer was then copolymerized with the functionalized diol monomer catalyzed by DBTDL at room temperature for 24 hours. The resulting polymer was deprotected with 4N HCl in 1,4-dioxane/CH_2Cl_2 to give the final product. Similarly, the non-functionalized Tecoflex® was synthesized by copolymerizing the prepolymer with 1,4-butanediol under the same conditions. The chemical structure of the polymers was

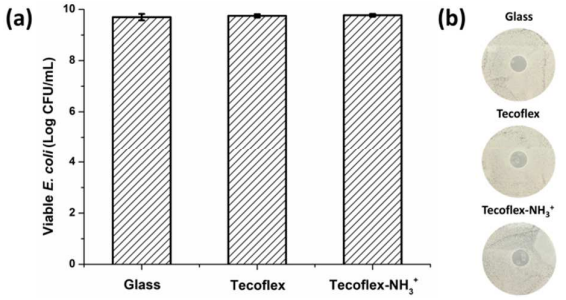

Figure 3. (a) Bactericidal activity of polymer coatings against planktonic *E. coli*; (b) Zone of inhibition was not observed for the polymer coatings

confirmed by IR and ^{1}H NMR spectroscopy (Figures S1-S3). As determined by DMF GPC, the molecular weights of Tecoflex® and Tecoflex-NH_3^+ used in this work are 41.5 and 30.7 kDa, respectively (Table S1). The Tecoflex-NH_3^+ surface exhibited lower contact angle (80°) compared to the non-functionalized Tecoflex® surface (92°), which can be attributed to the presence of cationic groups on the surface. As shown by the DSC data (Figure S4), both Tecoflex® and Tecoflex-NH_3^+ showed similar glass transition temperatures (T_g) (~-75 °C). Tecoflex® exhibited a melting peak (T_m) at 17°C, while Tecoflex-NH_3^+ was amorphous due to the incorporated pendant functional groups. Since the T_m of Tecoflex® is lower than room temperature, the effect of the different crystallization behavior on the performance of the two polymers is likely to be minimal because both polymers were amorphous under the test condition (37°C). To further confirm this, we performed ^{1}H solid-state magic-angle spinning (MAS) NMR spectroscopy at 298K (Figure S5) to evaluate the crystallinity of the two polymers. Typically, crystalline polymers will show broad peaks while amorphous polymers will show narrow peaks, reflecting the mobility of amorphous chains. ^{1}H MAS NMR spectra for both samples showed only narrow peaks, demonstrating the amorphous nature of both samples at the test conditions. [Fig. S5; see peaks for PTMO at 3.5 (-OCH_2) and 1.7 (-CH_2) ppm].

Catheter-associated urinary tract infections are frequently caused by fecal *E. coli* and as a proof-of-concept we tested the ability of polyurethane coatings to prevent *E. coli* biofilm formation.[2,4] The biofilm forming *E. coli* strain 25922, was used to evaluate biofilm formation.[22] To determine the effect of the polyurethane coatings on *E. coli* colonization, polymer coated coverslips were incubated with an *E. coli* suspension in M9 minimal medium at 37 °C for 24 hours. After 1 day, bacterial colonization and viability were determined by live-dead staining using fluorescence microscopy, in which live bacteria fluoresce green and dead bacteria fluoresce red. As shown in Figure 1(a), a large area of viable biofilm was seen on the Tecoflex® surface, as shown by the green fluorescence. In contrast to the non-functionalized surface, there were significantly fewer bacterial colonies on the functionalized surface (Tecoflex-NH_3^+), and most of the attached bacteria were dead, as evidenced by red fluorescence in Figure 1(b).

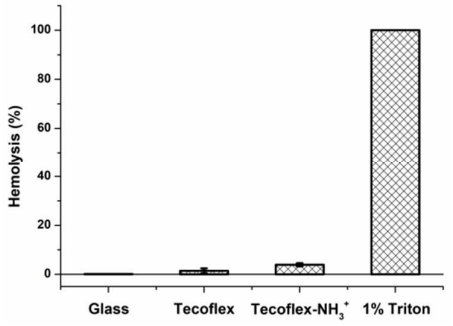

Figure 4. Hemolytic activity of Tecoflex® (1.4±1.0 %) and Tecoflex-NH_3^+ (3.9±0.7 %)

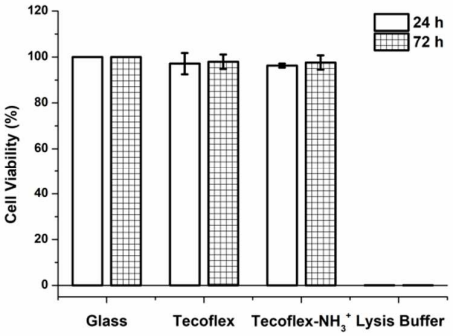

Figure 5. Cell viability of NIH-3T3 fibroblast cells seeded on the polymer coated coverslips after 24 and 72 hours of growth

To investigate whether the antimicrobial activity of the surface is due to residual small molecules or oligomers leaching out from the polymer coatings, polymer coated coverslips were incubated in an *E. coli* suspension for 24 h and the planktonic *E. coli* viability was determined. As shown in Figure 3(a), there was no significant difference between the various treatment groups (blank coverslip, Tecoflex® coated coverslip, and Tecoflex-NH_3^+ coated coverslip) in the number of viable planktonic bacteria in the media, which means that the polymer coatings did not have any discernable negative effects on planktonic bacteria. In addition, a zone of inhibition test was performed to confirm that direct contact is required for the observed killing mechanism; as shown in Figure 3(b), no zone of inhibition was observed around the coverslip for any sample. These results indicate that the mechanism of bacterial killing for this cationic polyurethane is a contact-killing mechanism.

To examine the contact-killing effects of this antimicrobial coating on biofilm formation and microbial growth, the progress of biofilm formation was monitored over a longer time period (1 day, 3 days, and 5 days) by SEM in order to visualize the attached bacteria on the surface. As shown in Figure 2, Tecoflex® coating showed a large coverage of bacteria at day 1, and the thickness of the biofilm significantly increased at days 3 and 5. In contrast to the non-functionalized Tecoflex® coating, there were far fewer bacteria on the amine functionalized Tecoflex® coating even after 5 days of incubation, which confirmed the effective anti-biofilm properties of the coating. It is worth noting that the amount of

bacteria on Tecoflex® and Tecoflex-NH_3^+ coated coverslips observed by SEM is relatively less than that observed by fluorescence microscopy (after 24 h incubation). This difference could be attributed to the additional dehydration steps using a series of ethanol/water mixtures required for the SEM sample preparation.

A hemolysis assay was performed to determine the potential toxicity of the cationic polymer coatings toward mammalian cell membranes. In this assay, the amount of hemoglobin released from lysed red blood cells was quantified and used as an indication of cell lysis, with 1% Triton X-100 as a positive control for full red blood cell lysis. The hemolytic activity of Tecoflex® coating and the functionalized Tecoflex-NH_3^+ coating was analyzed and as shown in Figure 4, the coatings did not show any discernable hemolytic activity.

The cytotoxicity of the polymer coatings to mammalian cells was further evaluated by determining the viability of NIH-3T3 fibroblast cells (ATCC CRL-1658) seeded on the polymer coated coverslips after 24 and 72 hours. A lactate dehydrogenase (LDH) assay was performed to determine the viability of the cells. As shown in Figure 5, the polymer coatings showed minimal cytotoxicity toward the NIH-3T3 cells after 24 and 72 hours of growth. The results from both the hemolysis assay and LDH assay indicate that the cationic polyurethane Tecoflex-NH_3^+ has excellent selective toxicity toward *E. coli* over mammalian cells. Such selectivity could arise due to the overall negative charge of bacterial membranes, leading to electrostatic interactions with the cationic polyurethanes.[2323]

In conclusion, a cationic antimicrobial polyurethane was designed and developed by incorporating an amine functionalized N-substituted diol to obtain a compositional variant of the commercial polyurethane Tecoflex®. This novel cationic polyurethane, Tecoflex-NH_3^+, exhibited a contact-killing mechanism and showed excellent anti-biofilm properties against *E. coli* even after 5 days. In addition, this cationic polyurethane has good selectivity against *E. coli* over mammalian cells, as depicted by very low hemolytic activity and cytotoxicity. The described method of incorporating the N-substituted diol can be applied to other step-growth polymer platforms *via* copolymerization to confer antimicrobial properties. Small structural changes in the polymer compositions used for catheter fabrication, such as shown here, can provide significant antibacterial properties and this strategy could be an important arsenal in the fight against such infections.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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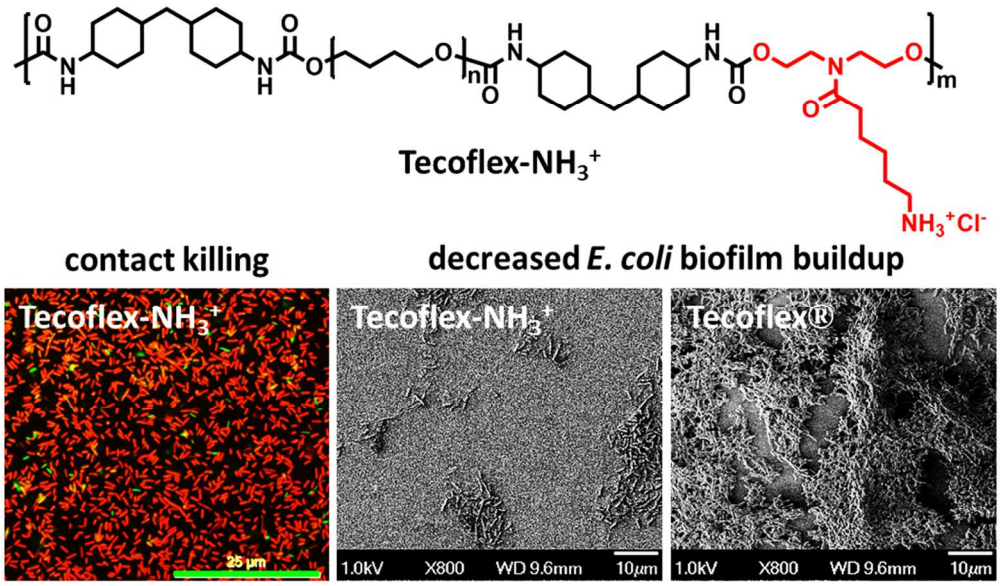

Tecoflex-NH_3^+ exhibited a contact-killing mechanism and showed excellent anti-biofilm properties against *E. coli* even after 5 days.

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