Polyurethane coated with polyvinylpyrrolidones via triazole links for enhanced surface fouling resistance

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Abstract
Surfaces with hydrophilic and antimicrobial properties are very attractive for cardiovascular device-associated applications. The aim of this study was to prepare and coat a hydrophilic polymer containing a functional group capable of forming triazole functionality onto the surface of polyurethane (PU). The modified surfaces were assessed with cell adhesion, bacterial adhesion and bacterial viability. Mouse fibroblast cells (NIH-3T3) and three bacterial species were used for assessment. The results showed that the modified surface not only exhibited a significant reduction in cell adhesion with a 25%–59% decrease to mouse fibroblast but also showed a significant reduction in bacterial attachment with 26%–67%, 24%–61% and 23%–57% decrease to Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, respectively, as compared with original PU. Furthermore, the polymer-modified surface exhibited a significant antibacterial function by inhibiting bacterial growth with reduction of 49%–84%, 44%–79% and 53%–79% to S. aureus, E. coli and P. aeruginosa, respectively, as compared with original PU. These results indicate that covalent polymer attachment enhanced the antibacterial and antifouling properties of the PU surface.

KEYWORDS
bacterial adhesion and inhibition, cell adhesion, polymer coating, polyurethane

1 | INTRODUCTION

The surface of polymeric biomaterials plays a key role in a variety of biomedical applications [1–3]. When implantable biomaterials are used inside the body, their surfaces are the main target for cells, proteins and other biological species [1, 4]. Meanwhile, their surfaces are also more likely contaminated or infected with bacteria [1, 5, 6]. It is known that most synthetic polymeric materials for biomedical applications are extremely susceptible to cell, protein and bacterial attachment due to their inherent hydrophobic nature [1]. To enhance the survival rates of these implanted biomaterials and reduce the potential cell or bacterial adhesions, surfaces of biomaterials must be improved or treated to minimise potential cell/protein and bacterial attachment [1, 2].

For reducing cell/protein attachment, the surface should be modified to be very hydrophilic, so that neither amphiphilic proteins nor cells are able to attach to the surface [1, 2]. Regarding bacterial contamination, two strategies were applied [2, 5]: (1) providing a highly hydrophilic surface which can minimise bacterial attachment and thus reduce the risk of bacterial contamination, and (2) preparing a highly antibacterial surface which contains an antibacterial agent that can either inhibit bacterial growth or kill the attached bacteria [1, 7].

Polyurethane (PU) is an elastic and tough polymer. Medical grade PU has been used in the biomedical device industry for decades due to its superior biocompatibility and excellent elasticity [1]. This biocompatible polymer is particularly attractive for cardiovascular device-associated applications such as artificial blood vessels, catheters, dialysis pump, etc.
due to its tough and elastic properties that are similar to those of real blood vessels [8]. However, like most synthetic polymers, PU is very hydrophobic [1]. When it is in contact with body fluids or blood, amphiphilic proteins and cells in bloodstream would absorb or adhere to its surface, causing clot formation or blood blockage [4]. On the other hand, during surgical implantation, bacterial adhesion can lead to bacteria-associated contamination or infection [6]. To help PU find a wider application in cardiovascular areas, its surface must be modified or treated to be compatible with body fluids and/or blood. To modify the surface of PU, its inert surface must first be activated to be reactive. Since PU contains urethane hydrogen on its surface, it can be chemically activated through a urea formation between urethane and isocyanate groups [9]. After surface activation, other reactive agents or polymers can then be coated onto its surface [9]. It is known that most effective nucleophiles and their associated reactions are often conducted in polar organic solvents because nucleophiles and their reactions are only viable and effective under these solvents. As a result, the modified surfaces would inevitably be altered or damaged, although most people have rarely addressed this issue. To keep polymer surface intact during coating, water is one of the best choices to be used as reaction media. On the other hand, however, water is a strong competitor to most nucleophiles, which can ultimately fail the surface coating reactions. In this study, we proposed to coat a hydrophilic polymer on PU surface through a click reaction using water as a reaction medium with concurrent triazole formation.

Click chemistry and its associated chemical reaction have emerged as a fast and efficient approach for synthesizing novel heterocyclic compounds in the last decade [10, 11]. Click reaction has been used to bridge two molecules or two polymers with one containing an azide group and the other having an alkyne group in the presence of the appropriate catalysts in water or water/organic solvent mixture [11, 12], forming a 1,2,3-triazole link. The formed 1,2,3-triazole is a nitrogen-containing heterocycle capable of forming hydrogen bonds, which can improve its solubility in water and ability to interact with numerous biomolecular targets [13]. There has been a growing interest in the formed triazoles and their applications [10–14]. Triazoles and their derivatives have shown various pharmacological properties such as antimicrobial [15], antiinflammatory [18], antiinflammatory, analgesic [19] and antiviral [20] functions. Therefore, the triazole linkages formed through a click reaction would exhibit a certain degree of antibacterial functions. It is our hypothesis that modifying PU surface with a hydrophilic Poly(N-vinylpyrrolidone) (PVP) polymer via a click reaction would not only provide a hydrophilic surface to PU but also make the modified surface antibacterial.

The objective of this study was to use a highly hydrophilic and biocompatible polymer to modify PU surface with the help of a click reaction in a mild reaction condition and evaluate cell adhesion, bacterial adhesion, and bacterial properties of the modified surface.

2 | MATERIALS AND METHODS

2.1 | Materials

Propargyl alcohol, glycidyl methacrylate, N-ethylmaleimide (NVP), dibutyltin dilaurate, 1,6-diisocyanatohexane, 2,2’-azo-bisisobutyronitrile, sodium azide, dioxane, tetrahydrofuran, dimethylformamide, ether, copper sulphate, tetrabutylammonium bromide, sodium ascorbate, anhydrous magnesium sulphate, and sodium chloride were used as received from Sigma-Aldrich Co. without further purifications. Thermoplastic PU, TT-1075D-M, was received from Lubrizol Co.

2.2 | Surface modification

2.2.1 | Synthesis of reactive functional polymer

Synthesis of a reactive functional polymer was conducted based on the two steps: GMA and poly(NVP-co-GMA) or PVPGMA preparations. (1) GMA synthesis [21]: In short, to a solution of glycidyl methacrylate (GM, 0.1 mol) and ammonium chloride (0.1 mol) in tetrahydrofuran, sodium azide (0.1 mol) was added. The reaction was conducted at 60°C for 24 h, followed by filtering out the solids, adding distilled water, extracting with ether, washing with brine, drying with anhydrous magnesium sulphate, and removing the solvent by a rotary evaporator. (2) PVPGMA synthesis [22]: the polymer was synthesised similarly to our published procedure. Briefly, 2,2’-azo-bisisobutyronitrile (1% by mole) was added to a solution containing a total of 5 g of NVP and GMA at a molar ratio of 95/5, 90/10, 85/15, 80/20, 75/15 and 70/30 in dimethylformamide. After the reaction was carried out under a N2 blanket at 64°C for 24 h, the formed polymer was purified with ether and dried in a vacuum oven.

2.2.2 | Surface modification of PU

Polyurethane surface modification was completed based on the steps below: (1) PU circular specimen preparation [23]: briefly, PU Tecothane was dissolved in a mixture of dioxane and tetrahydrofuran. The formed solution was poured into a Teflon petri dish and conditioned in an oven at room temperature overnight, followed by maintaining it at 40°C for 4 h and then at 60°C overnight. After removing it from the dish, the polymer membrane (thickness = 0.25 mm) was cut to small circular specimens with surface area of 78.5 mm². (2) Isocyanate group introduction: the specimens were placed in a vial containing dibutyltin dilaurate (0.3%), 1,6-diisocyanatohexane (5%) in hexane. After stirring at 50°C for 2 h [24], the specimens were washed with hexane and ether, respectively, to remove excessive dibutyltin dilaurate and 1,6-diisocyanatohexane, to form PUI. (3) Acetylene group introduction: immediately after the above step, the isocyanate-containing specimens were immersed in a vial having dibutyltin dilaurate (0.3%) and propargyl alcohol (5%) in ether. After refluxing at 50°C for 4 h, the modified specimens were washed.
with ether several times, to form PUPA. (4) Surface modification with the synthesised polymers: the modified PU circular specimens were placed in a container with PVPGMA (6%), tetrabutylammonium bromide (1%), sodium ascorbate (0.5%) and copper sulphate (2%) in distilled water. After stirring at 50°C for 3 h, the specimens were washed with distilled water, resulting in the specimens having the coated polymers on the surfaces, to form PU-PVPGMA.

2.3 | Characterisation

The synthesised functional monomer, polymer and surface-modified circular specimens were characterised with Fourier transform-infrared (FT-IR) spectroscopy. The surface functional groups of the modified PU were identified with the help of attenuated total reflectance FT-IR. FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR1000).

2.4 | Evaluation

2.4.1 | Cell adhesion test

Mouse fibroblasts (NIH-3T3) were obtained to assess cell adhesion on the PU discs (modified or original) [22]. Briefly, cells were cultured in a mixture containing high glucose Dulbecco’s Modified Eagle Medium (DMEM, Lonza), supplemented with 10% foetal bovine serum (FBS, Invitrogen) and 5 mg/ml streptomycin as well as 5 mg/ml penicillin (Invitrogen Inc.). After incubating at 37°C under a humidified atmosphere of 5% CO₂ for 24 h, the cells were harvested by adding 5.3 mM trypsin-EDTA (ThermoFisher Scientific) solution in PBS, followed by centrifuging at 1200 rpm for 3 min, removing trypsin and re-suspending the cell pellets in DMEM medium supplemented with 10% FBS to a density of 5 × 10⁴ cells/mL. The formed cell suspension (1 ml) was then placed in a 24-well plate with each having a disc specimen and incubated for 48 h, followed by washing the disc with PBS to remove non-adherent cells. The cells attached to the surface of the disc specimen were harvested with trypsin, followed by imaging with an inverted microscope (Nikon Ti-E) and counting. Triplicate samples were used to obtain a mean value for each material.

2.4.2 | Bacterial adhesion test

The bacterial adhesion was assessed according to the published procedures [22]. Three bacterial species (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) were used for assessment. Briefly, colonies of bacteria were suspended in 5 ml of tryptic soy broth supplemented with 1% sucrose (to form a suspension with 10⁸ CFU/ml of bacteria) and incubated for 24 h. To the above bacteria in tryptic soy broth, the disc specimen, which was washed with 70% ethanol for 10 s and sterile water three times, was added. The suspension was incubated at 37°C under 5% CO₂ for 24 h, followed by washing with sterile PBS solution and de-ionised water (to remove non-adherent bacteria). The adhered bacteria were eluted from the disc specimen to 1 ml sterile PBS by ultrasonic treatment for 10 min. Bacterial CFU was enumerated by counting the colonies in the agar plate. The data represent a mean value for each material based on triplicate samples.

2.4.3 | Bacterial viability test

The bacterial viability was assessed based on the protocol elsewhere [25]. Three bacterial species (S. aureus, E. coli and P. aeruginosa) were assessed. In short, bacterial colonies which were suspended in tryptic soy broth (5 ml) supplemented with 1% sucrose, to form a suspension with 10⁸ CFU/ml of bacteria, were incubated for 24 h. To the above bacteria in tryptic soy broth, the disc specimen, which was washed with 70% ethanol for 10 s and sterile water three times, was added. The suspension was incubated at 37°C under 5% CO₂ for 48 h. Then, to 1 ml of the above bacterial suspension, 3 μL of a green/red (1:1 v/v) dye mixture (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc.) was added, followed by vortexing for 10 s, sonication for 10 s, vortexing for another 10 s and keeping in the dark for about 15 min before analysis. Finally, to a glass slide, 20 μL of the stained bacterial suspension was added, followed by imaging viable bacteria (green) with an inverted fluorescence microscope (EVOS FL, AMG). A bacterial suspension without incubating with any disc specimen was used as control and viable bacteria counts from it were used as 100%. The viability was analysed by counting from the recorded images. The data represent a mean value for each material based on triplicate samples.

2.4.4 | Statistical analysis

One-way analysis of variance (ANOVA) with the post hoc Tukey-Kramer multiple-range test was used to determine significant differences of each measured property or activity among the materials in each group. A level of α = 0.05 was used for statistical significance.

3 | RESULTS AND DISCUSSION

3.1 | Characterisation

Figure 1 shows a set of FT-IR spectra for PVPGMA synthesis: (a) GM, (b) GMA, (c) NVP and (d) PVPGMA. By comparison of spectra (a) and (b), both exhibited two strong peaks at 1721 for the carbonyl group and at 1636 for the carbon-carbon double bond group. In contrast, two strong new peaks at 3490 for the hydroxyl group and at
2103 for the azido group appear in spectrum (b), confirming the GMA formation. By comparison of spectra (b), (c) and (d), the peaks at around 1929–1636 for the carbon–carbon double bond group disappear in spectrum (d), corresponding to those shown in spectra (b) and (c). The peak at 2101 for the azido group appears in spectrum (d), corresponding to that shown in spectrum (b). A broader peak at 3434 appears in spectrum (d), corresponding to both the imide from NVP in spectrum (c) and the hydroxyl group from GMA in spectrum (b). These changes confirmed the PVPGMA formation.

Figure 2 shows a set of FT-IR spectra in absorbance for PU surface coating: (a) PU, (b) PUI, (c) PUPA and (d) PU-PVPGMA. By comparison of spectra (a) and (b), the appearance of a strong new peak at 2262 for the isocyanate group confirmed that 1,6-diisocyanatohexane was successfully attached onto the PU surface by reacting with hydrogen from the urethane group to form the urea group on the PU surface. By comparison of spectra (b) and (c), a new peak at 2121 for the acetylene group and a broader peak at 3292 for the overlapped urethane and the urea groups appear, and the peak at 2263 for the isocyanate group disappears in spectrum (c), confirming that PA was successfully attached onto the PU surface by forming a urethane link between the isocyanate and the hydroxyl groups. By comparison of spectra (c) and (d), the disappearance of the peak at 2121 for acetylene group confirmed that PVPGMA was successfully attached onto the PU surface by a click reaction between the extended acetylene groups from the modified surface and the azido groups from PVPGMA.

3.2 | Evaluation

Unlike conventional medical or pharmaceutical supplies, the implantable medical devices being used in cardiovascular or body-fluid contact applications require low cell attachment and/or minimum microbial contamination [1, 2, 4]. This demands the implantable materials or devices to have antifouling and antibacterial functions, meaning that surface of the materials or devices must be either hydrophilic or antifouling [1, 2, 8, 26]. To achieve this goal, we proposed to coat the surface in this study by using a newly prepared biocompatible and hydrophilic polymer by a click reaction in the presence of water. By doing this, not only the hydrophilic polymer would be coated on the hydrophobic surface of PU but also the antibacterial components would be incorporated onto the surface of PU.

It is known that hydrophilic surfaces can minimise protein adsorption, cell adhesion and bacterial attachment [1, 2]. Thus surface coated with hydrophilic polymer would no doubt enhance antifouling function of hydrophobic surfaces. Polyurethane is a very popular biocompatible polymer being used in cardiovascular areas due to its similarity in elasticity to human blood vessels [8]. Surface modification of PU would enhance its applications in biomedical research. Poly(N-vinylpyrrolidone) is a very biocompatible polymeric material that has been used as hydrogel building blocks in tissue engineering and blood substitute for many years [1, 27, 28]. Applying PVP onto the surface of medical devices no doubt would improve their antifouling properties. To minimise bacterial contamination or infection, two strategies have often been used, that is,
antifouling and antibacterial [29–34]. The former utilises the antifouling surface to minimise bacterial adhesion, thus reducing the risk of biofilm formation [29–31], whereas the latter prevents bacteria by inhibiting bacterial growth and/or killing bacteria [32–34]. In this study, we used a click reaction between azido group and acetylene group to couple a hydrophilic polymer onto hydrophobic surface, with concurrent formation of triazole groups. The formed 1,2,3-triazole groups not only would provide antifouling function but also contribute antibacterial functions to the PU surfaces. The following results and discussion describe the outcome from this study.

Figure 3 shows the effect of the PVGA polymers on 3T3 mouse fibroblasts adhesion to the PU surface. The cell adhesion was in the decreasing order of PU > PUPA > PVPGMA955 > PVPGMA7030 > PVPGMA9010 > PVPGMA7525 > PVPGMA8515 > PVPGMA8020, where there were statistically significant differences among all the tested surfaces (p < 0.05). Hydrophobic surfaces are known to show higher affinity to proteins, cells and even bacteria [1, 8]. PU is a highly antifouling or hydrophobic polymeric material. The modified PUPA showed a reduced cell adhesion of 16% as compared with original PU, probably due to introduced polar urethane groups. Regarding the PVPGA surface modification, all the modified surfaces exhibit a significant reduction in adhesion with 59%, 56%, 49%, 49%, 40%, 30% and 25% reduction for PVPGMA8020, PVPGMA8515, PVPGMA7525, PVPGMA9010, and PVPGMA955, respectively. Each individual component from PVPGA possesses qualities contributing to overall functionality. NVP is a hydrophilic monomer and its constructed polymeric material has been used as blood substitutes for many years due to its excellent blood compatibility [1, 27, 28]. GMA is also a hydrophilic molecule with attached hydroxyl group and polar azido group. Interestingly, the result from Figure 3 demonstrated that neither the surface with the highest NVP content nor the one with the highest GMA content showed the lowest cell adhesion. It is the one with PVPGMA8020 that showed the lowest cell adhesion, indicating that the proper molar ratio of NVP/GMA in the polymer plays a key and synergistic role in cell adhesion reduction. Therefore, the formulation optimisation is very important.

Figure 4 shows the effect of the PVPGA polymers on bacterial adhesion to the PU surface. Three bacterial species were investigated in this study. Bacterial adhesion demonstrated a similar pattern to that of 3T3 fibroblast adhesion, as shown in Figure 3. Taking adhesion to PU as 100%, we have found that the bacterial adhesion was observed in the following decreasing order: PU > PUPA > PVPGMA955 > PVPGMA7030 > PVPGMA9010 > PVPGMA7525 > PVPGMA8515 > PVPGMA8020, where there were statistically significant differences among all the tested surfaces with all three different bacteria (p < 0.05). The modified surfaces showed a significant reduction in bacterial adhesion of 67%, 53%, 49%, 42%, 32%, 26% and 13% with S. aureus, 61%, 46%, 45%, 36%, 30%, 24% and 16% with E. coli, and 57%, 51%, 42%, 39%, 26%, 23% and 16% with P. aeruginosa, for PVPGMA8020, PVPGMA8515, PVPGMA7525, PVPGMA9010, PVPGMA7030, PVPGMA 955 and PUPA, respectively, as compared with original PU. In addition, S. aureus showed the lowest adhesion, followed by
**FIGURE 3** Cell adhesion on polyurethane (PU) surfaces with and without coatings.

**FIGURE 4** Bacterial adhesion on polyurethane (PU) surfaces with and without coatings.
P. aeruginosa and E. coli. Again, PU is a highly hydrophobic polymeric material and that is likely the reason why it showed the highest bacterial adhesion. The acetylene-attached PU showed a reduction in bacterial adhesion, probably due to introduced polar urethane groups. The PVPGMA-modified PU exhibited a further reduction in bacterial adhesion. Similar to the results shown in Figure 3 for 3T3 cell adhesion, the one with the proper molar ratio of NVP/GMA at 80/20 showed the lowest bacterial adhesion.

Figure 5 shows the effect of the PVPGMA polymers on viability of three bacterial species in the supernatant of bacterial suspensions containing the disc specimens. Bacterial viability was found to be in the following decreasing order: PU > PUPA > PVPGMA955 > PVPGMA9010 > PVPGMA8515 > PVPGMA8020 > PVPGMA7525 > PVPGMA7030. S. aureus showed slightly lower viability than E. coli and P. aeruginosa. PUPA showed 14%, 21% and 19% reduction in S. aureus, E. coli and P. aeruginosa viability, suggesting that PUPA also has some bacterial inhibition activity, as compared to original PU. The antibacterial activity demonstrated by PVPGMA can be well attributed to the triazole moieties produced from the click reaction between acetylene groups from PUPA and azido groups pendant on PVPGMA. The triazole moieties have been found to have an antibacterial function and are being added to a variety of pharmaceutical formulations nowadays. With increasing GMA content, S. aureus, E. coli and P. aeruginosa displayed reduction values of 49%, 44% and 53% for PVPGMA955%, 62%, 54% and 60% for PVPGMA9010, 64%, 60% and 64% for PVPGMA8515, 72%, 72% and 69% for PVPGMA8020, 75%, 74% and 76% for PVPGMA7525, and 84%, 79% and 79% for PVPGMA7030, respectively, as compared to original PU. Apparently, GMA itself has nothing to do with any antibacterial function. It has been reported that triazoles show low cytotoxicity to human cells as compared to other antibacterial compounds or functionalities [35]. This suggests that triazoles formed from our surface modification may provide a novel route for developing an antibacterial surface or material with minimum cytotoxicity.

Figure 6 shows a set of micrographs of the viability of three bacteria species after incubating with original PU and modified PU specimens. The images from Figure 6a to 6h represent (a) PU with E. coli, (b) PUPA with E. coli, (c) PU-PVPGMA955 with E. coli, (d) PU-PVPGMA7030 with E. coli, (e) PU-PVPGMA955 with P. aeruginosa, (f) PU-PVGA7030 with P. aeruginosa, (g) PU-PVGA55 with S. aureus, and (h) PU-PVGA7030 with S. aureus. For E. coli, obviously, the original PU showed the highest numbers of bacteria, followed by PUPA, PVPGMA955 and PVPGMA7030. The result was consistent with those from Figure 5. For P. aeruginosa and S. aureus, similar to the images for E. coli (6d and 6e), the images for PVPGMA7030 (6f and 6h) showed less bacterial numbers than those for PVPGMA955 (6c and 6g), which are attributed to the triazole contents.

![Graph](image-url)  
**Figure 5** Bacterial viability on polyurethane (PU) surfaces with and without coatings.
4 | CONCLUSIONS

A novel hydrophilic polymer containing a functional group capable of forming triazole functionality was successfully synthesised and coated onto a hydrophobic PU surface. The modified surface not only exhibited a significant reduction in cell adhesion with a 25%–59% decrease to 3T3 fibroblast but also showed a significant reduction in bacterial attachment with 26%–67%, 24%–61% and 23%–57% decrease to *S. aureus*, *E. coli* and *P. aeruginosa*, respectively, as compared with original PU. Furthermore, the polymer-modified PU surface exhibited a significant antibacterial function by inhibiting bacterial growth with reduction of 49%–84%, 44%–79% and 53%–79% to *S. aureus*, *E. coli* and *P. aeruginosa*, respectively, as compared with original PU. These results hold much promise in preventing or reducing medical device-associated adhesion.
and infections. Future studies will focus on optimisation of the polymers and their formulations as well as preparation protocols.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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