COATING OF POLYVINYLCHLORIDE FOR REDUCED CELL/BACTERIAL ADHESION AND ANTIBACTERIAL PROPERTIES

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LIST OF ACRONYMS

PVC	Original PVC
AZ	PVC tethered with azido groups
PA	PVC tethered with hydroxyl groups
IC	PVC tethered with isocyanate groups
AM	PVC tethered with amino groups
PVPA1	PVC coated with PVPA at a molar ratio of VP/SA = 95/5 and synthesized at AIBN = 0.5%
PVPA2	PVC coated with PVPA at a molar ratio of VP/SA = 95/5 and synthesized at AIBN = 1.0%
PVPA3	PVC coated with PVPA at a molar ratio of VP/SA = 95/5 and synthesized at AIBN = 3.0%
PVPA4	PVC coated with PVPA at a molar ratio of VP/SA = 90/10 and synthesized at AIBN = 1.0%
PVPA5	PVC coated with PVPA at a molar ratio of VP/SA = 85/15 and synthesized at AIBN = 1.0%

ABSTRACT

Almousa, Rashed Abdulaziz R. M.S.B.M.E., Purdue University, May 2019. Coating of Polyvinylchloride for Reduced Cell/Bacterial Adhesion and Antibacterial Properties. Major Professor: Dong Xie.

A Polyvinylchloride surface was modified by coating a biocompatible, hydrophilic and antibacterial polymer by a mild surface modification method. The surface was first activated and then functionalized, followed by coating with polymer. The surface functionality was evaluated using cell adhesion, bacterial adhesion and bacterial viability for polymers with antibacterial properties. 3T3 mouse fibroblast cells were used for cell adhesion, *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus* were used for bacterial adhesion in the first study, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used for bacterial adhesion and antibacterial activity in the second study.

Chapter 2 reports how we synthesized, immobilized and evaluated a novel hydrophilic polymer with anti-fouling properties onto surface of polyvinylchloride via an effective and mild surface coating technique. The polyvinylchloride surface was first activated by azidation as well as amination, and then tethering a newly synthesized hydrophilic and biocompatible polyvinylpyrrolidone having pendent reactive succinimide functionality onto the surface. Results show that the coated hydrophilic polymer significantly reduced the 3T3 fibroblast cell adhesion as well as the adhesion of the three bacterial species.

Chapter 3 reports how we prepared, immobilized and evaluated an antibacterial and anti-fouling polymer onto polyvinylchloride surface following an efficient and simple method of surface modification. The surface coated with a terpolymer constructed with N-vinylpyrrolidone, 3,4-Dichloro-5-hydroxy-2(5H)-

furanone derivative and succinimide residue was evaluated with cell adhesion, bacterial adhesion and bacterial viability. Surface adhesion was evaluated with 3T3 mouse fibroblast cells and two bacterial species. Also, antibacterial activity was evaluated by bacterial viability assay with the two bacterial species. Results showed that the polymer-modified polyvinylchloride surface exhibited significantly decreased 3T3 fibroblast cell adhesion and bacterial adhesion. Furthermore, the modified polyvinylchloride surfaces exhibited significant antibacterial functions by inhibiting bacterial growth with bactericidal activity.

Altogether, we have successfully modified the surface of polyvinylchloride using a novel efficient and mild surface coating technique. The first hydrophilic polymer-coated polyvinylchloride surface significantly reduced cell adhesion as well as adhesion of three bacterial species. The second hydrophilic and antibacterial polymer-coated polyvinylchloride surface demonstrated significant antibacterial functions by inhibiting bacterial growth and killing bacteria in addition to significantly reduced 3T3 fibroblasts and bacterial adhesions.

CHAPTER 1. INTRODUCTION

1.1 Background

Surface modification is very crucial to a variety of biomaterials applications. Some surfaces need to be modified to be cell- or tissue-integrated whereas others require modification to be anti-fouling, i.e., lower or no cell adhesion. In light of biomedical applications, two main factors limit use of polymeric materials in surface-related medical devices: their highly hydrophobic surface while being used in contact with body fluid and/or blood [1,2] and bacterial infection or contamination while bacteria attach and/or grow on surface [3]. Hydrophobic surface can cause cell adhesion, bacterial adhesion and nonspecific protein adsorption [4–10]. Cell adhesion and protein adsorption can lead to blood flow blockage if polymers are used internally [11,12]. Bacterial attachment and biofilm formation can cause biomaterials-relevant infections [13]. Some used a strategy to develop entire hydrophilic or amphiphilic polymers and use them to form medical devices, [14] but others focused on modifying surface of the formed medical devices [15–20]. Polyvinylchloride is a commonly used thermoplastic polymer for biomedical application, due to its low cost, easy processing and low toxicity [1]. This polymer has been used in making many cardiovascular devices such as catheters, blood vessels, artificial heart pump, dialysis device, and others [1,21]. However, like most other polymers, this polymer is very hydrophobic [1] which leads to cell adhesion and protein adsorption, if it contacts body fluid or blood, and bacteria contamination if it is not sterile. So far, few reports deal with surface modification of polyvinylchloride.

There is a pressing need for surface modification of polyvinylchloride in order for this conventional plastic to find wider applications in biomaterials and biomedical areas. Unitl now, there have been numerous studies on surface modification of

polymers for decreased cell adhesion and protein adsorption [11, 12, 14–18, 22–25]. Many of them were focused on polyurethanes, polyureas and other types of polymers [12,14–18,22–26], but some involved polyvinylchloride surface modification, specifically by plasma, UV, or chemicals [27–36]. Most modifications were limited to simple chlorine substitution through a nucleophilic reaction [32–36] or surface free radical formation by plasma or UV [27–31]. Although the chlorine atoms on polyvinylchloride are labile and can be substituted with a number of nucleophiles, substitution reactions often need to be carried out in polar organic solvents or polar organic solvent/water mixtures to make nucleophiles viable and effective [37–39]. On the other hand, polyvinylchloride and most nucleophiles are soluble in polar organic solvents. Thus, the reactions under polar organic solvents or polar organic solvent/water mixtures could absolutely alter or damage the surface morphology or surface topography, although this issue has not been really addressed in the published literature [37–39]. To keep polyvinylchloride surface intact, water is one of the best choices to be used as reaction media. However, water is a strong competitor to most nucleophiles. According to literature, chlorine on polyvinylchloride can be substituted with ethylenediamine, acromatic thiol, thiocyanate and amino groups in the presence of water or water/N, N'-dimethylformamide mixture [32–40]. Azidation in aqueous solution, with the help of a phase transfer agent [41, 42], is a good example of a technique for modifying the polyvinylchloride surface without worrying about dissolution and surface damage [35, 36, 41]. Up to date, there has been no report on surface modification of polyvinylchloride with biocompatible and extremely hydrophilic polyvinylpyrrolidone polymer using a simple and effective coating technology in the presence of water. Polyvinylpyrrolidone is a very biocompatible polymer which has been used as a blood substitute and a hydrogel building blocks for years [43,44]. Introducing this polymer onto the surface of medical devices would no doubt enhance their anti-fouling properties.

In terms of preventing bacterial infection, another approach is to create anti-microbial surfaces by chemically linking antibacterial compounds onto the surfaces,

which allows the attached compounds to kill or inhibit bacteria by simple contact. This strategy is thought to be unique in preventing long-term disinfection and reducing the risk for formation of antibiotic-resistant bacteria [45–49]. It is believed to one of the most effective strategies. Due to the fact that quaternary ammonium salts can be simply derivatized and easily incorporated into a polymer, their derivatives have been widely and extensively studied for contact-mediated microbial inhibition [45–48]. However, it was reported that interactions between quaternary ammonium salts and proteins can reduce antimicrobial effectiveness [36, 37]. Not long ago it has been found that the derivatized 2(5H)-furanone compounds exhibited significant antibacterial functions without proteins interference [38]. It has been validated this antibacterial effect on dental restoratives [49,50]. These derivatives were covalently linked to dental polymers or dental composites, resulting in killing bacteria or inhibiting bacterial growth by simple contact but not via release or leaching [51, 52]. This greatly reduces the potential cytotoxicity from the antibacterial derivatives to the surrounding tissues. In these studies, it is also found that the modified restoratives did not significantly interact with human saliva, limiting negative protein effects on antibacterial functions [51,52] unlike quaternary ammonium salt-containing materials [49, 50].

1.2 Hypothesis and Objectives

It is our hypothesis that immobilizing the novel anti-fouling and biocompatible polymers with and without antibacterial residues onto the surface of polyvinylchloride via an effective and mild surface coating technique would provide a novel route for surface modification to reduce cell/bacterial adhesion and bacterial infection. The objectives of the study in this thesis were to:

1. Synthesize and characterize a novel anti-fouling and biocompatible polymer with reduced cell/bacterial adhesion properties.

2. Synthesize and characterize a novel anti-fouling and antibacterial polymer with reduced cell/bacterial adhesion and antibacterial properties.

3. Modify the surface of polyvinylchloride with these polymers.

4. Evaluate the cell and bacterial adhesion of the coated polyvinylchloride.

5. Evaluate the antibacterial activity of the coated polyvinylchloride.

Chapter 2 primarily describes the process of synthesis, characterization, immobilization and evaluation of the novel hydrophilic polymer with anti-fouling properties onto the surface of polyvinylchloride. Chapter 3 primarily describes the synthesis, characterization, immobilization and evaluation of the antibacterial and anti-fouling polymer onto the polyvinylchloride surface. Cell adhesion, bacterial adhesion and bacterial viability were examined.

CHAPTER 2. COATING POLYVINYLCHLORIDE SURFACE FOR IMPROVED ANTI-FOULING PROPERTY

2.1 Introduction

Anti-fouling surfaces are specifically crucial to cardiovascular applications. In this study, a polyvinylchloride (PVC) surface was modified by a coating a biocompatible and hydrophilic polymer by a mild coating technique. The PVC surface was first activated and then functionalized, followed by coating with the polymer. Results show that the coated hydrophilic polymer significantly reduced 3T3 fibroblast cell adhesion as well as bacteria adhesion. The 3T3 cell adhesion to the polymer-coated surface was reduced to 52-66% as compared to the original PVC surface. Bacterial adhesion to the polymer-coated surface was reduced to 61-80% for *Pseudomonas aeruginosa*, 65-81% for *Staphylococcus aureus*, and 73-85% for *Escherichia coli*, as compared to the original PVC surface. It appears that this novel polymer-coated PVC surface has an anti-fouling property.

2.2 Materials and Methods

2.2.1 Materials

N-vinylpyrrolidone (VP), acryloyl chloride, N-hydroxysuccinimide, triethylamine, 4-methoxyphenol, sodium carbonate, 2,2'-azobisisobutyronitrile (AIBN), sodium azide, tetrabutylammonium bromide, propargyl alcohol, copper sulfate, sodium ascorbate, 1,6-diisocyanatohexane, dibutyltin dilaurate, hexane, tetrahydrofuran, N,N'-dimethylformamide and diethyl ether were used as received from Sigma-Aldrich Co. (Milwaukee, WI) without further purifications. Polyvinylchloride (PVC) sheet (0.5 mm thick) was received from Interstate Plastics (Sacramento, CA).

2.2.2 Surface Coating

2.2.2.1 Synthesis of Functional Hydrophilic Polymer

Synthesis of N-acryloyl Succinimide. N-acryloyl succinimide (AS) was prepared similarly to our previous publication [53]. In short, triethylamine (0.1 mol) in tetrahydrofuran was slowly added to a solution containing N-hydroxysuccinimide (0.1 mol), acryloyl chloride (0.1 mol) and 4-methoxyphenol (0.1 mol% of acryloyl chloride) in tetrahydrofuran. The reaction was carried out at 24 °C for 24 h and then the white by-product triethylamine-hydrogen chloride was filtered. The final white solids (yield = 93%) were obtained by removing tetrahydrofuran using a rotary evaporator and drying *in vacuo*. The scheme for synthesis is shown in Fig. 2.1A.

Synthesis of Poly(VP-co-AS). To coat hydrophilic polyvinylpyrrolidone on PVC surface, we incorporated a reactive moiety AS onto the polymer chain by co-polymerization of VP with AS. Poly(VP-co-AS) or PVPA was prepared similarly to our published procedures [51]. Briefly, AIBN (1% by mole) in N,N'-dimethylformamide was added to a solution containing VP and AS at a molar ratio of 95 to 5 in N,N'-dimethylformamide. After the reaction was conducted under N_2 purging at 64 °C for 24 h, the polymer was precipitated with diethyl ether and then dried *in vacuo*. The scheme for synthesis is also shown in Fig. 2.1B. The PVPA polymers with different molar ratios of VP/AS (90/10 and 85/15) at the same AIBN, i.e., 1% (by mole) and different initiator concentrations (0.5% and 3%) at the same molar ratio (95/5) were prepared similarly to the procedures described above.

2.2.2.2 Surface Activation of PVC

PVC sheet was cut into disks 7 mm in diameter. Then disks were placed in a container with sodium azide (20%, w/v), tetrabutylammonium bromide (2%, w/v) and 10 mL distilled water with stirring. After running the reaction at 80 °C for 7 h, the disks (designated as AZ) were washed three times with distilled water,

followed by placing in a container with propargyl alcohol (16%), copper sulfate (2%), tetrabutylammonium bromide (1%), sodium ascorbate (0.5%) and distilled water (15 mL). The reaction was conducted at 50 °C for 3 h and then washed three times with distilled water, to form disks (designated as PA) having hydroxyl groups on the surface. The modified PVC disks were then placed in a container with 1,6-diisocyanatohexane (20%), dibutyltin dilaurate (1%) and hexane (10 mL) with stirring. After running the reaction at 40 °C for 1.5 h, the disks (designated as IC) were washed with hexane three times, followed by placing in a container with sodium carbonate and distilled water. After soaking at 24 °C overnight, the disks were washed three times with distilled water and then dried *in vacuo* before the next steps. The disks (designated AM) were finally modified with amino groups on the surface, as shown in Fig. 2.1C.

2.2.2.3 Surface Preparation with PVPA Polymers

10% (w/w) of PVPA polymer in distilled water was added to PBS (pH = 8.5) [53, 54]. Then the amine-modified PVC disks were added upon dissolution of the polymer. The reaction was conducted at 24 °C for 30 min, followed by washing the modified disks three times with distilled water before evaluation. The scheme for surface coating is shown in Fig. 2.1C.

2.2.3 Characterization

The synthesized polymer and surface-modified disks were characterized and evaluated with Fourier transform-infrared (FT-IR) spectroscopy. The functional groups of the modified PVC were identified by attenuated total reflectance FT-IR. FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR1000, Madison, WI). The viscosity values of the synthesized polymers were determined in DMF at 23 °C using a cone/plate viscometer (RVDV-II + CP, Brookfield Eng. Lab. Inc., Middleboro, MA). To characterize whether amino groups

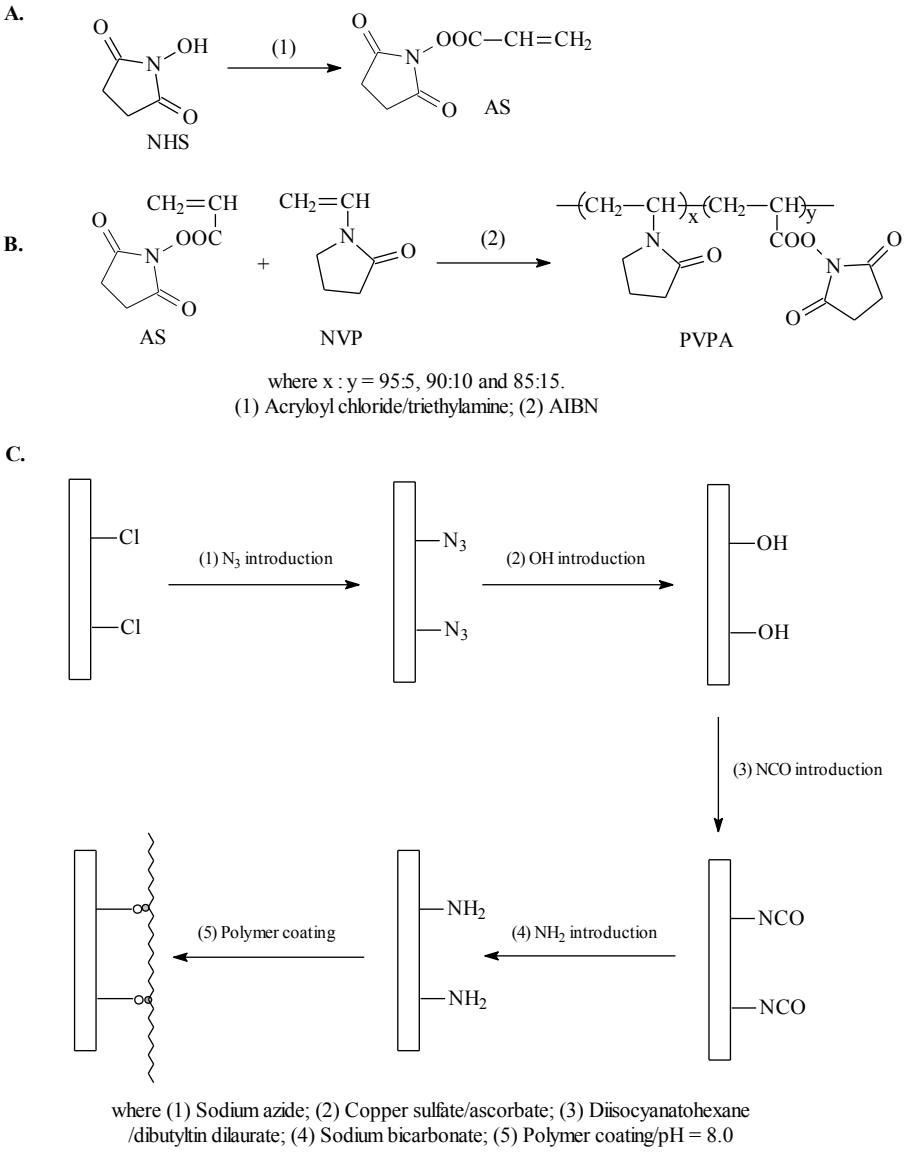


Fig. 2.1.: Schematic diagrams for synthesis of AS and PVPA and surface coating: (A) synthesis of AS; (B) synthesis of PVPA; (C) surface coating

and the synthesized polymers were attached onto the PVC surface, original and modified PVC disks were soaked in a fluorescent green/red (1:1 v/v) dye mixture (LIVE/DEAD BacLight bacterial viability kit L7007, Molecular Probes, Inc., Eugene, OR, USA) in the dark for 15 min, followed by imaging with an inverted fluorescence microscope (EVOS FL, AMG, Mill Creek, WA, USA).

2.2.4 Evaluation

2.2.4.1 Cell Adhesion Test

NIH-3T3 mouse fibroblasts were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, Lonza) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 5 mg/mL penicillin and 5 mg/mL streptomycin (Invitrogen Inc., Singapore). After maintaining at 37 °C under a humidified atmosphere of 5% CO_2 for 24 h, the cells were harvested from the culture flask by the addition of a 5.3 mM trypsin-EDTA (ThermoFisher Scientific) solution in PBS and centrifuged at 1200 rpm for 3 min, followed by removing trypsin and re-suspending the cell pellets in DMEM medium supplemented with 10% FBS to a density of 5×10^4 cells/mL. The formed cell suspension (1 mL) was then added to each well containing the disk specimen in a 24-well plate and cultured for 48 h, before the disk was washed with PBS to remove non-adherent cells. The cells attached to the disk were harvested by the addition of trypsin, followed by counting and imaging with an inverted microscope (Nikon Ti-E, Melville, NY). Triplicate samples were used to obtain a mean value for each material.

2.2.4.2 Bacterial Adhesion Test

The bacterial adhesion test was conducted following published procedures with slight modification [55]. Briefly, colonies of bacteria were suspended in 5 mL of tryptic soy broth, supplemented with 1% sucrose, to make a suspension with 10^8 CFU/mL of bacteria, and cultured for 24 h. Three bacterial species, *Pseudomonas aeruginosa,*

Escherichia coli and *Staphylococcus aureus*, were assessed. After washing with 70% ethanol for 10 s and sterile water three times, the disk specimen was incubated with bacteria in tryptic soy broth at 37 °C for 24 h under 5% CO_2. Then the disk was rinsed with sterile PBS and de-ionized water to remove non-adherent bacteria. The adhered bacteria were eluted from the surfaces by ultrasonic treatment in 1 ml sterile PBS for 10 min. Bacterial CFU was enumerated by agar plate counts. Data represent a mean value for each material based on triplicate samples.

2.2.4.3 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 $\alpha = 0.05$ was used for statistical significance.

2.3 Results and Discussion

2.3.1 Surface Coating and Characterization

Fig. 2.2 shows the FT-IR absorbance spectra data for PVPA polymer synthesis: (1) VP, (2) AS and (3) PVPA. Comparing all three spectra, appearance of the two small peaks at 1812 and 1782 for imide corresponds to 1805 and 1775 from AS, appearance of the peak at 3450 for amide and imide corresponds to 3565 from VP and 3590 from AS, appearance of the peak at 1703 for carbonyl group corresponds to 1713 from AS, appearance of the peak at 1664 for amide corresponds to 1654 on AS, and disappearance of the peak at 1640 for carbon-carbon double bond corresponds to 1628 from VP and 1638 from AS. These data confirmed the successful formation of PVPA.

Fig. 2.3 shows the FT-IR absorbance spectra for PVC and modified PVC surfaces: (1) PVC, (2) AZ, (3) PA, (4) IC, (5) AM, and (6) PVPA. In comparison with spectra

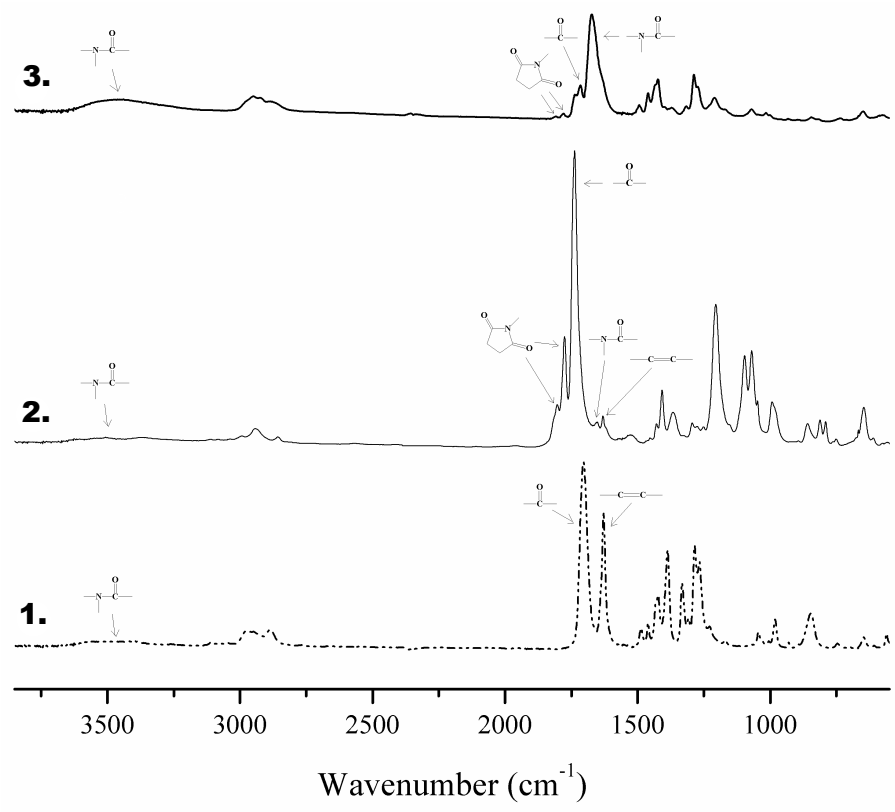


Fig. 2.2.: FT-IR spectra for PVPA synthesis: (1) VP; (2) AS; (3) PVPA

(1) and (2), a strong new peak formation at 2104 for azido group confirmed that azido groups were successfully attached onto the PVC surface by replacing some chlorine groups. In comparison with spectra (2) and (3), the azido peak disappears and a broad new peak between 3000 and 3700 appears, suggesting the hydroxyl group formation on the PVC surface. Regarding spectra (3) and (4), a strong new peak formation at 3340 and 1650 for urethane group and at 2261 for isocyanate group confirmed that isocyanate groups were successfully attached onto the PVC by the reaction between hydroxyl and isocyanate groups. Regarding (4) and (5), a broad peak formation at 3400 and isocyanate group disappearance at 2261 confirmed the formation of amino groups on the PVC surface. In the case of (5) and (6), it is hard to tell the difference between the spectra; however, the significant difference was identified by the following fluorescence image (Fig. 2.5) and cell and bacterial adhesion tests.

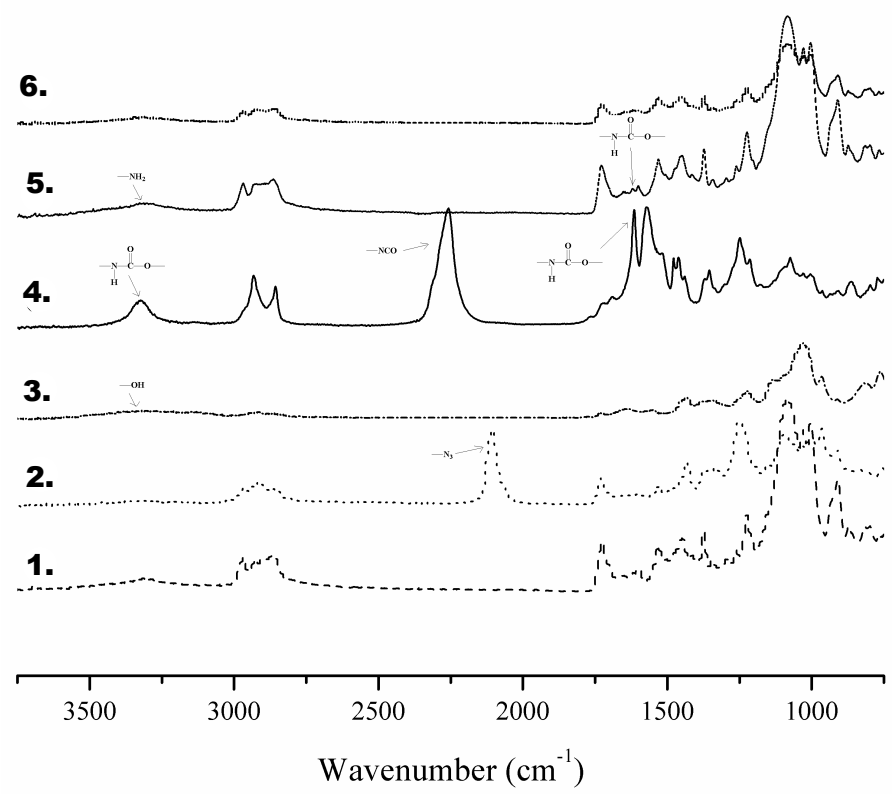


Fig. 2.3.: FT-IR spectra for PVC surface coating: (1) PVC; (2) AZ; (3) PA; (4) IC; (5) AM; (6) PVPA

Fig. 2.4 shows the viscosity of the synthesized PVPA polymers. It is known that viscosity of polymer can be correlated to molecular weight (MW) of polymer, and initiator concentration of polymerization affects MW of the formed polymer [56]. A lower initiator concentration can produce a polymer with higher MW and a higher viscosity value means a higher MW. PVPA1 showed the highest viscosity, followed by PVPA2, PVPA5, PVPA4 and PVPA3. PVPA5, PVPA4 and PVPA2 were synthesized using the same AIBN concentration (1%), whereas PVPA1 and PVPA3 were prepared using 0.5% and 3% AIBN, respectively. Furthermore, PVPA1, PVPA2 and PVPA3 had the same VP/AS molar ratio (95/5). It is known that higher the viscosity of the polymer, higher the molecular weight it has [56]. Therefore, it is plausible that the polymer PVPA1 with the lowest initiator concentration showed the highest viscosity

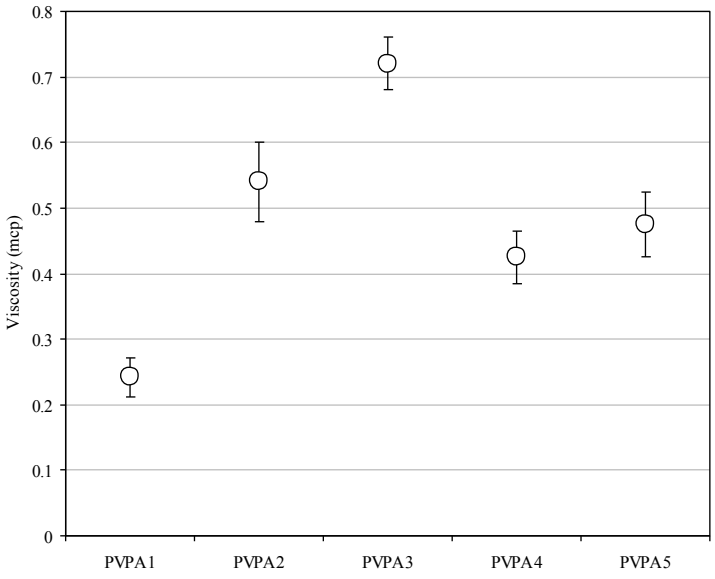


Fig. 2.4.: Viscosity of the synthesized PVPA polymers

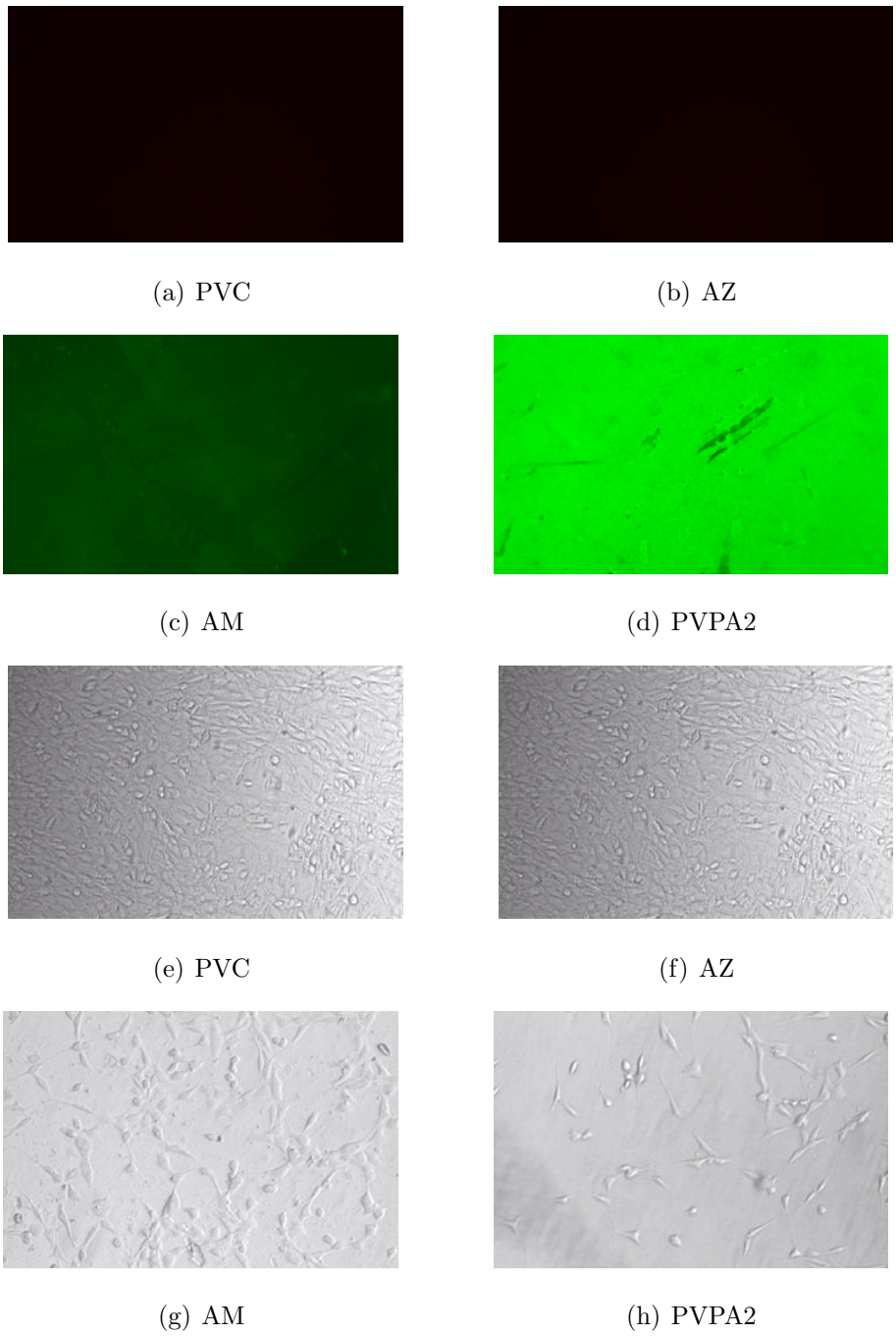
or highest MW, followed by PVPA2 and PVPA3. The polymers PVPA2, PVPA4 and PVPA5 were close to each other in their viscosities, because they were polymerized under the same AIBN concentration.

2.3.2 Evaluation

Surface modification is very critical to polymer-based medical devices such as cardiovascular grafts, catheters and others, especially when they are used for the areas in association with body fluid or blood [2, 57]. The devices being used in these applications require minimum microbial adhesion and low cell attachment [2, 13]. To achieve this goal, we propose to modify the surface using a newly synthesized hydrophilic and biocompatible polymer containing N-vinylpyrrolidone residue. It is well known that polyvinylpyrrolidone polymer (PVP) is a very hydrophilic biocompatible polymer and has been used as a blood compatible polymer and a blood substitute for many years [43, 44]. In this study, we used a very efficient and mild coating technique, i.e., using N-acryloyl succinimide (AS) which is pendent on PVPA to covalently link

PVPA onto the amino-containing PVC surface in the presence of water at pH = 8.0 [53, 54]. This technique has been widely used in protein coupling reaction in biology and biomedical applications [58]. The reaction was accomplished between amino groups on the PVC surface and acryloyl succinimide groups on the PVPA polymer by forming amide linkages, concurrent with losing N-hydroxysuccinimide in water. Fig. 2.5 (a-d) shows a set of optical images to demonstrate that both amino groups and PVPA-coated polymers have been successfully attached onto the PVC surface. The images in Fig. 2.5 (a-d) represent PVC and AZ-, AM- and PVPA-modified surfaces after staining with a green/red two-color dye kit. It is known that the fluorescent green dye emits the green color based on the reaction between dye molecules and amino groups [59]. Since both PVC and AZ had no amino groups attached, the expected black color is observed (Fig. 2.5a and Fig. 2.5b). On the other hand, both AM- and PVPA-modified surface showed fluorescent green color because both contain amino groups (Fig. 2.5c and Fig. 2.5d). It is noticed that the surface attached with PVPA showed a significant white color coating on the green surface, indicating successful completion of polymer coating.

Fig. 2.6 shows the effect of the hydrophilic PVPA polymers on cell surface adhesion by 3T3 mouse fibroblasts. The cell adhesion was in the decreasing order of PVC > AZ > AM > PVPA5 > PVPA4 > PVPA3 > PVPA2 > PVPA1 > PA, where there were no statistically significant differences between PVPA1 and PVPA2 and among PVPA3, PVPA4 and PVPA5 ($p > 0.05$). It is known that hydrophobic surfaces have higher affinity to proteins, cells and even bacteria [1, 57]. PVC is a very hydrophobic or biofouling material. The modified AZ and AM showed 14% and 39% cell adhesion reduction, respectively, as compared to original PVC, probably due to significant decreased hydrophobility. Azido functionality is known for its polarity whereas amino group is hydrophilic. By looking at the results for the surfaces modified with the PVPA polymers, an even more dramatic change is observed. The reduction in cell adhesion is observed from 52 to 66% with different polymers. Although it is not significant, there seems to be a trend on the synthesized polymers: (1) the



(a) PVC (b) AZ

(c) AM (d) PVPA2

(e) PVC (f) AZ

(g) AM (h) PVPA2

Fig. 2.5.: Optical photomicrographs of PVC and its modified surfaces after staining with fluorescent dye kit (LIVE/DEAD BacLight) (a to d) and cell images of 3T3 mouse fibroblasts after incubating with original and modified PVC surfaces (e to h)

polymer synthesized with a lower initiator concentration showed a lower adhesion, which may be explained by molecular weight of the polymers. High molecular weight of the polymer means longer polymer chain. Longer hydrophilic polymer chains may exert steric repulsion effects for enhanced cell exclusion [60]; (2) the more PVP on the surface the lower the cell adhesion, e.g., PVPA2 < PVPA5 < PVPA6 in cell adhesion; (3) AM also showed lower cell adhesion than original PVC, due to its hydrophilic amino group. Surprisingly PA showed the lowest cell adhesion with 82% reduction as compared to original PVC. This may be attributed to the well distributed hydroxyl groups on the surface.

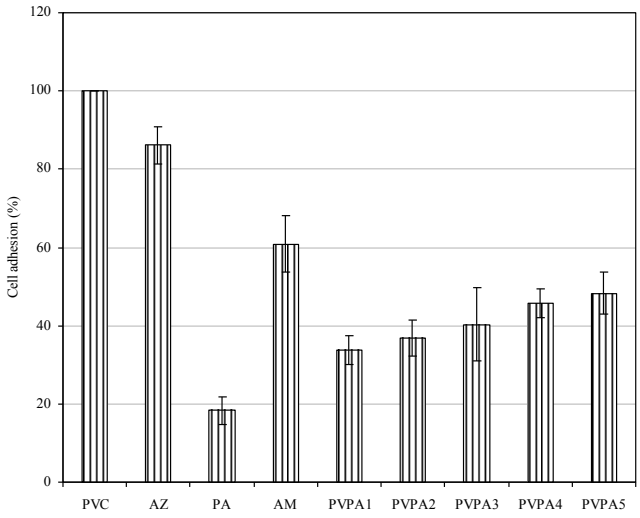
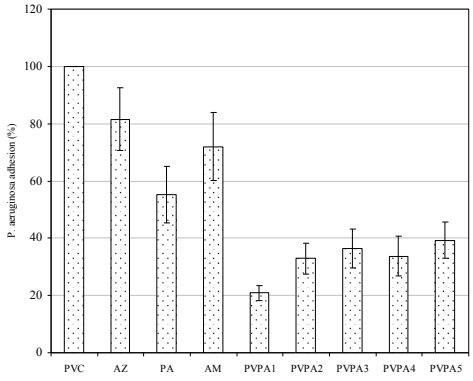
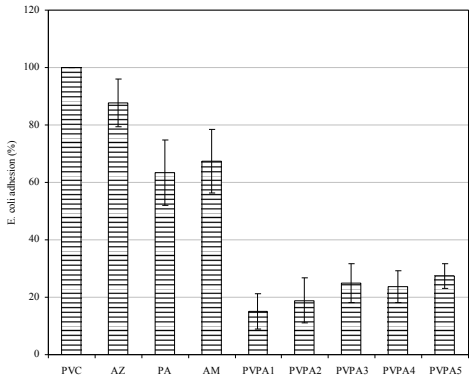


Fig. 2.6.: 3T3 mouse fibroblast adhesion on original and modified PVC surfaces with different coatings

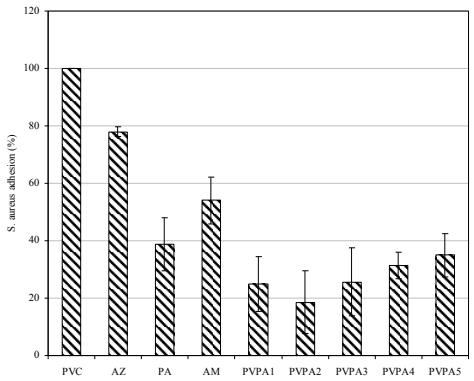
Fig. 2.5 (e-f) also show a set of photomicrographs of original PVC and modified PVC surfaces with 3T3 mouse fibroblasts adhesion, corresponding to the result from Fig. 2.6. The images from Fig. 2.5 e to h represent PVC, AZ, PA, AM and PVPA2. Apparently, the image of original PVC is almost full of cells among which many have an elongated and spindle shape (Fig. 2.5e). With both AZ and AM, the reduced cell numbers are observed with the latter being more significant (Fig. 2.5f and 2.5g).



(a) *P. aeruginosa*



(b) *E. coli*



(c) *S. aureus*

Fig. 2.7.: Bacterial adhesion on original and modified PVC surfaces with different coatings

For the PVPA-coated PVC, adhered cell numbers were more reduced with only a few attached 3T3 fibroblasts observed on the image (Fig. 2.5h). The results were pretty consistent with those cell adhesion number values shown in Fig. 2.6.

Fig. 2.7 shows the effect of the PVPA polymers on surface bacterial adhesion. *P. aeruginosa, E. coli*, and *S. aureus* were investigated, as shown in Fig. 2.7 a, b and c, respectively. Bacterial adhesion exhibited a pattern similar to that of 3T3 fibroblast adhesion shown in Fig. 2.6. After 24 h incubation with bacteria, original PVC and its modified surfaces were evaluated. From Fig. 2.7, if we take original PVC as 100% bacterial adhesion, the bacterial adhesion was in the decreasing order of (1) *P. aeruginosa*: PVC > AZ > AM > PA > PVPA5 > PVPA3 > PVPA4 = PVPA2 > PVPA1, where there were no statistically significant differences among PVPA2, PVPA3, PVPA4 and PVPA5 ($p > 0.05$); (2) *E. coli*: PVC > AZ > AM > PA > PVPA5 > PVPA3 > PVPA4 > PVPA2 > PVPA1, with no statistically significant differences between PA and AM as well as PVPA2 and PVPA1, and among PVPA3, PVPA4 and PVPA5; (3) *S. aureus*: PVC > AZ > AM > PA > PVPA5 > PVPA4 > PVPA3 = PVPA1 > PVPA2, where there were no statistically significant differences among PA, PVPA4 and PVPA5, PVPA1, PVPA2 and PVPA3, as well as PVPA1, PVPA3 and PVPA4. *S. aureus* showed bacterial adhesion reduction of 22% for AZ, 61% for PA, 46% for AM, and 65 to 81% for the PVPA-coated surfaces. *P. aeruginosa* showed adhesion reduction of 18% for AZ, 45% for PA, 28% for AM, and 61 to 80% for the PVPA-coated surfaces. *E. coli* showed adhesion reduction of 12% for AZ, 37% for PA, 33% for AM, and 73 to 85% for the PVPA-coated surfaces. It seems that PVPA affected *E. coli* slightly more than *S. aureus* and *P. aeruginosa*. PVC is a highly hydrophobic polymer so it showed the highest bacterial adhesion. The azido-modified PVC (AZ) showed reduced bacterial adhesion, indicating that the azido group is more hydrophilic than original PVC probably due to high polarity of azido group. After the azido was converted to hydroxyl group, the bacterial adhesion was reduced much more due to the hydrophilic nature of the hydroxyl group. When hydroxyl groups were converted to amino groups, bacterial adhesion appeared to

be increased. This can be attributed to the introduction of hydrophobic six-carbon chain due to incorporation of 1,6-diisocyanatohexane, a necessary coupling agent, to allow PVPA to attach onto the PVC surface. Regarding the PVPA-modified surfaces, the bacterial adhesion was further reduced. All the polymer-coated surfaces showed significantly reduced bacterial adhesion, with PVPA1 which has the highest viscosity or highest MW exhibiting the lowest adhesion. The results shown in this study exhibit a new route to prepare the PVC surface with anti-fouling property.

2.4 Conclusions

In this study we have successfully modified the surface of polyvinylchloride by using a novel efficient and mild surface coating technique. The hydrophilic polymer-coated PVC surfaces significantly reduced 3T3 fibroblast cell adhesion as well as adhesion of three bacterial species. The 3T3 cell adhesion to the polymer-coated surface was reduced to 52-66% as compared to the original PVC. Bacterial adhesion to the polymer-coated surface was reduced 61-80% for *P. aeruginosa*, 65-81% for *S. aureus*, and 73-85% for *E. coli*, as compared to original PVC. Molecular weight of the coated polymer slightly enhances anti-fouling property. The more hydrophilic the polymer is on the PVC surface, the lower the cell adhesion will be. Future work will focus on further improvement of anti-fouling property and antibacterial property and quantitative characterization of the coated polymer on surface.

CHAPTER 3. A MODIFIED POLYVINYLCHLORIDE SURFACE WITH ANTIBACTERIAL AND ANTI-FOULING FUNCTIONS

3.1 Introduction

Surfaces with antibacterial and hydrophilic properties are very attractive to cardiovascular applications. The objective of this study was to synthesize and immobilize a novel antibacterial and hydrophilic polymer onto surface of polyvinylchloride via an effective and mild surface coating technique. The surface coated with a terpolymer constructed with N-vinylpyrrolidone, 3,4-Dichloro-5-hydroxy-2(5H)-furanone derivative and succinimide residue was evaluated with cell adhesion, bacterial adhesion and bacterial viability. 3T3 mouse fibroblast cells and two bacterial species were used to evaluate surface adhesion and antibacterial activity. Results showed that the polymer-modified polyvinylchloride surface exhibited not only significantly decreased 3T3 fibroblast cell adhesion with a 66-87% reduction but also significantly decreased bacterial adhesion with 69-87% and 52-74% reduction of *Pseudomonas aeruginosa* and *Staphylococcus aureus* attachment, respectively, as compared to original polyvinylchloride. Furthermore, the modified polyvinylchloride surfaces exhibited significant antibacterial functions by inhibiting bacterial growth (75-84% and 78-94% inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, as compared to original polyvinylchloride) and killing bacteria. These results demonstrate that covalent polymer attachment conferred anti-fouling and antibacterial properties to the polyvinylchloride surface.

3.2 Materials and Methods

3.2.1 Materials

Acryloyl chloride, N-hydroxysuccinimide, triethylamine, 4-methoxyphenol, 2-hydroxyethyl acrylate, 3,4-dichloro-5-hydroxy-2(5H)-furanone, p-toluenesulfonic acid, toluene, 4-methoxyphenol, sodium azide, tetrabutylammonium bromide, 1,6-diisocyanatohexane, propargyl alcohol, dibutyltin dilaurate, 2,2'-azobisisobutyronitrile, N-vinylpyrrolidone (NVP), poly(ethyleneimine) (PEI), tetrahydrofuran, dimethylformamide, diethyl ether, copper sulfate, sodium ascorbate, sodium chloride, anhydrous magnesium sulfate and sodium bicarbonate were used as received from Sigma-Aldrich Co. (Milwaukee, WI) without further purifications. Polyvinylchloride (PVC) sheet (0.5 mm thick) was received from Interstate Plastics (Sacramento, CA).

3.2.2 Surface Modification

3.2.2.1 Synthesis of Functional Antibacterial Hydrophilic Polymer

Synthesis of functional antibacterial hydrophilic polymer was carried out in three steps, i.e., synthesis of N-succinimidyl acrylate (SA), synthesis of 5-acryloylethyleneglycol-3,4-dichloro-2(5H)-furanone (ADCF) and synthesis of poly(NVP-ADCF-SA) or PVDCS. (1) Synthesis of SA: SA was synthesized similarly to our previous publication [53]. In short, acryloyl chloride (0.1 mol) was slowly added to a solution containing N-hydroxysuccinimide (0.1 mol), triethylamine (0.1 mol), 4-methoxyphenol (0.1 mol% of triethylamine) and tetrahydrofuran. The reaction was conducted at 23 ^{o}C for 24 h and the by-product triethylamine-hydrogen chloride was filtered. The product, white solid, was recovered after removing tetrahydrofuran with a rotary evaporator and drying *in vacuo*. (2) Synthesis of ADCF: ADCF was prepared based on the published protocol in principle with some modification [51]. Briefly, a mixture of 3,4-dichloro-5-hydroxy-2(5H)-furanone (0.1 mol), 2-hydroxyethyl acrylate (0.12 mol), 4-methoxyphenol (0.1 mol %), toluene and p-toluenesulfonic acid (2

mol %) was refluxed at 100-110 °C for 3-4 h. After toluene was removed via the rotary evaporator, the recovered crude product ADCF was dissolved in diethyl ether, washed with saturated sodium bicarbonate solution, brine and distilled water, and dried with anhydrous magnesium sulfate, followed by removing solvent by the rotary evaporator. (3) Synthesis of PVDCS: PVDCS was polymerized similarly to our published procedures [51]. Briefly, 2,2'-azobisisobutyronitrile (1% by mole) was added to a solution containing N-vinylpyrrolidone, ADCF and SA at a molar ratio of 87/2/8, 82/10/8, 77/15/8 or 72/20/8 in N,N'-dimethylformamide. After the reaction was carried out under a N_2 blanket at 64 °C for 24 h, the PVDCS polymer was purified with diethyl ether and dried *in vacuo*. The scheme for synthesis is shown in Fig. 3.1A.

3.2.2.2 Surface Modification of Polyvinylchloride

Polyvinylchloride (PVC) sheet was cut into 7-mm diameter disks. Then disks were placed in a container with sodium azide (20%, w/v), tetrabutylammonium bromide (2% w/v) and 10 ml distilled water with stirring [41]. After running the reaction at 80 °C for 7 h, the disks were washed three times with distilled water (formation of PVC with azido groups: $PVCN_3$), followed by placing them in a container with propargyl alcohol (16%), copper sulfate (2%), tetrabutylammonium bromide (1%), sodium ascorbate (0.5%) and distilled water (15 ml). The reaction was conducted at 50 °C for 3 h and then the disks were washed three times with distilled water, resulting in the disks having hydroxyl groups on the surfaces (formation of PVC with hydroxyl groups: PVCPA). The modified PVC disks were then placed in a container with 1,6-diisocyanatohexane (20%), dibutyltin dilaurate (1%) and hexane (10 ml) with stirring. After running the reaction at 40 °C for 1.5 h, the disks were washed three times with hexane (formation of PVC with isocyanate group: PVCNCO), followed by placing them in a container with 5% PEI solution. After coating at 23 °C overnight, the disks were washed three times with distilled water (formation of PVC coated with PEI

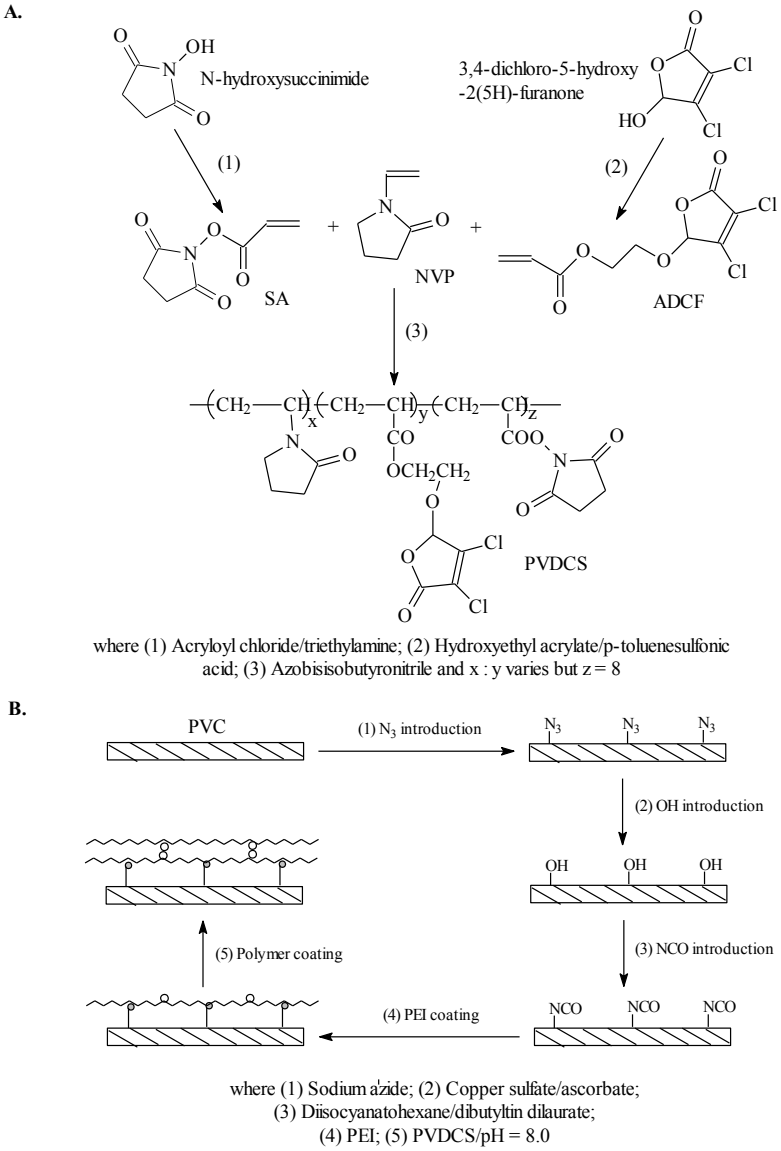


Fig. 3.1.: Schematic diagrams for synthesis of PVDCS and surface modification: (A) synthesis of PVDCS; (B) surface modification

having amino groups on the surface: PVCPEI) and then dried in an oven. Finally the antibacterial and hydrophilic PVDCS polymer was coated onto the PVCPEI surface. Briefly, 10% (wt/wt) of the synthesized PVDCS in distilled water was added to a solution containing buffer (pH = 8.5) and acetone (1:1 v/v) [54]. Then the amine-modified PVC disks were added upon dissolution of the polymer. The reaction was conducted at 24 °C for 30 min, followed by washing the modified disks three times with distilled water before evaluation. The scheme for modification is shown in Fig. 3.1B.

3.2.3 Characterization

The synthesized polymer and surface-modified disks were characterized and evaluated with Fourier transform-infrared (FT-IR) spectroscopy. The surface functional groups of the modified PVC were characterized with attenuated total reflectance FT-IR. FT-IR spectra were acquired on a FT-IR spectrometer (Mattson Research Series FT/IR1000, Madison, WI).

3.2.4 Evaluation

3.2.4.1 Cell Adhesion Test

NIH-3T3 mouse fibroblasts were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, Lonza) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 5 mg/ml penicillin and 5 mg/ml streptomycin (Invitrogen Inc., Singapore). After maintaining at 37 °C under a humidified atmosphere of 5% CO_2 for 24 h, the cells were harvested from the culture flask by the addition of a 5.3 mM trypsin-EDTA (ThermoFisher Scientific) solution in PBS and centrifuged at 1200 rpm for 3 min, followed by removing trypsin and re-suspending the cell pellets in DMEM medium supplemented with 10% FBS to a density of 5×10^4 cells/mL. The formed cell suspension (1 mL) was then added to each well containing the disk specimen in a

24-well plate and cultured for 48 h, before the disk was washed with PBS to remove non-adherent cells. The cells attached to the disk were harvested by the addition of trypsin, followed by counting and imaging with an inverted microscope (Nikon Ti-E, Melville, NY). Triplicate samples were used to obtain a mean value for each material.

3.2.4.2 Bacterial Adhesion Test

The bacterial adhesion test was conducted following the published procedures with slight modification [55]. In short, colonies of bacteria were suspended in 5 mL of tryptic soy broth, supplemented with 1% sucrose, to form a suspension with 10^8 CFU/mL of bacteria and cultured for 24 h. *P. aeruginosa* and *S. aureus* were assessed. After washing with 70% ethanol for 10 s and sterile water three times, the disk specimen was incubated with bacteria in tryptic soy broth at 37 °C for 24 h under 5% CO_2. Then the disk was rinsed with sterile PBS and de-ionized water to remove non-adherent bacteria. The adhered bacteria were eluted from the surfaces by ultrasonic treatment in 1 ml sterile PBS for 10 min. Bacterial CFU was enumerated by agar plate counts. Data represent a mean value for each material based on triplicate samples.

3.2.4.3 Bacterial Viability Test

The bacterial viability test was carried out based on the protocol elsewhere [61]. In short, bacterial colonies were suspended in 5 mL of tryptic soy broth, supplemented with 1% sucrose, to form a suspension with 10^8 CFU/mL of bacteria and incubated for 24 h. Both *P. aeruginosa* and *S. aureus* were assessed. The disk specimen was sterilized with 70% ethanol for 10 s and incubated with the bacterial suspension in tryptic soy broth at 37 °C for 48 h under 5% CO_2. 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., Eugene, OR, USA) was added, followed by vortexing for 10 s, sonicating for 10 s, vortexing for another 10 s and

keeping in dark for about 15 min before analysis. Then 20 μL of the stained bacterial suspension was added onto a glass slide and viable bacteria (green) were imaged with an inverted fluorescence microscope (EVOS FL, AMG, Mill Creek, WA, USA). A bacteria suspension without disks was used as control and viable bacteria counts form the suspension were used as 100%. The viability was analyzed by counting from the recorded images. Triplicate samples were used to obtain a mean value for each material.

3.2.4.4 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 $\alpha = 0.05$ was used for statistical significance.

3.3 Results and Discussion

3.3.1 Characterization

Fig. 3.2 shows a set of FT-IR spectra for SA (a), ADCF (b), NVP (c) and PVDCS (d). In comparison with all the four spectra, the peaks around 1620-1655 for C=C group disappear in spectrum d, which corresponds to those at 1652 and 1629 for SA from spectrum a, 1639 for ADCF from spectrum b as well as 1629 for NVP from spectrum c. A broader and stronger peak at 3200 for amide group appears in spectrum d, which corresponds to that for NVP from spectrum c. Two small peaks at 1805 and 1778 for succinimidyl group (amide I) appear in spectrum d, which corresponds to the peaks at 1805 and 1776 for SA from spectrum a. A small peak at 750 for C-Cl group appears in spectrum d, which corresponds to that for ADCF from spectrum b. These changes confirmed the PVDCS formation.

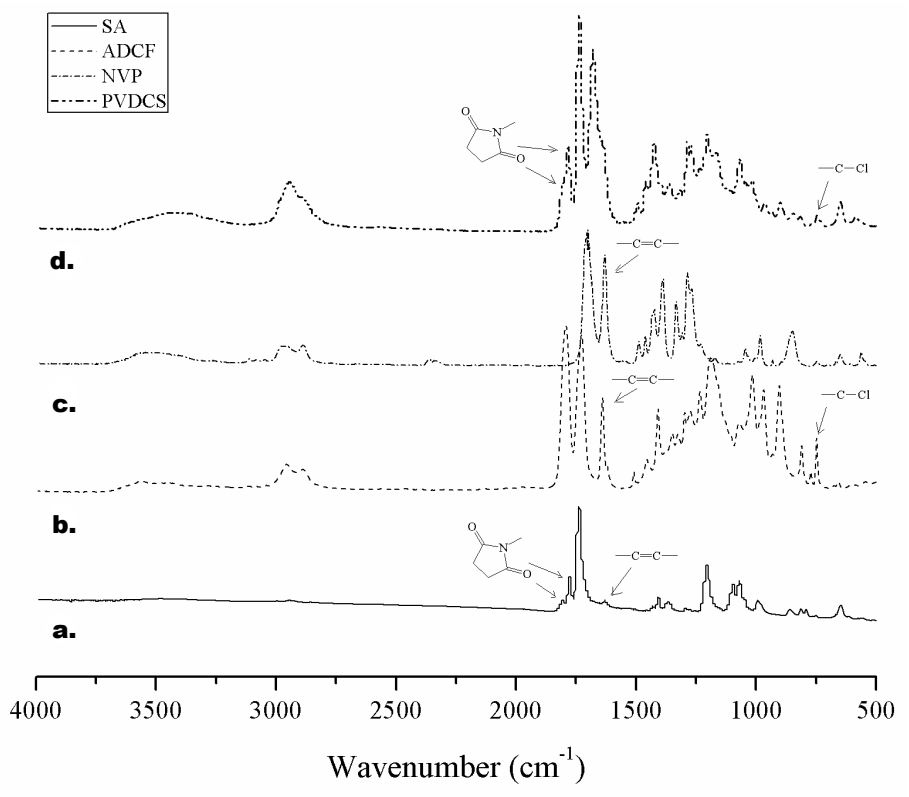


Fig. 3.2.: FT-IR spectra for synthesized PVDCS: (a) SA; (b) ACDF; (c) NVP; (d) PVDCS

Fig. 3.3 shows a set of FT-IR spectra for PVC (a), $PVCN_3$ (b), PVCPA (c), PVCNCO (d) and PVCPEI (e). In comparison with spectra a and b, the appearance of a strong new peak at 2104 for azido group confirmed that azido groups were successfully attached onto the PVC surface by replacing some chlorine groups. By comparing spectra b and c, the azido peak disappeared and a broad new peak appeared between 3000 and 3700, indicating the hydroxyl group formation on the PVC surface. In comparison with spectra c and d, the appearance of new peaks at 3340 and 1650 for urethane group and at 2261 for isocyanate group confirmed that isocyanate groups were successfully attached onto the PVC surface by the reaction between hydroxyl and isocyanate groups. In comparison with d and e, appearance of

a broad peak at 3400 and disappearance of isocyanate group at 2261 confirmed the successful coating of PEI on the PVC surface.

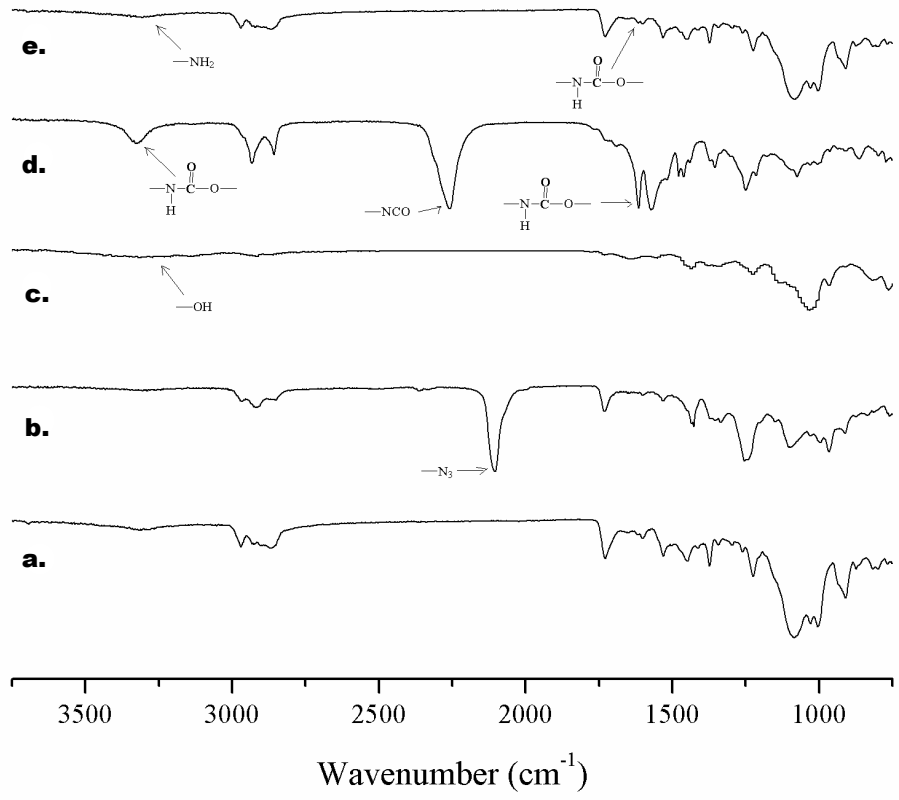


Fig. 3.3.: FT-IR spectra for PVC surface modification: (a) PVC (b) $PVCN_3$; (c) PVCPA; (d) PVCNCO; (e) PVCPEI

3.3.2 Evaluation

The medical devices being used in cardiovascular applications require minimum microbial adhesion and low cell attachment [2, 13, 57]. To achieve this, we proposed to coat the surface by using a newly prepared polymer containing both hydrophilic and antibacterial moieties, which not only can prevent mammalian cell adhesion but also reduce or prevent bacteria from infection. We applied a very simple and effective coupling technique that has been broadly applied for protein coupling,

i.e., coupling carboxyl with primary amino groups in water at pH = 8.0 with N-hydroxysuccinimide [53, 54].

It is well known that medical device-associated microbial infections are the most popular problems for the implantation. These infections are associated with almost each type of medical device. The examples include but not limited to catheters, vascular grafts and ureteral stents. The research concepts on killing or inhibiting bacteria by touch or simple contact has attracted a special attention recently [45–49]. The quaternary ammonium salts and their derivatives, due to their potent antimicrobial functions, have been studied extensively and used for a number of biomedical and pharmaceutical applications [45–48]. On one hand, these materials have shown capability of inhibiting and/or killing those bacteria that demonstrate resistance to cationic antibacterial compounds [13]. On the other hand, however, these potent compounds have also shown some weakness while interacting with proteins such as human saliva. It was reported that oral saliva can significantly and negatively affect the antibacterial activity of these compounds. It has been attributed to electrostatic interactions between these quaternary ammonium salts and proteins in saliva [49, 50]. Another type of new antimicrobial compounds, furanone-containing molecules, have been reported to show a broad spectrum of biological and physiological properties including but not limited to antibiotic, antitumor, haemorrhagic and insecticidal activities [43, 44, 62] although their biological mechanisms are still under investigation [63]. Our previous studies using the 3,4-dichloro-5-hydroxy-2(5H)-furanone-containing polymer-composed dental composites have been found effective in inhibiting the growth of the oral bacterium *Streptococcus mutans* [51, 52] To explore the application of 3,4-dichloro-5-hydroxy-2(5H)-furanone in surface modification research, we proposed to introduce 3,4-dichloro-5-hydroxy-2(5H)-furanone through a polymerizable molecule 2-hydroxyethyl methacrylate via a covalent bond linkage into the hydrophilic PVDCS, covalently link the PVDCS to the activated PVC surface, and investigate the effect of the attached polymer on anti-fouling and antibacterial functions of the modified surface. The discussion below

demonstrates how the attached PVDCS polymers affected the surface antibacterial and cell adhesion functions.

Fig. 3.4 shows the effect of the PVDCS polymers on cell surface adhesion by 3T3 mouse fibroblasts. The cell adhesion was in the decreasing order of PVC > $PVCN_3$ > PVCPA > PEI > PVDCS72208 > PVDCS77158 > PVDCS82108 > PVDCS8758 ($p < 0.05$). It is known that a hydrophobic surface has higher affinity to proteins, cells and even bacteria [1,57]. PVC is a very hydrophobic or biofouling material. The modified $PVCN_3$, PVCPA and PVCPEI showed significantly reduced cell adhesion (24%, 40% and 55% reduction, respectively, as compared to original PVC), probably due to significantly decreased hydrophobicity. Azido group is known for its polarity. Both hydroxyl groups on PVCPA and amino groups on PVCPEI are hydrophilic. The surfaces modified with the antibacterial and hydrophilic polymers exhibit a further significant decrease in adhesion: PVDCS72208, PVDCS77158, PVDCS82108 and PVDCS8758 exhibited 66%, 70%, 80% and 87% cell adhesion reduction, respectively. The individual components of PVDCS each possess qualities contributing to overall functionality. NVP is very hydrophilic monomer and its formed polymers have been used for blood substitutes for years due to their excellent blood-compatibility [43,44]. ADCF exhibits antimicrobial and antitumor functions [62]. SA has been used for coupling amino groups with carboxyl groups in protein chemistry [58]. PVDCS8758 represents a molar ratio of 87/5/8 for NVP/ADCF/SA, which contains the highest ratio of NVP (hydrophilic component) and lowest ratio of ACDF (antibacterial component) whereas PVDCS72208 contains the lowest hydrophilic component but the highest antibacterial component. Apparently the more NVP on the surface the lower the surface adhesion of the 3T3 cells.

Fig. 3.5 shows the effect of the PVDCS polymers on surface bacterial adhesion. Two bacterial species were investigated. Bacterial adhesion exhibited a pattern similar to that of 3T3 fibroblast adhesion, as shown in Fig. 3.4. After 24 h incubation with bacteria, PVC and its modified surfaces were evaluated, considering adhesion to PVC as 100%. We found that bacteria attached to the disks in the

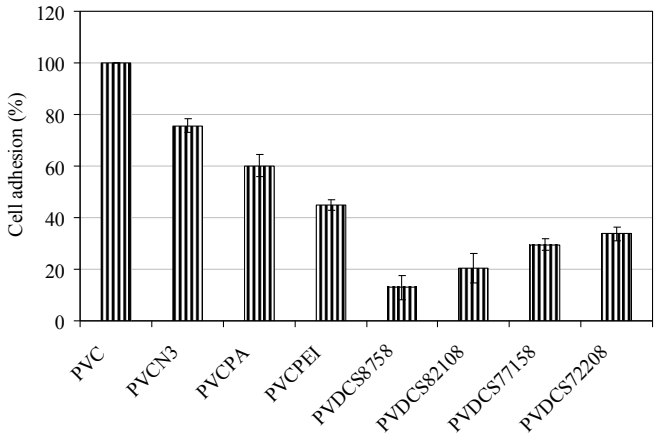


Fig. 3.4.: 3T3 mouse fibroblast adhesion on PVC and surface-modified PVC with different polymer coatings

following decreasing order: PVC > $PVCN_3$ > PVCPA > PVCPEI > PVDCS72208 > PVDCS77158 > PVDCS82108 > PVDCS8758. The modified surfaces showed a significant bacterial adhesion reduction of 21%, 42%, 57%, 87%, 80%, 73% and 69% with *P. aeruginosa* and 16%, 32%, 45%, 74%, 67%, 60% and 52% with *S. aureus* for $PVCN_3$, PVCPA, PVCPEI, PVDCS8758, PVDCS82108, PVDCS77158 and PVDCS77208, respectively, as compared to original PVC. In addition, *S. aureus* showed higher adhesion than *P. aeruginosa*. Again, PVC is a highly hydrophobic polymer and that is likely why it showed the highest bacterial adhesion. The azido-modified PVC showed reduced bacterial adhesion, indicating that the azido group is more hydrophilic than PVC. After the azido group was converted to hydroxyl group and then amino group, the bacterial adhesion was further reduced due to hydrophilic nature of both hydroxyl and amino groups. The PVDCS-modified PVC displayed further reduced bacterial adhesion. Similar to the results shown in Fig. 3.4, the one with the highest content of NVP (PVDCS8758) showed the lowest bacterial adhesion but the one with highest ADCF showed the highest bacterial adhesion, although the adhesion value was still significantly lower than for PVC, $PVCN_3$, PVCPA and PVCPEI.

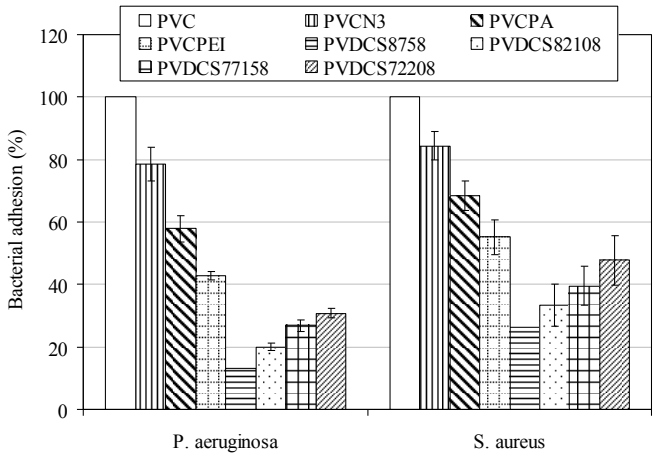


Fig. 3.5.: Bacterial adhesion on PVC and surface-modified PVC with different polymer coatings: (1) *P. aeruginosa* and (2) *S. aureus*

Fig. 3.6 shows the effect of the PVDCS polymers on viability of two bacterial species in the supernatant above the disks. Bacterial viability in the presence of the disk was found in the following decreasing order: PVC > $PVCN_3$ > PVCPA > PVCPEI > PVDCS8758 > PVDCS82108 > PVDCS77158 > PVDCS72208. *S. aureus* showed lower viability than *P. aeruginosa*. Although $PVCN_3$, PVCPA and PVCPEI did not contain any antibacterial residues, they still showed significantly decreased *P. aeruginosa* viability with reduction of 24%, 62% and 65% for $PVCN_3$, PVCPA and PVCPEI and *S. aureus* viability with reduction of 23%, 42% and 55% for $PVCN_3$, PVCPA and PVCPEI, as compared to original PVC. The result suggests that $PVCN_3$, PVCPA and PVCPEI have some type of bacterial inhibition capability. It was reported that $PVCN_3$ showed bacterial inhibition activity [41]. The amine-containing polymers such as polyimine and polylysine were also reported to have antibacterial function [64, 65]. The antibacterial activity exhibited by PVCPA can be attributed to the triazole moieties produced from the reaction between acetylene groups from propargyl alcohol and azido groups on $PVCN_3$. The triazole moieties have been found to have an antimicrobial activity and are incorporated to many pharmaceutical formulations nowadays [66]. By comparing with $PVCN_3$, PVCPA

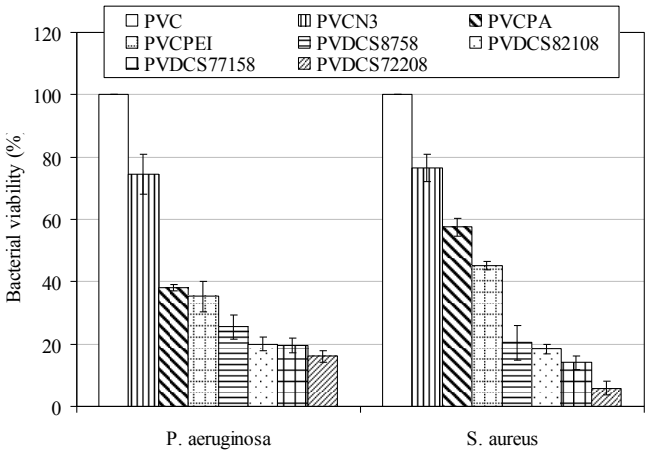
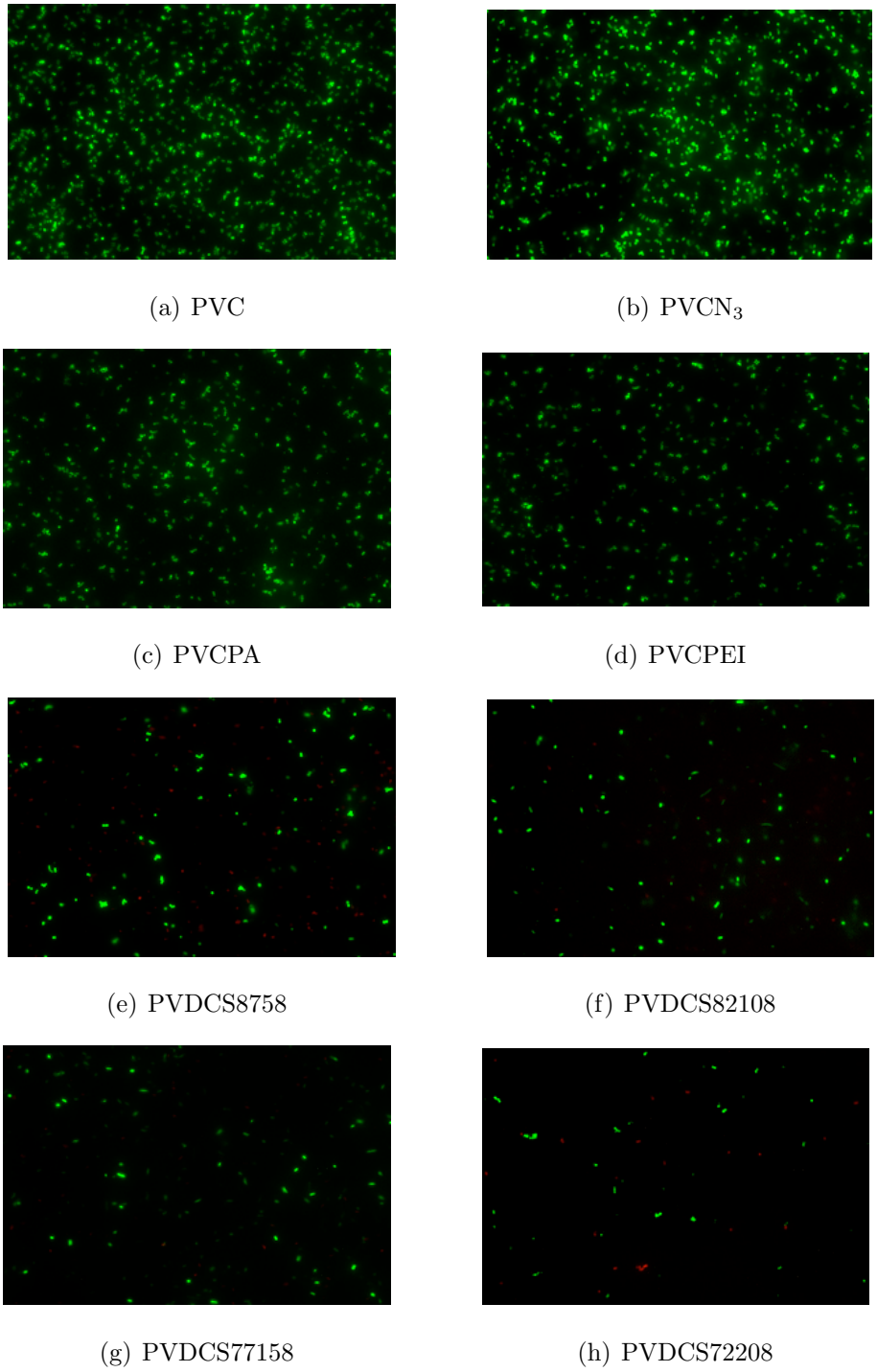


Fig. 3.6.: Bacterial viability after incubating with PVC and its surface-modified PVC with different polymer coatings: (1) *P. aeruginosa* and (2) *S. aureus*

and PVCPEI, the surfaces modified with our antibacterial and hydrophilic polymers exhibited a dramatic viability reduction. *P. aeruginosa* and *S. aureus* displayed reduction values of 75% and 80% for PVDCS8758, 80% and 78% for PVDCS82108, 81% and 86% for PVDCS77158, and 84% and 94% for PVDCS72208, respectively, as compared to original PVC. The result is plausible because the more antibacterial component on the polymer or on the PVC surface, the lower the viability or higher bacterial inhibition is observed. It was also noticed that *S. aureus* seems more vulnerable to the PVDCS polymer than *P. aeruginosa*. These results suggest that the polymer-coated surfaces can kill bacteria by contact.

Fig. 3.7 shows a set of photo-images of *S. aureus* viability after incubating with original PVC and modified PVC disks. The images from Fig. 3.7a to Fig. 3.7h represent (a) PVC, (b) $PVCN_3$, (c) PVCPA, (d) PVCPEI, (e) PVDCS8758, (f) PVDCS82108, (g) PVDCS77158 and (h) PVDBS72208. It is clear that original PVC showed the highest numbers of bacteria (green dots), followed by $PVCN_3$, PVCPA, PVCPEI, PVDCS8758, PVDCS82108, PVDCS77158 and PVDCS72208. Nearly no red bacteria (dead cells) are observed from Fig. 3.7a to Fig. 3.7d for PVC, $PVCN_3$, PVCPA and PVCPEI. On the other hand, however, red bacteria (dead cells) are



(a) PVC

(b) $PVCN_3$

(c) PVCPA

(d) PVCPEI

(e) PVDCS8758

(f) PVDCS82108

(g) PVDCS77158

(h) PVDCS72208

Fig. 3.7.: Images of *S. aureus* after incubating with PVC and its surface-modified PVC disks

observed from Fig. 3.7e to Fig. 3.7h. The images of PVDCS72208 showed only a few living bacteria cells (green) but more dead cells (red). The results are plausible and can be explained below. Because $PVCN_3$, PVCPA and PVCPEI did not contain any antibacterial substances on the surfaces, they only inhibited bacterial growth but did not actively kill bacteria. With the antibacterial and hydrophilic polymer-coated PVC, however, not only bacteria growth were inhibited but also bacteria were actively killed, which led to significantly reduced living bacteria numbers and increased dead bacteria. Furthermore, increasing antibacterial component ADCF on polymers further decreased the living bacteria and increased the dead bacteria. Combining the results from Figs. 3.4, 3.5, 3.6 and 3.7, the PVDCS polymer-coated PVC surfaces demonstrated an attractive anti-fouling property with significantly decreased mammalian cell and bacterial adhesion. Meanwhile, the polymer-coated surfaces also exhibited the capability of not only inhibiting bacterial growth but also killing bacteria, which would enhance antimicrobial activity of PVC and may also reduce the risk to bacterial infection due to insufficient sterilization.

3.4 Conclusions

We have successfully synthesized a novel anti-fouling and antibacterial polymer and immobilized the polymer onto hydrophobic surface of polyvinylchloride. The modified surface not only exhibited significantly reduced cell adhesion with a 66-87% decrease to 3T3 fibroblast but also showed significantly decreased bacterial attachment with 69-87% and 52-74% decrease to *P. aeruginosa* and *S. aureus*, respectively, as compared to original PVC. Furthermore, the polymer-modified PVC surface demonstrated significant antibacterial functions by inhibiting bacteria growth with reduction of 75 to 84% to *P. aeruginosa* and 78 to 94% to *S. aureus*, as compared to original PVC and killing bacteria as well. These results hold much promise in preventing medical device-related infections or complications. Future studies will focus on optimization of the polymers as well as preparation protocols.

CHAPTER 4. CONCLUSIONS

We have successfully modified the surface of polyvinylchloride by using an efficient and mild surface coating technique with polymers that either have only anti-fouling properties or have both anti-fouling and antibacterial properties. The coated polyvinylchloride with anti-fouling polymers showed significant reduction of 3T3 fibroblast cell adhesion as well as bacterial adhesion. The 3T3 cell adhesion was reduced to 52-66% whereas bacterial adhesion of three different species was reduced to 61-85% as compared to the original polyvinylchloride surface. The surface coated with terpolymer constructed with N-vinylpyrrolidone, 3,4-Dichloro-5-hydroxy-2(5H)-furanone derivative and succinimide residue showed a 66-87% reduction of 3T3 fibroblast cell adhesion, 69-87% and 52-74% reduction of *Pseudomonas aeruginosa* and *Staphylococcus aureus* adhesion, respectively, as compared to polyvinylchloride. This coating also showed significant antibacterial functions by inhibiting 75-84% *Pseudomonas aeruginosa* growth and 78-94% *Staphylococcus aureus* growth, as compared to original polyvinylchloride. The results of the two studies demonstrate that the polymer coating by covalent attachment conferred bacterial prevention properties to the polyvinylchloride surface that could reduce bacterial infections.

LIST OF REFERENCES

LIST OF REFERENCES

[1] B. Ratner, A. Hoffman, F. Schoen, and J. Lemons, *Biomaterials Science: An Introduction to Materials in Medicine*, 3rd ed. CA: San Diego: Elsevier Academic Press, 2012.

[2] L. Shedden, K. Oldroyd, and P. Connolly, "Current issues in coronary stent technology," *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, vol. 223, no. 5, pp. 515–524, 2009.

[3] G. Donelli, "Vascular catheter-related infection and sepsis," *Surgical Infections*, vol. 7, no. 2, pp. S25–27, 2006.

[4] X. Cheng, H. Canavan, D. Graham, D. Castner, and B. Ratner, "Temperature dependent activity and structure of adsorbed proteins on plasma polymerized n-isopropyl acrylamide," *Biointerphases*, vol. 1, no. 1, p. 6172, 2006.

[5] D. Huber, R. Manginell, M. Samara, B.-I. Kim, and B. Bunker, "Programmed adsorption and release of proteins in a microfluidic device," *Science*, vol. 301, no. 5631, pp. 352–354, 2003.

[6] Q. Yu, Y. Zhang, H. Chen, Z. Wu, H. Huang, and C. Cheng, "Protein adsorption on poly(n-isopropylacrylamide)-modified silicon surfaces: Effects of grafted layer thickness and protein size," *Colloids and Surfaces B: Biointerfaces*, vol. 76, no. 2, pp. 468–474, 2010.

[7] L. Chen, M. Liu, H. Bai, P. Chen, F. Xia, D. Han, and L. Jiang, "Antiplatelet and thermally responsive poly(n-isopropylacrylamide) surface with nanoscale topography," *Journal of the American Chemical Society*, vol. 131, no. 30, p. 1046710472, 2009.

[8] K. Uchida, K. Sakai, E. Ito, O. H. Kwon, A. Kikuchi, M. Yamato, and T. Okanob, "Temperature-dependent modulation of blood platelet movement and morphology on poly(n-isopropylacrylamide)-grafted surfaces," *Biomaterials*, vol. 21, no. 9, pp. 923–929, 2000.

[9] C. Alarcn, T. Farhan, V. L. Osborne, W. Huck, and C. Alexander, "Temperature-dependent modulation of blood platelet movement and morphology on poly(n-isopropylacrylamide)-grafted surfaces," *Journal of Materials Chemistry*, vol. 15, pp. 2089–2094, 2005.

[10] D. Cunliffe, C. Alarcn, V. Peters, J. R. Smith, and C. Alexander, "Thermoresponsive surface-grafted poly(nisopropylacrylamide) copolymers: effect of phase transitions on protein and bacterial attachment," *Langmuir*, vol. 19, no. 7, pp. 2888–2899, 2003.

[11] D. Tan, Z. Li, X. Yao, C. Xiang, H. Tan, , and Q. Fu, "The influence of fluorocarbon chain and phosphorylcholine on the improvement of hemocompatibility: a comparative study in polyurethanes," *Journal of Materials Chemistry B*, vol. 2, pp. 1344–1353, 2014.

[12] I. You, S. M. Kang, Y. Byun, and H. Lee, "Enhancement of blood compatibility of poly(urethane) substrates by mussel-inspired adhesive heparin coating," *Bioconjugate Chemistry*, vol. 22, no. 7, p. 12641269, 2011.

[13] T. Garrett, ManmohanBhakoo, and Z. Zhang, "Bacterial adhesion and biofilms on surfaces," *Progress in Natural Science*, vol. 18, no. 9, pp. 1049–1056, 2008.

[14] J. Jiang, Y. Fu, Q. Zhang, X. Zhan, and F. Chen, "Novel amphiphilic poly(dimethylsiloxane) based polyurethane networks tethered with carboxybetaine and their combined antibacterial and anti-adhesive property," *Applied Surface Science*, vol. 412, pp. 1–9, 2017.

[15] Z. Ren, G. Chen, Z. Wei, L. Sang, and M. Qi, "Hemocompatibility evaluation of polyurethane film with surfacegrafted poly(ethylene glycol) and carboxymethylchitosan," *Journal of Applied Polymer Science*, vol. 127, no. 1, pp. 308–315, 2011.

[16] K. Sask, L. Berry, A. Chan, and J. Brash, "Modification of polyurethane surface with an antithrombinheparin complex for blood contact: Influence of molecular weight of polyethylene oxide used as a linker/spacer," *Langmuir*, vol. 28, no. 4, pp. 2099–2106, 2012.

[17] Y. Wang, W. Xu, and Y. Chen, "Modification of polyurethane surface with an antithrombinheparin complex for blood contact: Influence of molecular weight of polyethylene oxide used as a linker/spacer," *Colloids and Surfaces B: Biointerfaces*, vol. 81, no. 2, p. 1046710472, 2010.

[18] M. Tan, Y. Feng, , H. Wang, L. Zhang, M. Khan, J. Guo, Q. Chen, and J. Liu, "Immobilized bioactive agents onto polyurethane surface with heparin and phosphorylcholine group," *Macromolecular Research*, vol. 21, no. 5, p. 541549, 2013.

[19] Y. Chung, J. W. Choi, J. Lee, and B. Chun, "The control of molecular interactions between polyurethane copolymers by grafted malic acid and its impact on polymer characteristics," *Journal of Applied Polymer Science*, vol. 126, no. S2, pp. E225–E232, 2012.

[20] Y. Lu, L. Shen, F. Gong, J. Cui, J. Rao, J. Chen, and W. Yang, "Polycarbonate urethane films modified by heparin to enhance hemocompatibility and endothelialization," *Polymer International*, vol. 61, no. 9, pp. 1433–1438, 2012.

[21] D. Banoriya, R. Purohit, and R. Dwivedi, "Advanced application of polymer based biomaterials," *Materials Today: Proceedings*, vol. 4, no. 2, pp. 3534–3541, 2017.

[22] I. Francolini, C. Vuotto, A. Piozzi, and G. Donelli, "anti-fouling and antimicrobial biomaterials: an overview," *Journal of Pathology, Microbiology and Immunology*, vol. 125, no. 4, pp. 392–417, 2017.

[23] I. Banerjee, R. Pangule, and R. Kane, "anti-fouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms," *Advanced Materials*, vol. 23, no. 6, pp. 690–718, 2011.

[24] L. Upadhyaya, X. Qian, and S. Wickramasinghe, "Chemical modification of membrane surface overview," *Current Opinion in Chemical Engineering*, vol. 20, pp. 13–18, 2018.

[25] J. Ayyavoo, T. Nguyen, B.-M. Jun, In-ChulKim, and Y.-N. Kwon, "Protection of polymeric membranes with anti-fouling surfacing via surface modifications," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 506, pp. 190–201, 2016.

[26] V. Kochkodan, D. Johnson, and N. Hilala, "Polymeric membranes: Surface modification for minimizing (bio)colloidal fouling," *Advances in Colloid and Interface Science*, vol. 206, pp. 116–140, 2014.

[27] H. Kaczmarek, J. Kowalonek, A. Szalla, and A. Sionkowska, "Surface modification of thin polymeric films by air-plasma or uv-irradiation," *Surface Science*, vol. 507-510, pp. 883–888, 2002.

[28] S. M. Hosseini, S. S. Madaeni, A. R. Khodabakhshi, and A. Zendehnam, "Preparation and surface modification of pvc/sbr heterogeneous cation exchange membrane with silver nanoparticles by plasma treatment," *Journal of Membrane Science*, vol. 365, no. 1-2, pp. 438–446, 2010.

[29] K. Bazaka, M. Jacob, R. Crawford, and E. Ivanova, "Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment," *Acta Biomaterialia*, vol. 7, no. 5, pp. 2015–2028, 2011.

[30] C. Mao, W. Zhao, A. Zhu, J. Shen, and S. Lin, ". a photochemical method for the surface modification of poly(vinyl chloride) with o-butyrylchitosan to improve blood compatibility," *Process Biochemistry*, vol. 39, no. 9, pp. 1151–1157, 2004.

[31] H. Melndez-Ortiz, C. Alvarez-Lorenzo, A. Concheiro, V. Jimnez-Pez, and E. Bucio, "Modification of medical grade pvc with n-vinylimidazole to obtain bactericidal surface," *Radiation Physics and Chemistry119*, vol. 119, pp. 37–43, 2016.

[32] S.Moulay, "Chemical modification of poly(vinyl chloride)still on the run," *Progress in Polymer Science*, vol. 35, no. 3, pp. 303–331, 2010.

[33] C. McCoy, N. Irwin, J. Hardy, S. Kennedy, L. Donnelly, J. Cowley, G. Andrews, and S. Pentlavalli, "Systematic optimization of poly(vinyl chloride) surface modification with an aromatic thiol," *European Polymer Journal*, vol. 97, pp. 40–48, 2017.

[34] J. Lafarge, N. Kbir, D. Schapman, and F. Burela, "Design of self-disinfecting pvc surfaces using the click chemistry," *Reactive and Functional Polymers*, vol. 73, pp. 1464–1472, 2013.

[35] N. James and A. Jayakrishnan, "Surface thiocyanation of plasticized poly(vinyl chloride) and its effect on bacterial adhesion," *Biomaterials*, vol. 24, no. 13, pp. 2205–2212, 2003.

[36] A. Jayakrishnan and M. Sunny, "Phase transfer catalysed surface modification of plasticized poly(vinyl chloride) in aqueous media to retard plasticizer migration," *Polymer*, vol. 37, no. 23, pp. 5213–5218, 1996.

[37] J. Sacristn, H. Reinecke, and C.Mijangos, "Surface modification of pvc films in solventnon-solvent mixtures," *Polymer*, vol. 41Polymer, no. 15, pp. 5577–5582, 2000.

[38] S. Bigot, G. Louarn, N. Kbir, and F. Burel, "Facile grafting of bioactive cellulose derivatives onto pvc surfaces," *Applied Surface Science*, vol. 283, pp. 411–416, 2013.

[39] M. Herrero, R. Navarro, Y. Grohens, H. Reinecke, and C. Mijangos, "Controlled wet-chemical modification and bacterial adhesion on pvc-surfaces," *Polymer Degradation and Stability*, vol. 91, no. 9, pp. 1915–1918, 2006.

[40] J. Allan, L. Prest, and E. Easton, "The sulfonation of polyvinyl chloride: Synthesis and characterization for proton conducting membrane applications," *Journal of Membrane Science*, vol. 489, pp. 175–182, 2015.

[41] S. Lakshmi, S. Kumar, and A. Jayakrishnan, "Bacterial adhesion onto azidated poly(vinyl chloride) surfaces," *Journal of Biomedical Materials Research*, vol. 61, no. 1, pp. 26–32, 2002.

[42] M. Takeishi, Y. Naito, and M. Okawara, "Surfactant effects on heterogeneous polymer reaction: nucleophilic substitution of poly(vinyl chloride) in water," *Macromolecular Materials and Engineering*, vol. 28, no. 1, pp. 111–119, 1973.

[43] E. Moffitt, "Blood substitutes," *Canadian Anaesthetists' Society Journal*, vol. 22, no. 1, pp. 12–19, 1975.

[44] M. Teodorescu and M. Bercea, "Poly(vinylpyrrolidone) a versatile polymer for biomedical and beyond medical applications," *Polymer-Plastics Technology and Engineering*, vol. 54, no. 9, pp. 923–943, 2015.

[45] S. Imazato, R. Russell, and J. McCabe, "Antibacterial activity of mdpb polymer incorporated in dental resin," *Journal of Dentistry*, vol. 23, no. 3, pp. 177–181, 1995.

[46] B. Gottenbos, H. van der Meia, F. Klatter, P. Nieuwenhuis, and H. Busscher, "In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber," *Biomaterials*, vol. 23, no. 6, pp. 1417–1423, 2002.

[47] H. Murata, R. Koepsel, K. Matyjaszewski, and A. Russell, "Permanent, non-leaching antibacterial surfaces2: how high density cationic surfaces kill bacterial cells," *Biomaterials*, vol. 28, no. 32, pp. 4870–4879, 2007.

[48] D. Xie, Y. Weng, X. Guo, J. Zhao, R. Gregory, and C. Zheng, "Preparation and evaluation of a novel glass-ionomer cement with antibacterial functions," *Dental Materials*, vol. 27, no. 5, pp. 487–496, 2011.

[49] N. Ebi, S. Imazato, Y. Noiri, and S. Ebisu, "Inhibitory effects of resin composite containing bactericide-immobilized filler on plaque accumulation," *Dental Materials*, vol. 6, no. 17, pp. 485–491, 2001.

[50] S. Imazato, N. Ebi, Y. Takahashi, T. Kaneko, S. Ebisu, and R. Russell, "Antibacterial activity of bactericide-immobilized filler for resin-based restoratives." *Biomaterials*, vol. 24, no. 20, pp. 3605–3609, 2003.

[51] Y. Weng, L. Howard, V. Chong, J. Sun, R. Gregory, and D. Xie, "A novel furanone-modified antibacterial dental glass ionomer cement," *Acta Biomaterialia*, vol. 8, no. 8, pp. 3153–3160, 2012.

[52] Y. Weng, L. Howard, X. Guo, V. Chong, R. Gregory, and D. Xie, "A novel antibacterial resin composite for improved dental restoratives," *Journal of Materials Science: Materials in Medicine*, vol. 23, no. 6, pp. 1553–1561, 2012.

[53] D. Xie, C. Smyth, C. Eckstein, G. Bilbao, J. Mays, D. Eckhoff, and J. Contreras, "Cytoprotection of peg-modified adult porcine pancreatic islets for improved xenotransplantation," *Biomaterials*, vol. 26, no. 4, pp. 403–412, 2005.

[54] D. Xie, "Surface coatings for biological implants and prostheses," US Patent 9 550 011B2, 2017.

[55] H. Yuan, B. Qian, H. Chen, and M. Lan, "The influence of conditioning film on anti-fouling properties of the polyurethane film modified by chondroitin sulfate in urine," *Applied Surface Science*, vol. 426, pp. 587–596, 2017.

[56] P. Painter and M. Coleman, *Fundamentals of Polymer Science*, 2nd ed. FL; Boca Raton: CRC Press, 1997.

[57] S. Guelcher and J. Hollinger., *An Introduction to Biomaterials*. FL; Boca Raton: CRC Press, 2006.

[58] G. Mattson, E. Conklin, S. Desai, G. Nielander, M. D. Savage, and S. Morgensen, "A practical approach to crosslinking," *Molecular Biology Reports*, vol. 17, no. 3, p. 167183, 1993.

[59] A. Kenworthy, "Imaging protein-protein interactions using fluorescence resonance energy transfer microscopy," *Methods*, vol. 24, no. 3, pp. 289–296, 2001.

[60] M. Amiji and K. Park, "Surface modification of polymeric materials with poly(ethylene oxide), albumin, and heparin for reduced thrombogenicity," *Journal of Biomaterials Science, Polymer Edition*, vol. 4, no. 3, pp. 217–234, 1993.

[61] Y. mi Kim, S. Farrah, and R. H. Baney, "Membrane damage of bacteria by silanols treatment," *Electronic Journal of Biotechnology*, vol. 10, no. 2, p. 252259, 2007.

[62] E. Lattmann, S. Dunn, S. Niamsanit, and N. Sattayasai, "Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5h)-furanones," *Bioorganic & Medicinal Chemistry Letters*, vol. 15, no. 4, pp. 919–921, 2005.

[63] C. Freij-Larsson, P. Jannasch, and B. Wessln, "Polyurethane surface modified by amphilic polymers: effect on protein adsorption," *Biomaterials*, vol. 21, no. 3, pp. 307–315, 2000.

[64] K. Gibney, I. Sovadinova, A. Lopez, M. Urban, Z. Ridgway, G. Caputo, and K. Kuroda, "Poly(ethylene imine)s as antimicrobial agents with selective activity," *Macromolecular Bioscience*, vol. 12, no. 9, pp. 1279–1289, 2012.

[65] S. Shima, H. Matsuoka, T. Iwamoto, and H. Sakai, "Antimicrobial action of epsilon-poly-l-lysine. j. antibiot," *The Journal of Antibiotics*, vol. 37, no. 11, pp. 1449–1455, 1984.

[66] P. Zoumpoulakis, C. Camoutsis, G. Pairas, M. Sokovi, J. Glamolija, C. Potamitis, and A. Pitsas, "A. synthesis of novel sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles, as potential antibacterial and antifungal agents, biological evaluation and conformational analysis studies," *Bioorganic & Medicinal Chemistry*, vol. 20, no. 4, pp. 1569–1583, 2012.