LAYER-BY-LAYER POLYPHOSPHAZENE COATINGS FOR BIOMEDICAL APPLICATIONS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Layer-by-Layer Polyphosphazene Coatings for Biomedical Applications

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This work explores antibiotic-containing coatings constructed utilizing polyphosphazenes (PPz) and designed to release antibiotics only in the presence of bacteria. Specifically, polymer coatings of anionic PPzs with trifluoroethoxy as fluorinated groups and carboxylatophenoxy or sulfophenoxy as ionic groups were directly assembled with cationic antibiotics and compared. The effect of charge density, binding strength, and polymer fluorination degree on several film properties including growth, swelling, hydrophobicity, stability/pH response, mechanical adhesion, and antibacterial efficacy was determined. All PPzs enabled electrostatic layer-by-layer deposition with a wide range of cationic antibiotics, including Polymyxin B, Colistin, Gentamicin, and Neomycin. Sulfo-containing PPzs coatings exhibited more linear growth than carboxy-containing PPzs, while all types of PPz antibiotic coatings exhibited strong mechanical adhesion of the coating to the silicon wafer substrate. Wettability and swelling ratio of coatings in pH 7.5 PBS at 37 °C were shown to be dependent on the degree of fluorination and binding strength. The two types of coatings were designed to have drastically different response to pH lowering, which is usually associated with *E. coli* bacteria, with carboxy-containing PPzs being pH responsive and

sulfo-containing PPzs resistant to pH change. Bacterial performance is dependent on the type of PPz but can prevented high colonization with bacteria with concentrations up to 10^7 CFU per cm^2. Altogether, these findings show how the properties of coatings can be controlled by the composition of PPzs, which can lead to creating biocompatible self-defensive antibacterial coatings.

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NOMENCLATURE

PPz	Polyphosphazene
CA	Contact Angle
PB	Phosphate Buffer
PBS	Phosphate Buffered Saline
BPEI	Branched Polyethyleneimine
LbL	Layer-by-Layer Deposition
PCPP	Poly[di(carboxylatophenoxy)phosphazene]
FP77	Poly[(carboxylatophenoxy)(trifluoroethoxy)phosphazene]
FP20	Poly[(carboxylatophenoxy)(trifluoroethoxy)phosphazene]
FPS	Poly[(sulfophenoxy)(ethylphenoxy)phosphazene]
Poly B	Polymyxin B
Gent	Gentamicin
Neo	Neomycin
Col	Colistin

CHAPTER I

INTRODUCTION

1.1 Antimicrobial Coatings

Bacterial colonization of surfaces has always been a hindrance to the biomedical and material's community. Issues such as medical device failure, prolong patient recovery time, and infection is onset by these bacterial colonies [1]. Antimicrobial surfaces became highly desirable and researched to combat biofilm formation. Antimicrobial surfaces are material that contains an antimicrobial agent that could inhibit the ability of microorganisms to form colonies (biofilm) *via* two main mechanisms, repelling or killing [2]. A repelling antimicrobial surface utilizes techniques such as steric repulsion, electrostatic repulsion, or low surface energy to force bacterial away from the surface [2]. A killing antimicrobial surface utilizes biocide releasing or killing by biocide contact [3]. These bacteria will be killed if it comes near or contacts the coating and therefore prevent any surface colonization. This research will be focus on antimicrobial surfaces that can prevent biofilm via bacteria killing. Antibacterial properties are usually imparted to surfaces by deposition of antimicrobial coatings. Antimicrobial coatings have been an enormous topic in current research, and it has shown to be effective in numerous applications [4].

1.2 Importance of Preventing Biofilm

Biofilm formation is a multi-industrial problem that has caused issues such as medical device failure, food poisoning, and infections on living tissue [5]. Biofilm is the formation of a community of bacteria on a material surface [6]. This community of bacteria, once adhered to a surface, will produce an extracellular matrix to protect itself. This biofilm matrix is the main issue as the bacteria in the biofilm are highly resistant to antibiotics, unlike the planktonic bacteria [6].

Prevention of the formation of biofilm has become the utmost priority as it has caused a loss of billions of dollars in the healthcare sector [5]. Also, this community of bacteria has caused a rise in morbidity and mortality [5]. Biofilm can be produced from both gram-negative, gram-positive bacterial and can be found in living tissues, medical implants, food processing machinery, and hydrophilic environments [6].

1.3 Objective of Project

In this project, antimicrobial coatings are prepared via direct assembly of polymers and antibiotics. We explore how nature of charged groups and hydrophobicity of polymers affects a broad ranges of film properties such as growth, surface tension, wettability, adhesion to a substrate, and bacterial efficacy. The characterization of the coatings will be analyzed with ellipsometry, contact angle, optical microscopy, mass spectrometry, and a biofilm prevention test. A growth curve will be obtained from a measurement of thickness to determine the rate of growth per bilayer. Surface tension, swelling, adhesion to a substrate, and antibiotic content will provide insight into the properties of the film. Lastly, these coatings will be exposed to matching bacterial associated with the antibiotic it is grown with to show how effective they are. The ultimate goals are (a) to develop a antibiotic coating that is effective at preventing biofilm formation without eluting antibiotics under normal conditions and (b) to understand the role of type of PPz coating with antibiotics in response of these coatings to bacterial colonization.

1.4 Polyphosphazenes (PPzs)

Polyphosphazenes (PPzs) are a class of polymer that consists of an inorganic phosphorous-nitrogen backbone with two side groups on each repeat unit [7]. This polymer is tunable in many aspects such as glass transitional temperature (T_g), degradation rate, surface wettability, and tensile strength [7]. These polymers have been used as flame retardants, biomedical scaffold material,

drug and gene delivery transporters [8]. The side groups on the polymer have a significant influence on polymer properties, including hydrophobicity, biocompatibility, and degradability [9]. PPzs have been used in a wide diversity of applications but have recently been emerged as promising biomedical materials. PPzs are bioinert and demonstrated good performance in subcutaneous applications [8]. Polyphosphazenes have been shown to form layer-by-layer films with cationic polymers and antibiotics, and these coatings are also biocompatible and thus highly suitable for life science applications [10].

1.5 Subclasses of PPzs

PPzs have tunable side groups and depending on chemistry of the functional groups, can exhibit different properties. These side groups have effects on physical and chemical properties and can ultimately determine the function of the coating. One common application for PPzs is in biomedical materials. These side groups make PPz unique and allow for direct alteration of the polymer properties by adjusting the synthesis process [6]. It has been shown, for example, that through adjusting the side group chemistry and its density in a polymer chain, PPzs can impart hydrophobicity to surface coatings [10]. The focus of this research is explore and compare the physicochemical and antibacterial properties of the coatings made from carboxylate- or sulfo-containing fluorinated PPz with antibiotics.

1.6 Layer-by-Layer Deposition

Layer-by-Layer (LbL) deposition is a thin film fabrication technique that allows for a formation of a multilayer coating of controlled thickness [11]. This technique results in coatings with controlled composition, thickness, and architecture [11]. LbL is used in developing nanoelectronics, optics, surface coatings, and controlled drug delivery [12]. LbL can be accomplished *via* dipping or spraying while utilizing electrostatic interactions or hydrogen

bonding as its binding force [11]. Solution dipping usually enables a denser and less rough films than those produced by spraying [11], and thus was chosen here as the multilayer fabrication method using anionic fluorinated PPzs and cationic antibiotics.

1.7 PPz Coatings

Polyphosphazene coatings have recently been shown to have a significant potential in life science applications [10]. Recently PPzs were assembled with a cationic polymer, branched polyethyleneimine (BPEI). This is significant as not all polymer can enable LbL and form multi-layer films [10]. A carboxy variant of PPz with fluorinated side groups have also been proven to be biocompatible [10]. Coatings constructed with PPzs with carboxylic side groups have been previously shown to be effective against a high concentration of gram-negative and gram-positive bacteria [13]. The effectiveness was attributed to the coating pH responsiveness, i.e. to the ability of the coating to release antibiotics in response to local pH lowering caused by bacterial presence. However, these coatings partially lost antibiotics upon post-assembly exposure to normal physiological conditions (PBS, pH 7.5) [13]. [14]. Leaching of antibiotics during normal condition can potentially increase bacterial resistance. Therefore, other variants of PPzs which stronger bind cationic antibiotics, such as sulfonated PPzs, were explored in this work.

1.8 Polyphosphazene and Antibiotic Coatings

LbL films studied in this work were constructed based on the ability of anionic PPzs to electrostatically interact with cationic antibiotics [10]. Using two types of PPzs and several types of cationic antibiotics, many LbL types were constructed. PPz . The properties of both PPz and antibiotics can influence release mechanism, antimicrobial properties, and ultimately determine how the coating will function in its specific application [10]. It is the purpose of this research to determine these characteristics of the PPz and antibiotic coatings and determine the correlations

between chemistry the side groups of the PPz and physicochemical and antibacterial characteristics of the coatings.

CHAPTER II

METHODS

2.1 Polymer and Antibiotic Solutions

Polyphosphazenes were synthesized by Dr. Alexander Andrianov's research group at the University of Maryland. The types of PPzs used in this project are PCPP, FP77, FP20, and FPS. All PPz are initially in powder/solid form and the Mw are as followed, Poly[di(carboxylatophenoxy)phosphazene] (PCPP) (800kDa), Poly[di(carboxylatophenoxy)(trifluoroethoxy)Polyphosphazene)] (FP77) (200kDa), Poly[di(carboxylatophenoxy)(trifluoroethoxy)Polyphosphazene)] FP20 (140kDa), and Poly[(sulfophenoxy)(enthlyphenoxy)phosphazene)] FPS (70.9kDa). PPz are dissolved in 0.01M PB with a pH of 7.5 and a concentration of 0.4 mg/ml. The exact weight is measure on a scientific scare to ensure accuracy. The polymer solid is put into a clean tube 15ml centrifuge tube as PB is adjusted on a pH meter to a pH of 7.5. Once calibrated, PB is poured into the tube with the PPz solid. All PPz solutions are dissolved overnight, and in addition, FPS is filtered with a 0.45 micrometer pore size filter. BPEI 0.2 mg/ml is made from dissolving 750kDa BPEI in Milli-Q water and dissolved overnight. Antibiotic solutions (Polymyxin B, Gentamicin, Colistin, and Neomycin) are dissolved in Milli-Q water at a pH of 7.5 with a concentration of 0.5 mg/ml. The chemical structure of these antibiotics is shown in Figure 1.

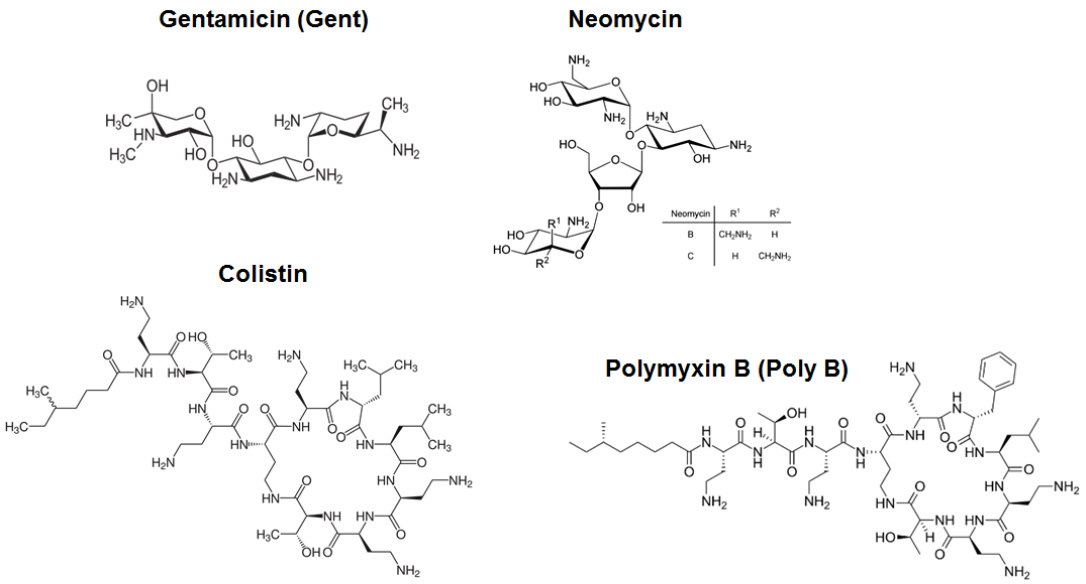

Figure 1. Chemical Structures of Gentamicin, Neomycin, Colistin, and Polymyxin B

2.2 pH Adjustment and Maintenance of Solutions

pH adjustment is made with a pH electrode and are calibrated biweekly. Solutions are pH adjusted with 1M, 0.1M, 0.01M HCL and NaOH. All pH measure within ±0.01 of the desired pH. To maintain a consistent pH throughout the experiment, all solutions are recalibrated daily. Polymer and antibiotic solutions conditions depend on the amount of usage but are typically replaced after two weeks. Solutions are parafilm and sealed up after use and check for contamination daily before usage.

2.3 Preparation of Substrates

Silicon wafers and titanium sheets are the two substrates utilized. Silicon disks are obtained from University Wafer and prepared into 1x1-cm^2 wafers. These wafers are cut via a diamond tip pen and a ruler to ensure accuracy. These square wafers will be remeasured to check if the dimensions are within 1x1-cm^2 ± 0.1-cm^2. These wafers are then exposed to UV for 24 hours to clean the surface. After UV, the wafers are soaked in sulfuric acid (H2SO4) for 45 minutes. After soaking, the wafers are washed with Milli-Q water and dried with nitrogen. Titanium

substrates are made by cutting a titanium sheet into 1x1-cm^2 ± 0.1-cm^2 pieces. It will also be exposed to UV for 24 hours to clean the surface and then submerge in sulfuric acid (H2SO4) for 20 minutes.

2.4 Priming of Substrates

A prime layer is created to introduce positive charges on the surface. BPEI and PB are needed to complete this process. BPEI is pH adjusted from pH 10 to pH 9 while PB is adjusted to a pH of 7.5. BPEI is polymer that will provide the substrate with a single layer of positive charges while PB is used to rinse off extra BPEI on the surface [15]. The clean wafers are first soaked in the pH adjusted BPEI for 15 minutes and then rinsed in a beaker of 0.01M PB to get rid of the excess BPEI. After rinsing, the wafers are dried with nitrogen gas and now ready for the specific PPz to form a prime layer.

2.5 Layer-by-Layer of PPz and Antibiotic Films

Layer-by-Layer deposition is used to create a coating of both PPz and antibiotics with a deposition time of 10 minutes and a solution pH of 7.5. All solutions must be preadjusted to pH 7.5 prior to LbL. The BPEI primed substrates are submerged in PPz (anionic) for 10 minutes. After 10 minutes, the wafer is taken out of the PPz solution and rinsed with PB in a beaker and then submerged in the antibiotic (cationic) solution for another 10 minutes. After that, the wafer is again rinsed with PB, and the first bilayer is formed. The process is repeated until a desire thickness is met. The total time for a bilayer is 20 minutes, and this process can be paused with the completion of a bilayer. Nitrogen gas is utilized to dry the substrate. This process is demonstrated in Figure 1.

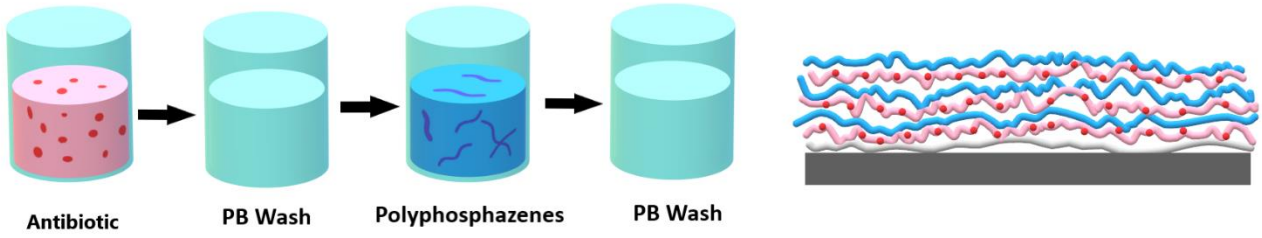

Figure 2. Layer-by-Layer Deposition of PPz and Antibiotic

2.6 Measurement of Thickness

The thickness of a film is measure with a J.A. Woollam ellipsometer. After the coating sample is raised with PB and dried with nitrogen, it can be put on the sample stage of the ellipsometer to be measured. Measurement angles of 45°, 55°, and 65° were used on the ellipsometry program. Measurements are only done after a completion of a bilayer and the sample can resume LbL deposition after being measured. This whole process will utilize the refraction index of the film to estimate a thickness. This process can be seen in Figure 2. As a coating gets thicker and goes above 150nm, the standard deviation of the measurements will increase. Thicker coatings will require a readjustment of the settings to calculate a thickness.

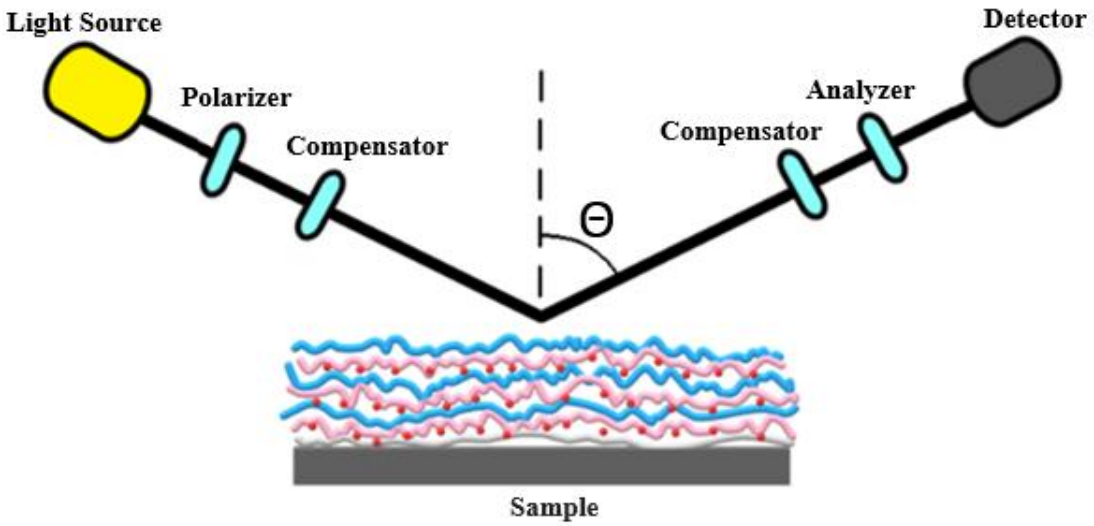

Figure 3. Ellipsometry: Measurement of LbL sample

2.7 Contact Angle of Films

Contact angle is used to characterize surface tension and is measured statically by applying a water droplet onto the surface of a film. Each film is tested a minimal of three times and dried with nitrogen in between each droplet. Each of the three-water droplets is dropped on different spots of the film to ensure an accurate contact angle value. Milli-Q Ultrapure water was used as the water droplet. Films were built up to a thickness of 10nm. Thickness is check with the ellipsometer first before conducting contact angle. The 10nm sample is set on the CA stage and both the camera and light source are turned on. Apply a droplet of Milli-Q water and the software is used to record a right, left, and average contact angle over 10 seconds. These contact angle measurements can be averaged, and a standard deviation is calculated from the results. The sample is dried with nitrogen gas and remeasure two more times on a different spot on the film.

2.8 Swelling of Films

Swelling was measured using a J.A. Woollam ellipsometer in a liquid heating cell. PBS is prepared before the experiment and adjusted to a pH of 7.5. The sample is first measured to determine an initial thickness. The ellipsometer sample stage is swap out for the liquid sample stage and set to have a constant temperature of 37° Celsius. These conditions are selected to simulate physiological conditions. A 100nm sample is inserted into the liquid sample stage to be submerged in PBS. PBS is injected into the chamber to submerge the sample fully. Additional PBS might need to be injected to remove air bubbles in the liquid cell. Swelling runs for one hour, and the change in thickness is calculated to determine how much the sample swells in physiological conditions. The difference between the swollen thickness and initial thickness is used to calculate the swelling ratio for each film.

2.9 Mass Spectrometry of Coatings

Mass Spec is utilized to determine the amount of antibiotic content in a coating. Samples of FPS with Poly B and FP77 with Poly B were built to 50nm, 100nm, and 150nm, and thickness is checked with an ellipsometer. To obtain a solution from the films, PBS is adjusted to a pH of 12 to destroy the films. The samples are first put into a well plate, and then 0.5ml PBS is poured on the surface of the film. The solution will then be collected after an hour. These solutions are then used to determine antibiotic content in the films.

2.10 Mechanical Adhesion to Substrate

A tape adhesion test was developed based on ASTM D3359 to test the mechanical stability of the coating to its substrate. A crosshatch pattern is cut on the film with a razor blade. The cuts on the film will expose the raw substrate, and the difference in the film will be used to determine the stability of the coatings. An optical microscope was used to image each step of cutting and tape

removal. After a film is cut, a piece of tape is applied to the surface and then ripped off. Samples of PCPP, FP77, and FP20 with Poly B were used in this experiment. These samples are first imaged on an optical microscope to document the samples before cutting. Using a razor blade, a crosshatch pattern is cut on the corner of each film. Images of the samples are taken to document before tape removal conditions. A piece of tape is applied to each of the samples and then ripped off instantaneously. A post taped sample picture is taken to determine if there changes on the surface. This experiment was repeated for another two sets of samples that were soaked in PBS at either pH 7.5 or pH 5.5. The samples soaked in pH 7.5 was to simulate physiological conditions while the samples soaked in pH 5.5 was to simulate bacteria environmental conditions.

2.11 Bacterial Test on Coatings

A bacterial experiment was conducted to test whether coatings can prevent bacterial colonization. *Escherichia coli* (*E.coli*) ATCC 25922 was chosen for analysis as a gram-negative strain with known susceptibility to Polymyxin B [16].

Coatings of FP77 and FPS with Poly B were tested to see if it can prevent 10^3, 10^5, 10^7 CFU per cm^2 of bacteria. These coatings were deposited to have a thickness of 10 nm and 70 nm. Prior to testing the coatings, *E. coli* was streaked on an agar plate and incubated for sixteen hours at 37°C temperature. Three mL of tryptic soy broth was inoculated with a single colony of *E. coli* from the plate and incubated for sixteen hours at 37°C temperature, 250 rpm. Optical density 600 was used to determine the concentration of bacteria and a dilution process was used to obtain 10^5, 10^7, 10^9 CFU per ml of bacteria in solution. 3M™ Petrifilm™ *E. Coli* Count Plates were rehydrated with 1 mL of sterile water prior to the assay. Samples are first put face up on the agar plate, and then five microliters of the desire bacterial concentration will be pipetted onto the surface of the coating. These agar plates will now be incubated for 24 hours. Once the incubation period

is over, the substrate can be removed from the agar plate and bacterial colonies that grew on the surface counted. To ensure the experiment did not have any contaminations, control samples of tryptic soy broth and water were also tested to see if there were any bacteria grow.

CHAPTER III

RESULTS

3.1 Polyphosphazenes of Interest

Polyphosphazenes have been used for a wide variety of applications and this was made possible due to their side groups. These side groups can dramatically impact the properties of the coatings made from these PPzs. Carboxylate PPz has been studied to form coatings with antibiotics and prevent bacterial colonization with its pH response release of antibiotics [10]. These coatings are very promising but has one crucial limitation of a onetime burst release upon exposure to physiological conditions. The goal of the research is to understand a new class of fluorinated, sulfonated PPz and how it compares to the carboxylate PPz. The newer sulfonated PPz have the potential to contain new coating properties and build upon the understanding of PPz and antibiotic coatings.

To ensure an in depth comparison of the several types of PPzs with different fluorination degree and type and density of ionic groups in their assemblies with antibiotics, this work will address film growth, surface tension, swelling, mechanical adhesion properties to substrates, and bacterial efficacy of the films. The structure of PPz polymers can be found in Figure 3. with PCPP, FP77, and FP20 being carboxylate PPz and FPS being sulfonate PPz. FPS will be compared to FP77 which has matched charge density but different charge type, as well as with FP20 which has matched fluorination degree. At the same time, nonfluorinated PCPP was used for construction of control coatings.

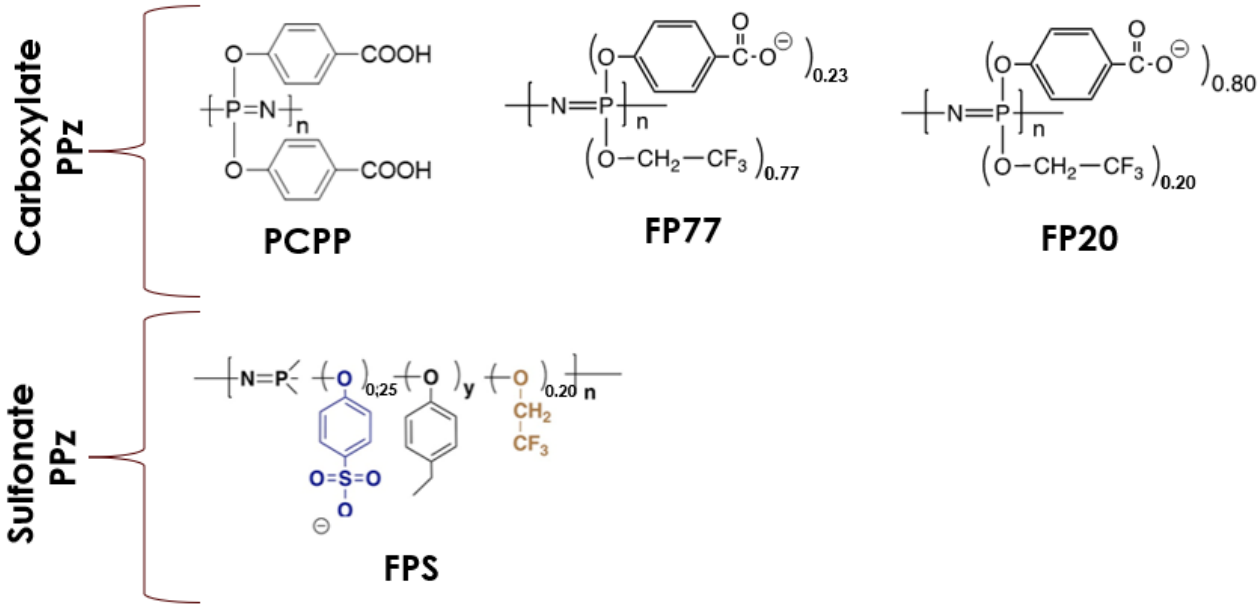

Figure 4. Chemical Structures of Polyphosphazenes

3.2 Growth Curves

PCPP, FP77, FP20, and FPS were used to form LbL films with Poly B, and the increase in film thickness per bilayer measured by ellipsometry was recorded to as a growth curve. These four PPzs are chosen to represent the effect of the carboxylate, sulfonated, and fluorinated side groups on antibiotic binding. The deposition conditions for all of these coatings were the same with deposition time set at 10 minutes and pH set at 7.5. Poly B was chosen as an antibiotic used in every growth experiment. The growth curves of PCPP, FP77, FP20, and FPS with Poly B are presented in Figure 4. There is a distinct thickness difference in film growth between the carboxylate PPz and sulfonate PPz. Specifically, carboxylate PPz show a more exponential growth with the antibiotic, while the sulfonate PPz shows a linear growth. All carboxylate PPz also achieves a thicker coating than sulfonate PPz at 20 bilayers. These trends can be linked to the fact that sulfonic acid group have stronger binding strength as compare to the carboxylic acid group [11]. Figure 4 also shows the impact of fluorinated groups. When comparing PCPP to the rest of

the PPzs, PCPP and strongly fluorinated FP77 are shown to have the fastest increase of thickness per bilayer. Ultimately these four PPzs are unique polymers with the ability to for multilayer films with cationic antibiotics since not all polymers or PPzs can form LbL films with antibiotics [10].

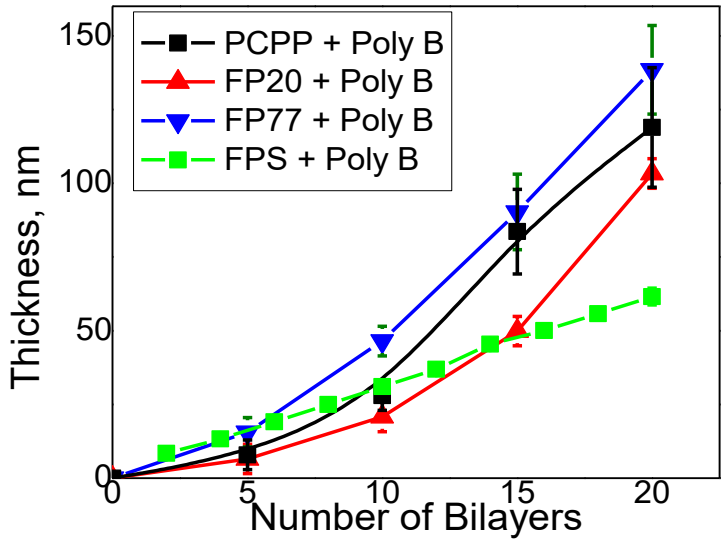

Figure 5. Growth Curve of PPzs with Poly B

As we compare the trends between carboxylate and sulfonate PPzs, FP77 and FPS are selected to form films with various antibiotics. Poly B, Colistin, Gent, and Neo were used as the antibiotics because these antibiotics represents both spectra of preventing gram-positive and gram-negative bacteria. Poly B and Colistin have generally been used as a last resort against gram-negative bacteria and therefore a good choice for *E. coli* [17]. Gent and Neo on the other hand are a type of aminoglycosides used to target gram-positive bacteria [18]. Figure 5 shows the growth trend of FP77 with the four antibiotics and Figure 6 shows the growth trend of FPS with the four antibiotics. With FP77, robust films could be deposited with all types of antibiotics, However, significantly thicker films were formed with Poly B and Colistin as compared to Gent and Neo. These differences are explained by the higher molecular mass of Poly B and Colistin

and their larger mass-to-charge ratio. As for the comparison between FP77 and FPS, the trends for FPS/antibiotic systems were similar, while bilayer thickness was smaller and film growth more linear than for FP77/antibiotic system. Moreover, films of FPS with Gent and Neo showed extremely slow growth, probably associated with incomplete surface coverage at each layer deposition step.

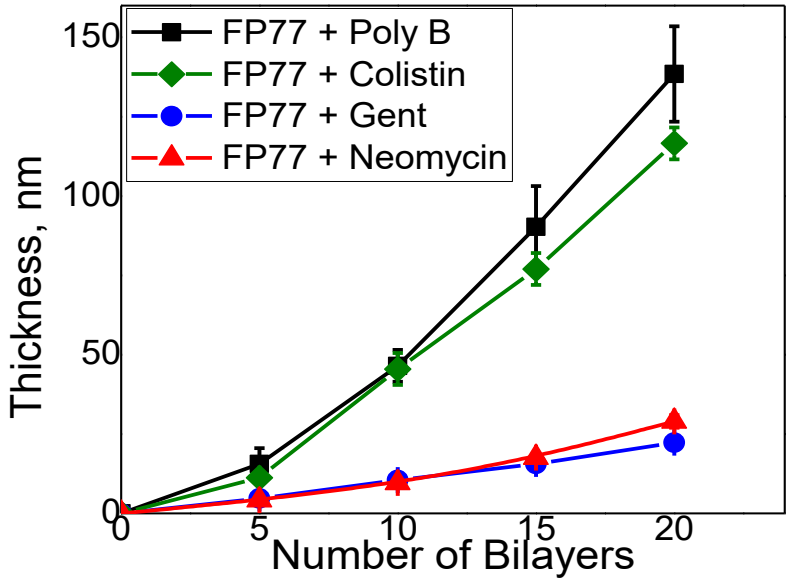

Figure 6. FP77 a carboxy PPz with various antibiotics

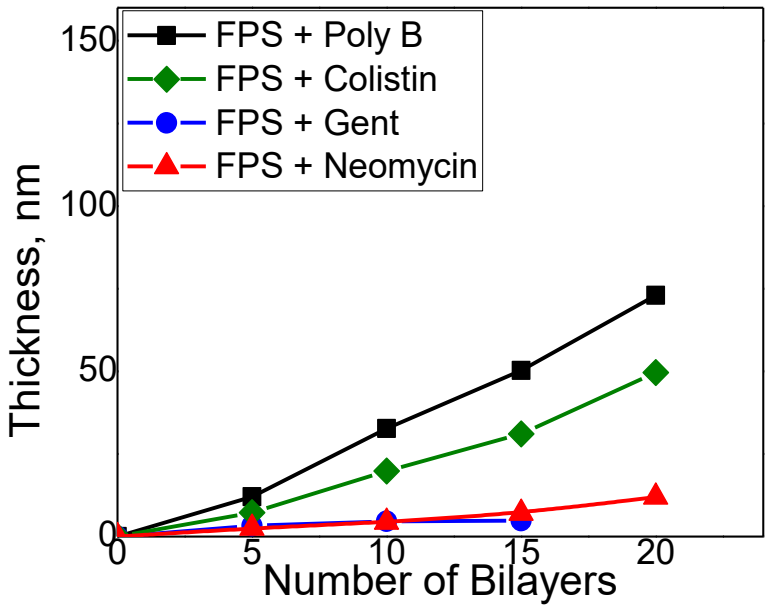

Figure 7. FPS a carboxy PPz with various antibiotics

3.3 Contact Angle

Contact angle is the measurement of surface tension. Surface tension is an important property when making a biomedical material and is crucial for these coatings if the intend of used is in physiological conditions. In our experiments, coatings constructed with PPzs of varied hydrophobicity were expected to have different contact angles. Contact angle measurements were performed with 10-nm film of PCPP with Poly B, FP77 with Poly B, and FPS with Poly B. Each measurement was done three times. The resultant contact angles of the coatings constructed with PCPP and FPS were around 70°, and those constructed with FP20 around 57°. There was no significant difference between the coatings built with carboxylate and sulfonate PPzs.

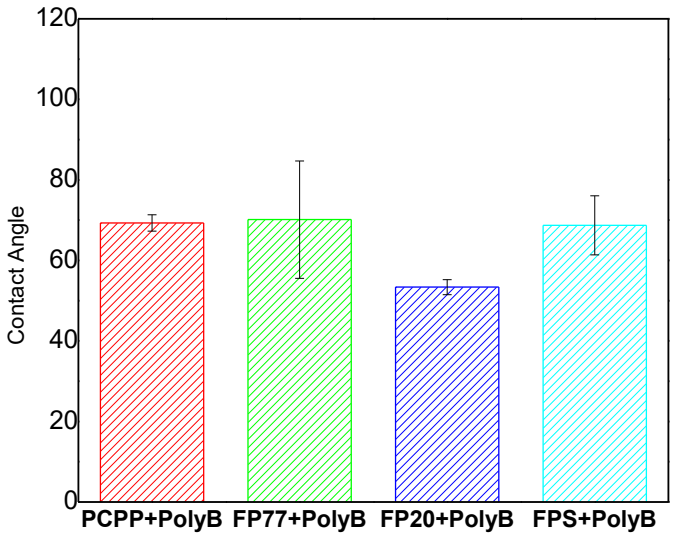

Figure 8. Contact angle measurement of PCPP with Poly B, FPS with Poly B, and FP77 with Poly B

3.4 Swelling

Swelling is an important biomaterial factor and it is shown from Figure 8. that the swelling ratio can dramatically change depending on the side group. Coatings of around 50nm was exposed to PBS at 37°C and a pH of 7.5 for one hour. The outcomes show that PCPP and FP20 with Poly B swell up to 1.6 times its original thickness while FP77 and FPS with Poly B only swell up to around 1.2 times its original thickness. This data trends suggests that there is not much of a difference between carboxylate or sulfonate PPz but instead was dependent on whether the PPz had fluorinated groups or not. PCPP was the only PPz out of the four that does not have any degree of fluorination and exhibit the highest swelling ratio with FP20 have a low fluorination degree of 20% also being second highest in swelling. It can be concluded that the hydrophobic nature of the fluorinated groups contributes to the coating not swelling as much as PCPP or FP20.

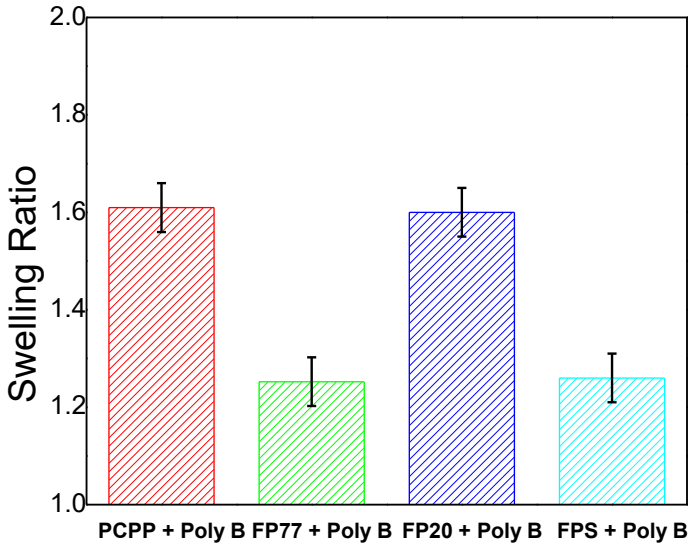

Figure 9. Swelling of PCPP with Poly B, FP77 with Poly B, and FPS with Poly B

3.5 Film Stability

Film stability was tested by soaking PCPP, FP77, and FPS films with Poly B in PBS at a condition of 37°C and pH of 7.5. This test was done to demonstrate how these films would withstand physiological conditions. The results show that the films containing sulfonate PPz had minimal to no change in thickness in this solution over time, while the films containing carboxylate PPz decreased in thickness. PCPP had the most drastic change of thickness over the first two days with a drop of 40% in thickness and FP77 had a change of 20% in thickness. All three films dis not show significant changes in thickness after the first day of exposure to PBS, suggesting the ionic-strength-induced reduction in the film thickness was fast. The results from Figure 9. demonstrate that sulfonate PPz has the ability to maintain its coating thickness and fully retain antibiotics while the carboxylate PPz tend to partially loose its antibiotic load from the coating upon exposure to salt solutions.

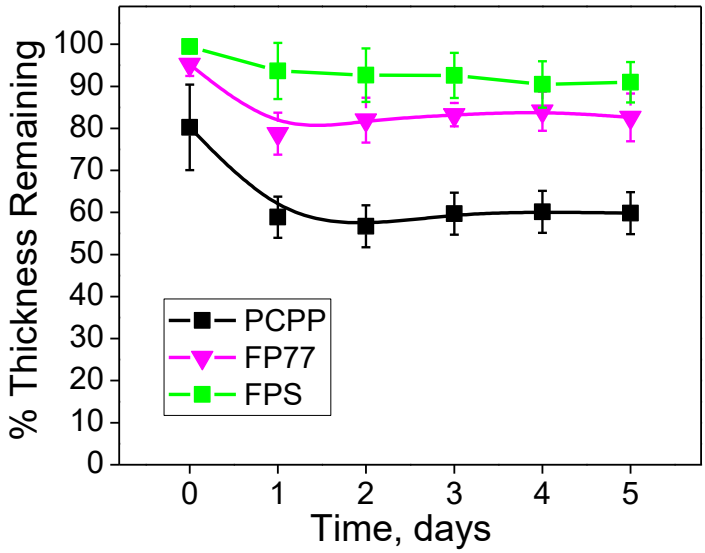

Figure 10. Thickness change in physiological conditions over time for PCPP FP77 and FPS with Poly B at an initial thickness of 100nm

3.6 Mass Spectrometry

Mass spectrometry was used to determine the total antibiotic content within the coating. The amount of antibiotic within a coating can influence how effective it is against bacteria colonization. Figure 11. shows the results of the mass spec measurements. The coatings of FP77 with Poly B and FPS with Poly B for 50-nm films showed a slight difference in antibiotic content. FPS with Poly B had a slightly higher Poly B content of 7.48±2.89 micrograms over the films of FP77 with Poly B which contained5.84±1.76 micrograms of antibiotic. This result suggests that films of sulfonate PPz contained more antibiotic per amount of thickness as compared to carboxylate PPzs. This result is likely

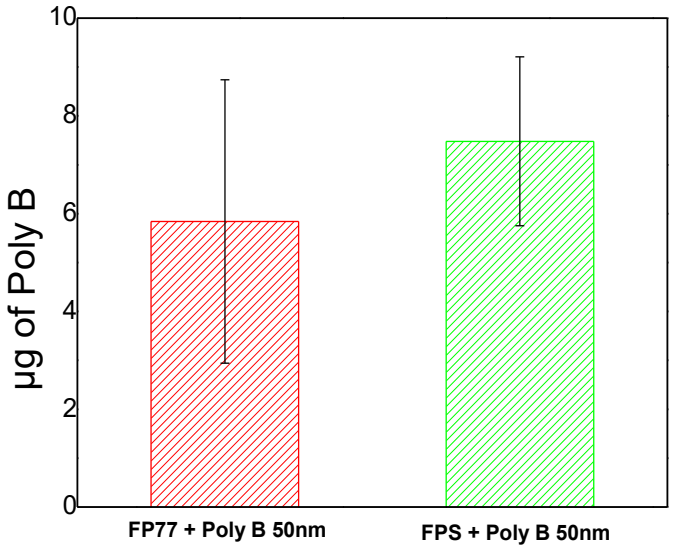

Figure 11. Antibiotic content of FP77+Poly B 50nm and FPS+Poly B 50nm

3.7 Bacterial Test

FPS with Poly B and FP77 with Poly B coatings were made and tested with bacteria in order to determine the efficacy of preventing bacterial colonization. By using FPS and FP77, a direct comparison can be done between antibacterial efficiency of films built with carboxylate PPz or sulfonated PPz. Two thicknesses of each type of coating were prepared in order to explore the effect of total content of antibiotic content on preventing bacteria colonization. *E. coli* was chosen as the strain of bacteria to test with Poly B. A table shown in Figure 12. details the type of coating and their effectiveness in preventing biofilm formation. FP77 with Poly B yielded the same results as the FPS coatings. Specifically, both FPS-based and FP77-based coatings with 10-nm thickness were effective at preventing bacterial colonization up till 10^5 CFU per cm^2, and failed at higher bacterial challenges, while increase in film thickness to 70 nm lead to prevention of bacterial colonization at bacterial concentration as high as 10^7 CFU per cm^2. The result is somewhat surprising since while the mass spec measurements indicate that these coatings have around the

same amount of antibiotic content, it was previously suggested that presence of pH-responsive moieties in the films is required to release antibiotics from the entire film thickness [19]. However, while FP77 is a part of the carboxylate PPz subgroup and utilize pH responsiveness to elute antibiotics, FPS as a sulfonate PPz does not have this property [11]. Since coatings of FPS and Poly B can prevent bacterial colonization, it can be suggested that FPS is preventing biofilm formation *via* contact killing. The ability of FPS to form films with cationic antibiotics which are highly stable and non-eluting in salt-containing physiological environments and efficiently prevent bacterial colonization makes these coatings promising candidates for antibacterial protection of biomedical devices.

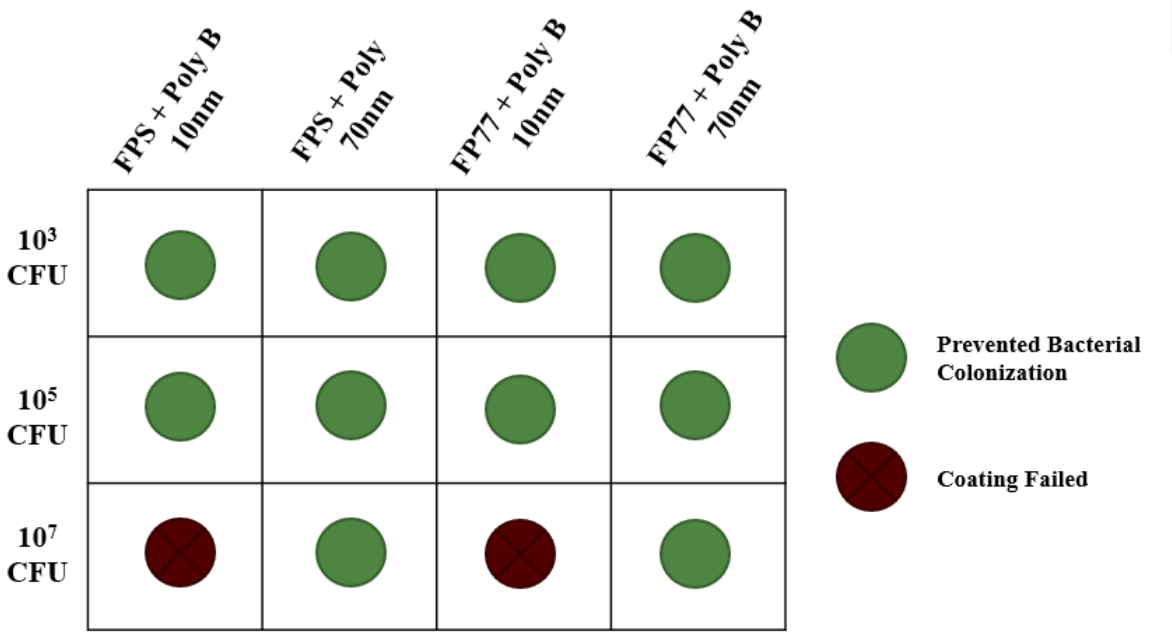

Figure 12. Bacterial experiment results for 10-nm and 70-nm FPS+Poly B films and FP77+Poly B films.

3.8 Mechanical Adhesion to Substrate

Maintaining bonding to a substrate is an important factor for coatings. To explore the adhesion capability of the coatings, coatings of PCPP, FP77 and FP20 with Poly B were cut through to the silicon substrate , and a tape is used to test delamination of the coatings. This test was performed with the coatings of 50-nm thickness at normal dry conditions, as well as after exposing them to PBS at pH 7.5 and 5.5. The dry film tape test for all three samples resulted in the same outcome. All three films stayed intact after a tape was placed on top of the coated substrates and then quickly removed from the surfaces. The cut on the samples did not expand and there was no visual change. The same samples were then soaked in a PBS solution at pH7 .5 to simulate physiological conditions, and dried. These post-soaked samples were then retested. There was not much change to the sample prior and after the tape test. This means that the film was still well adhered to the substrate after being exposed to physiological conditions. To further test the limits of these coatings, the same films were next exposed to PBS at a pH of 5.5 to simulate an acidic bacterial environment. After 12 hours, these films were dried and retested with tape. The films again exhibited good adhesion, showing no major changes to the areas exposed to the substrate. Minor pieces of the film missing from the substrate did not allow to make a definitive conclusion on whether they were removed with the tape or partially dissolve in the salty acidic conditions. PCPP with Poly B images are shown in Figure 9. FP77 and FP20 with Poly B images are shown in Figure 10.

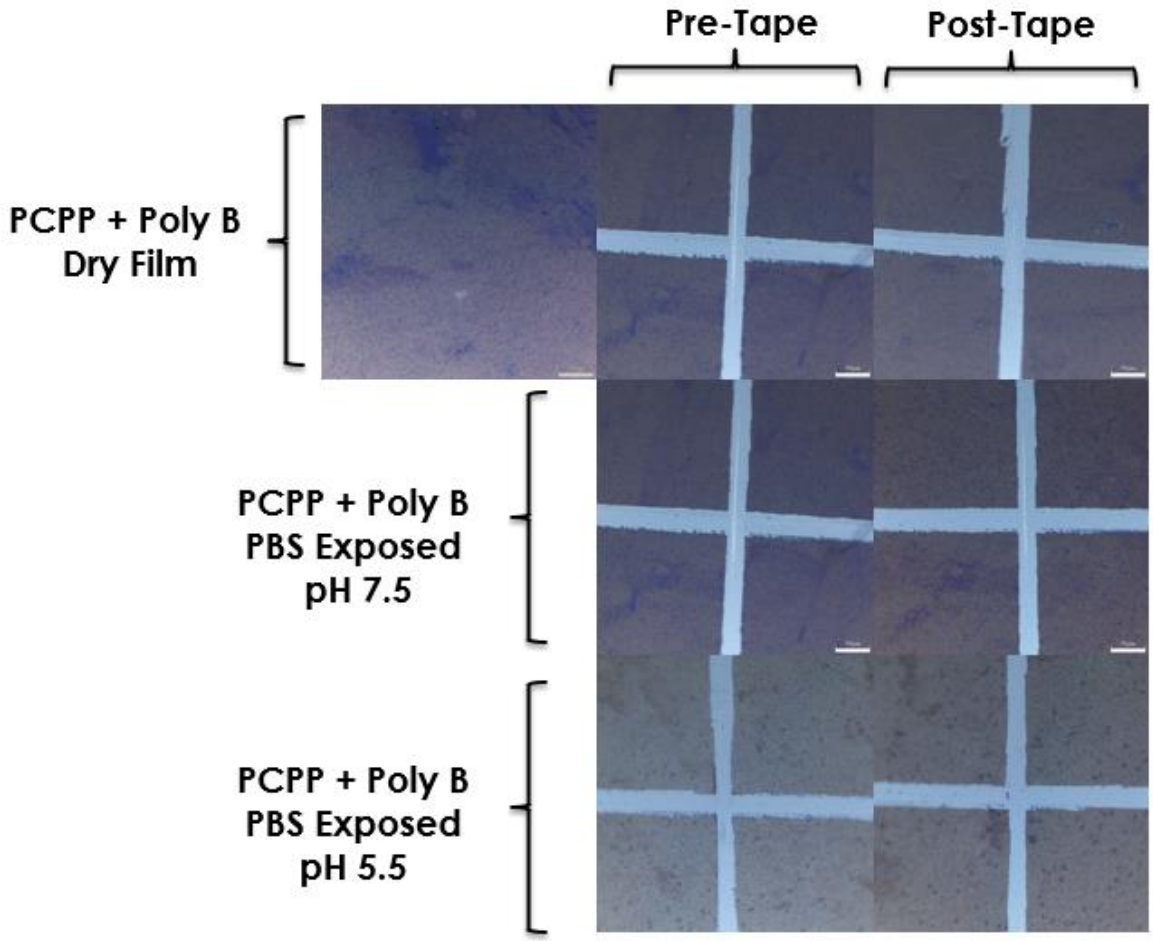

Figure 13. PCPP + Poly B Tape Test with dry film, PBS pH 7.5 soaked, and PBS pH 5.5 soaked

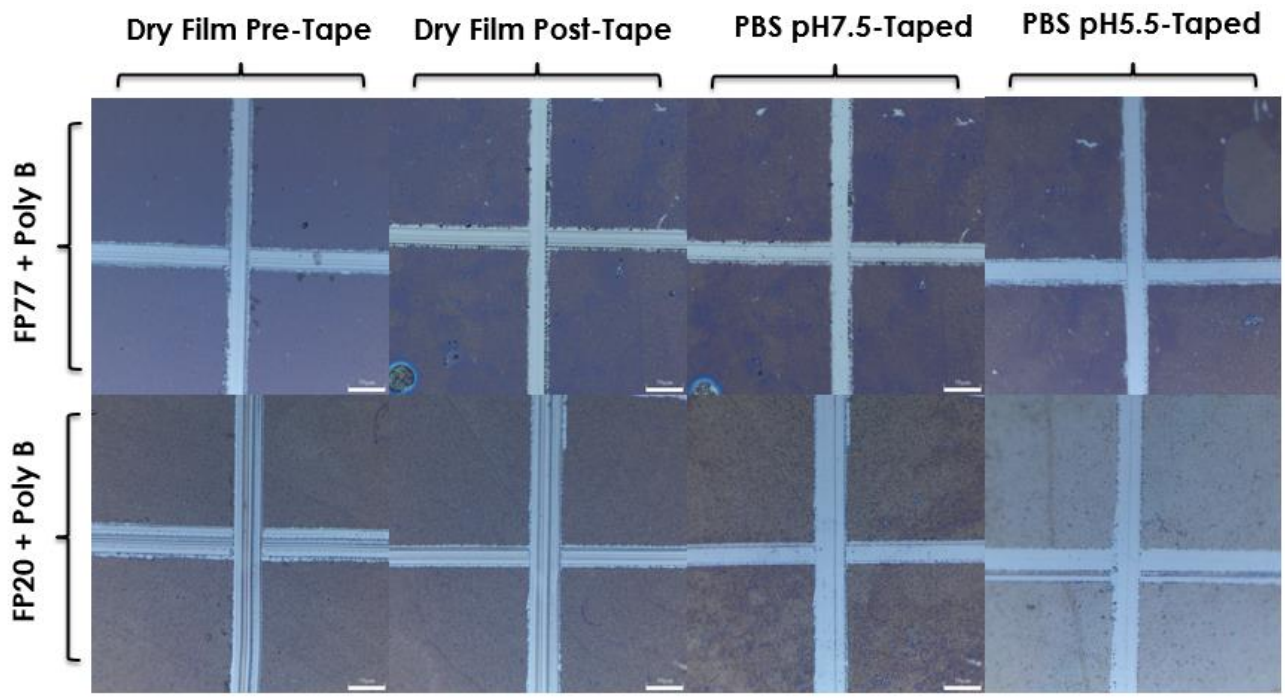

Figure 14. FP77+Poly B and FP20+Poly B tape adhesion test with dry film, PBS pH 7.5 exposure, and PBS pH 5.5 exposure

3.9 Coatings on Titanium Substrates

To demonstrate the versatility of these PPz and antibiotic coatings and their capability to be deposited on a variety of substrates, titanium was used as a substrate in place of silicon wafers. Titanium was chosen because of it is used more frequently as a biomaterial than silicon substrates [20]. PCPP and Poly B and FP 77 and Poly B coatings could be successfully deposited on titanium substrates, leading to changes surface appearance for 20-bilayer films as shown in Figure 13. Direct thickness measurements with ellipsometry was not possible because of poor surface reflection properties. These coatings were also tested with *E. coli* and resulted in prevention of bacteria of 10^5 CFU per cm^2 but not 10^7 CFU per cm^2. However, there was only one replicate of the titanium studies, so perhaps further investigation would help clarify the true extend of coatings on titanium.

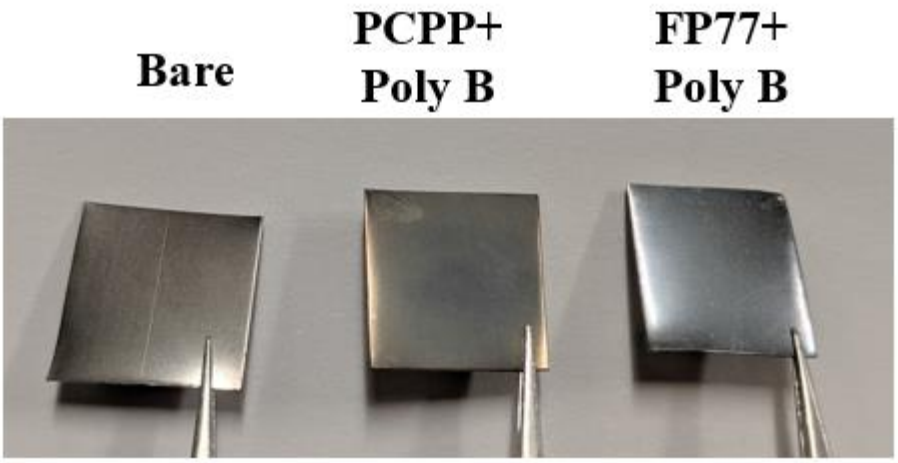

Figure 13 Titanium substrate from left to right: bare titanium, PCPP+Poly B on titanium, and FP77+Poly B on titanium

Figure 14 Bacterial test of FP77+Poly B on titanium substrates

CHAPTER IV

CONCLUSION

Polyphosphazene are unique polymers that enable their direct assembly with cationic antibiotics within LbL coatings of controlled thickness. These coatings has been demonstrated to be effective against bacterial colonization and have potential to be used as biomedical applications. Due to the adjustability of side groups on PPzs, characteristics such as thickness, surface hydrophobicity, swelling, and biocompatibility can be tailored to meet the needs of specific applications. Compared to antibiotic-containing films based on carboxylate PPz, sulfonate PPz have demonstrated more linear in growth and contained an equal or greater amount of antibiotic with the same thickness. More importantly, assemblies of sulfonate PPz with antibiotics improved stability of the coatings in normal physiological conditions and suppressed undesirable release of antibiotics in the absence of bacteria. This property is a significant improvement of performance of antibiotic-containing films which are based on carboxylate PPzs. The non-eluting nature of sulfonate PPz-based coatings, taken together with their equal to carboxylate PPz-based film antibacterial efficiency make this films promising antibacterial coatings which maintain their potency in bacteria-free normal physiological conditions, and efficiently deliver antibiotics upon bacteria attack in a manner that minimizes chances for the development of antibiotic resistance.

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