Stable Fluorinated Antimicrobial Coatings

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Stable Fluorinated Antimicrobial Coatings

A dissertation submitted in partial fulfillment of the requirement for the degree of
Doctor of Philosophy at Virginia Commonwealth University

by

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Abstract

Stable Fluorinated Antimicrobial Coatings

By Asima Chakravorty

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Major Director: Dr. Kenneth J. Wynne, Professor, Department of Chemical and Life Science Engineering

Contact antimicrobials for use in the medical device industry are being studied extensively to minimize the risk of hospital acquired infections, which are among the top ten leading causes of death in the US. Surfaces modified with quaternary ammonium containing side chains have been known to demonstrate excellent antimicrobial properties. Prior work has indicated that polyurethane surfaces with copolyoxetane soft blocks consisting of fluorinated and quaternary ammonium side chains can act as good antimicrobials. However, stabilizing the positive charge on the surface has been a challenge. The dissertation is aimed at creating a surface modifier that would confer a stable contact kill antimicrobial surface at very low modifier content, that is, less than 2 wt%. To achieve this objective, the study explored the introduction of a different fluorous group in the soft block to enhance stability. In particular, prior studies by other groups and early work by Kurt have shown that replacement of one of the terminal “chaperone” C-F bonds by C-H decreased surface tension. This led to the hypothesis that a –CF3H terminated “chaperone” group would be “amphiphilic” resulting in surface stability under both dry and wet conditions. Keeping this hypothesis in mind, a –CF2-CF2H (4F) terminal “chaperone” group was created in a modifier having two different 4F to quaternerary C12 ratios.

It was found that polyurethanes prepared with a 66:34 ratio of 4F:C12 as the diol, performed as a very good surface modifier with high zeta potentials over a long period of time compared to the –CF3 based modifier. Antimicrobial tests performed within one week and four
weeks after coating preparation have provided promising results that demonstrate improved biocidal stability.

Guided by improved antimicrobial properties obtained with surface modifier polyurethanes made from P[(4F)(C12)-66:34-Mn], a new concept was explored by end-capping the same diol with isocyanatopropyltriethoxysilane and blending the end-capped diol with base polyurethane along with a 10 wt % cross linker. These modifiers show excellent antimicrobial properties (100% kill of bacteria) over one month with no observable changes in the zeta potential or surface morphologies. XPS analysis confirms the presence of quaternary ammonium on the surface. Preliminary kinetic studies show excellent antimicrobial properties for a 2 wt% modifier and 100% kill within 1 hr.
Chapter 1.

Introduction – Antimicrobial polymeric surfaces

1.1 Introduction

Microbial infections have become increasingly problematic and sometimes fatal in the recent years with increased antibiotic resistant microbial strains. The number of antibiotic-immune strains is growing more rapidly than the number of usable antibiotics. In 2009, the US Center for Disease Control (CDC) reported that hospital acquired infections (HAIs) are among the top ten leading causes of death in the US, costing $20 billion annually. In the U.S. alone, two million hospital patients succumb to HAIs each year, that is, approximately 5 - 10% of hospitalized patients are affected with an estimated 99,000 deaths annually. Of these HAIs, 60% involve resistant bacteria. The majority of HAIs affect the urinary tract, bloodstream, surgical site and respiratory tract with urinary tract infections comprising of ~36% of total HAIs.

In principle hospital acquired infections could be prevented rather than treated by effective use of antimicrobial materials - biocidal polymers being one of them. One of the first studies in antimicrobial polymers was by Cornell and Donaruma who reported the biocidal properties of polymers and copolymers prepared from 2-methcryloxytropones. Over the past few decades antimicrobial solutions and coatings have been studied extensively. The background presented below is relevant to the present study which concentrates on developing surface antimicrobials and preparation of coatings with stable and effective antimicrobial activity.

Surfaces may act as reservoirs for microbes which could in turn lead to the spread of infection upon being touched, by either healthcare workers or patients. Hence, the spread of microbial infections could be largely arrested by imparting biocidal property to surfaces coming in
contact with biocides. Over the past three decades, a variety of antimicrobial polymers have been studied to prepare biocidal surfaces which can prevent spread of microbial infections. According to their mode of action, antimicrobial polymeric surfaces can be classified into three basic categories: 

*biocide release, oxidative kill and contact kill.*

1. **Biocide release polymers:** Polymeric coatings can be loaded with biocides that are released in the vicinity of microbial cells. Several approaches have been described including antibiotic\textsuperscript{6,7} or metal ion releasing\textsuperscript{8-11} systems. Such polymers have been found to be effective in the short term since a high concentration of biocides is released close to bacterial cells. However long term use is jeopardized since the biocide is depleted over time. Release of antibiotics clearly contributes to resistance build up among microorganisms.\textsuperscript{12,13} Increased exposure of microbes to these compounds inevitably leads to increased resistance to treatments.

   Release rate is another important issue as zero order release is ideal but not usually achieved. Existing systems generally display first or other non-zero order release due to transport limited diffusion.\textsuperscript{14} Non-zero order release is problematic and results in short term over-release and long term under-release. Another disadvantage is the release of toxins to the environment and their cytotoxic effects on human cells and tissues.\textsuperscript{15}

2. **Hydantoin based oxidative kill:** In 2006, Worley reported a method of coating silica gel particles with a hydantoinylsiloxane in either monomeric or polymeric form. Upon chlorination with aqueous solutions of household bleach, the coated silica gel particles became biocidal.\textsuperscript{16} The schematic of N-chloramine siloxane is given in Figure 1.2. This can be termed as a contact-release system wherein oxidative contact kill is achieved. Compared with the free halogens or hypochlorite,
Figure 1.1. Biocide releasing polymers-bacteria killed in the zone of release.

(Figure from “Antimicrobial polymers in solution and on surfaces: Overview and functional principles”- Felix Siedenbiedel and Joerg C. Tiller)²
Figure 1.2. N-chloramine biocidal silicone

(Figure from “Antimicrobial polymers: mechanism of action, factors of activity, and applications”- Larisa Timofeeva & Natalia Kleshcheva)
organic N-halamines are more stable, less corrosive, and have less tendency to generate halogenated hydrocarbons.

Gradual depletion of chlorine from the surface requires replenishment at regular intervals (treatment with bleach) to maintain efficacy. While these systems are useful for water purification,\textsuperscript{17,18} N-chloramine surface modification is not useful for applications that require biocompatibility since surfaces with –N-Cl functionality are cytotoxic.

3. Contact antimicrobials:

In the past decade, due to environmental concerns, emphasis has been on developing non-leaching surfaces that can kill microorganisms on contact. Because contact kill precludes the biocide entering the bacterial metabolic processes, elimination of bacterial resistance buildup may result.\textsuperscript{19}

\textit{Mechanism of contact kill}

Bacterial cells are typically 0.5-5 μm in diameter. Figure 1.3 depicts the broad classification of bacteria into Gram positive and Gram negative.

A. Gram positive bacteria have a thicker cell wall and a highly crosslinked peptidoglycan structure. The peptidoglycans are the targets of the antimicrobials.

B. Gram negative bacteria have relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.
Figure 1.3. Bacterial Cell Wall – Classification of gram negative and gram positive bacteria. 

An ordered structure is important for cell wall integrity since this provides structural strength. Bacterial cell walls have polar constituents and overall a net negative charge. While antibiotics generally target specific intracellular mechanisms including cell wall synthesis, protein synthesis and DNA replication, the working mechanism for contact kill is not fully understood. In 1983, a study by Gilbert on the interaction of polyelectrolyte salt polyhexamethylene biguanide chloride with E. coli showed disruption of outer membrane of the Gram negative bacteria. The proposed mechanism suggested strong attraction of the polyelectrolyte towards the negatively charged bacterial cell surface which impaired the integrity of the outer membrane. The polyelectrolyte then binds to the phospholipids of the inner membrane which increase the membrane permeability and eventually leads to precipitation of intracellular components. This mechanism was supported by the works of Ikeda and Moore. To understand the kill mechanism on biocidal surfaces, Klibanov and co-workers have used the two-color Live/Dead fluorescence test against S. aureus and E. coli, and concluded that surfaces covalently derivatized with N-hexyl,N-methyl-PEI (PEI with Mw 750 kDa) or painted with N-dodecyl,N-methyl-PEI (PEI with Mw 750 kDa) killed bacteria by rupturing their cellular membrane. Atomic force microscopic images of E. coli cells on glass surfaces, modified with PDMAEMA, also support the hypothesis of membrane disruption. Many examples of such antimicrobial polycations with quaternary ammonium, phosphonium, tertiary sulfonium and guanidinium functions are known.

**Surface modifications techniques**

Interest in contact kill has led to a number of studies on polymers with covalently bound alkylammonium/quaternary ammonium functional groups. One of the most common methods for making biocidal surfaces involves the process of treatment of a surface to render it amenable for attachment of antimicrobial functional groups. Plasma surface treatments (Figure 1.4 A) provide a
route to obtain free radical, hydroxyl, amino, and peroxide functional groups on the surface of a substrate which is then converted to an antimicrobial surface by immobilizing synthetic antimicrobial polymeric compounds like quats.\textsuperscript{30, 31}

Contact antimicrobial function is also accomplished by covalently bonding the biocide, onto a treated surface thereby promising stability over time (Figure 1.4 B). Graft polymerization of a monomer directly on a prepared glass surface or covalently attaching partially alkylated polymeric compounds to treated glass surfaces have been tried by Tiller.\textsuperscript{32,33} Preparation of non-covalent coatings by drip coating or painting surfaces with biocidal materials in solvents (Figure 1.4 C) has been tried by Park and Halder.\textsuperscript{25, 34}

These techniques, although effective, are difficult to commercialize due to the complex procedures involved which do not make them cost effective. Another approach for incorporating contact kill properties in medical devices is to incorporate antimicrobial agents in the bulk of the medical device. The agent might be (i) integrated in the original polymer used for the medical device,\textsuperscript{35} (ii) blended with the original polymer and extruded in the desired shape,\textsuperscript{36} or (iii) added to the original polymer as micro- or nanoparticles and crosslinked.\textsuperscript{37, 38} This approach is advantageous in terms of processability on an industrial scale and there is no need to process the medical device after manufacturing. Modification of polyurethanes – a common polymer in the medical device industry, by quaternising the pyridinium nitrogen in the hard block of the polyurethane has been reported by Cooper.\textsuperscript{35} Although this method was highly effective in terms antimicrobial efficacy (95% kill for \textit{S. aureus}) modification of hard block resulted in loss of mechanical property of the polymer. Modification of polyurethane soft blocks to enable quaternary ammonium containing side chains in soft blocks to migrate to the polyurethane surface (Figure 1.4 D) has been reported by
The present work uses this modification technique to develop contact antimicrobial polyurethane surfaces with increased stability of positive charge on the surface.

Among the three different categories, contact killing surfaces, have been studied widely as biomaterials for medical applications because these do not undergo biocide depletion. In principle the polycation approach is a greener solution due to non-release of harmful toxins.

1.2 Scope of work

The aim of this study is to identify a suitable biocidal polymeric composition that can be easily incorporated into commonly used medical polymers to render them antimicrobial without affecting bulk properties. The stability of the modified surface is a key goal. Polyurethanes are commonly used for medical applications because of toughness, biocompatibility and hemocompatibility. Polyurethanes can be strong elastomers or rigid plastics, and can be processed by extrusion, injection molding, film blowing, solution dipping, and two-part liquid molding. Versatility of polyurethanes is due to a unique chemistry. Polyurethanes consist of a soft segment that provides flexibility and a hard segment that provides strength. The hard segment is typically made up of a diisocyanate and a chain extender and the soft segment is a polyol.

The present study aims at modifying the surface of a commonly used polyurethane that is often referred to as base polyurethane. The structure of the base polyurethane is shown in Figure 1.7. H_{12}MDI (methylene bis-(p-cyclohexyl isocyanate)) is the diisocyanate, BD (1,4-butane diol) is the chain extender and PTMO (poly(tetramethylene oxide)) is the soft segment. The inherent immiscibility of the soft and hard segments is evident from the atomic force microscopy image shown in Figure 1.6. It is observed that at a high setpoint ratio, the surface is featureless but phase
Figure 1.4. Surface modification techniques: A. Plasma/ radiation surface treatments, B. Chemical methods- grafting quats onto treated surfaces, C. Painting, drip coating of quaternary ammonium containing groups, D. Polymeric blends- surface concentration of charge.
Figure 1.5. Depiction of polyurethane solid state morphology
Figure 1.6. TM-AFM phase image of base polyurethane. Scan size 500×500nm; Setpoint ratio \((A_{sp}/A_o)=0.9\)
separation becomes evident at lower setpoint ratios. This leads to the hypothesis that the surface is dominated by soft block. This feature has been utilized to incorporate a biocidal function in the soft block.

Kurt synthesized HMDI-BD based polyurethanes as the polymer surface modifiers containing a random P[AB] copolyoxetane soft block, wherein A is a fluorine based segment (3FOx), B contains a quaternary ammonium side chain and HMDI and BD comprise the hard block. The resulting P[AB] polyurethane was blended with an HMDI-BD-PTMO polyurethane. Figure 1.7 represents a model for the surface modification of base polyurethanes by this approach.

The basis for this model is thermodynamically driven surface concentration of the P[AB] soft block side chains where A is a short fluorinated side chain and B is a quaternary ammonium with a 12 member carbon chain. The soft block is represented as a bottle brush in Figure 1.7b. In this representation the A and B side chains are like short bristles emanating from the main chain. A cue was taken from the long established concentration of soft blocks at the air-polymer interface. It was hypothesized that due to its low surface energy, the fluorinated side chains of the soft block moiety will surface concentrate acting as hydrophobic “bristles” on the polymer surface. These short fluorinated side chains will in turn act as chaperone for the positively charged quaternary ammonium side chains and facilitate their surface concentration. Thus the flexible main chain (T_g’s, -40 to -60 °C) along with the short side chains constitute a bottle-brush like surface. This model targets two features of the bacterial cell wall - hydrophobicity and negative charge. A coating consisting of fluorinated polymer chains will favor interaction with the bacterial cell. The fluorinated side chain bristles provide the hydrophobic property to the bulk polymer surface. However, for these bristles to be erect on the surface, a repulsive bulk-modifier interaction is required. This is provided by the positively charged quaternary ammonium “brushes” which will
also serve the dual function of bacterial bioconjugation and disrupting the negatively charged bacterial cell wall with subsequent cell death.

Although Kurt’s study reported excellent antimicrobial properties for the P[AB] based soft segments, subsequent work by Brunson\textsuperscript{46} reported the loss of positive charge with time. This is discussed in detail in Chapter 2 of this dissertation.

**Chapter 1** presents the synthesis and characterization of novel fluorinated polyurethanes with side chains having –CF\textsubscript{2}H end groups. A unique model is proposed to account for the amphiphilic nature of these end groups. Based on enthalpic considerations that favor the presence of the –CF\textsubscript{2}H end groups at the outermost surface under both wet and dry conditions, surface modifiers containing these groups were sought for air/water stability.

**Chapter 2** focuses on synthesis and characterization of polyurethane based polymer surface modifiers with P[AB] soft segments where A consists of a side chain with –CF\textsubscript{2}H end group. A unique approach has been explored correlating surface active positive charge obtained by zeta potential measurements with biocidal performance.

**Chapter 3** presents an exciting new bottle-brush / nanoglass (BB-NG) concept which promises higher stability for quaternary surface charge and a simple surface modification method that has imparts broader applicability using the same P[AB] copolyoxetane diols discussed in Chapter 2.

**Chapter 4** explores the kill kinetics for modified surfaces including the optimum or the optimum exposure time to obtain 100% kill of bacteria under the spray challenge test conditions.

Finally, key future goals for the research has been discussed.
Figure 1.7. Model for surface modification of base polyurethane - a. Soft block for the surface modifier polyurethane, b. Surface concentration of surface modifier, c. Composition of the bulk (base polyurethane).
Chapter 2.

Novel Fluorinated polyoxetanes with –CF₂H end groups: Synthesis, Characterization and Applications

2.1 Introduction

Modification of surfaces with fluorous polymers has been widely studied to obtain surfaces with both hydrophobicity and oleophobicity. Much is known about surfaces with terminal CF₃ groups especially polyacrylates. Effects arising from replacement of a terminal fluorine atom, specifically with hydrogen have seen relatively little study, but the presence of -CF₂H end groups raises interesting consequences from C-H···X hydrogen bonding proposed by Ellison.

Zisman studied solid surface monolayers of acids having -CF₃ and -CF₂H end groups and reported 5° lower contact angles for the latter. For polystyrene-b-semifluorinated block copolymers, Ober found the presence of -CF₃ terminated side chains resulted in 21° lower advancing contact angles (θ_adv) compared to -CF₃ terminated analogs. Other studies on liquid crystalline structures showed surface energy differences as a result of replacement of -CF₃ by -CF₂H end groups. Not surprisingly, replacement of terminal -CF₃ by –CF₂H groups results in markedly decreased surface tensions.

While prior studies focused on wetting behavior for monolayers and glassy surfaces that include liquid crystalline systems well below order-disorder transition temperatures, we became interested in elucidating the wetting behavior of surfaces having –CF₂H terminated side chains in a relatively unconstrained amorphous domain. The near surface morphology of polyurethanes depicted in Figure 2.1 has been established by physical methods such as XPS, contact angle
measurements,\textsuperscript{58} and AFM imaging\textsuperscript{45,59} Thus, the wetting behavior for polyurethanes having soft blocks with short -CF\textsubscript{2}H terminated side chains is expected to be unconstrained by the formation of ordered domains characteristic of 8 and 10 carbon side chains.

Elaborating on preliminary work,\textsuperscript{60} polyoxetane diols P[4F], having a -CF\textsubscript{2}CF\textsubscript{2}H side chain and P[8F] with a -CF\textsubscript{2}(CF\textsubscript{2})\textsubscript{2}CF\textsubscript{2}H side chain were prepared by ring opening polymerization (ROP) of the respective monomers (4F and 8F) following the method of Malik.\textsuperscript{61} These polyoxetane diols were incorporated into polyurethanes that have been studied by XPS, AFM and determination of dynamic contact angles.

Interestingly, dynamic contact angle measurements (DCA, Wilhelmy plate) show wetting behavior that contrasts with previously investigated –CF\textsubscript{2}H systems.\textsuperscript{54-57} The differentiated wetting behavior is presented and discussed with regard to a model for high contact angle hysteresis.

2.2 Experimental Section

Materials: 2,2,3,3,-Tetrafluoropropan-1-ol and 2,2,3,4,4,5,5,-octafluoro-1-ol were generously provided by Daikin Industries, Yodogawa, Japan. 3-Bromomethyl-3-methyl oxetane (BrOx) was a gift from OMNOVA Solutions, Akron, OH. Isophorone diisocyanate (IPDI), tetrabutylammoniumbromide (TBAB), boron trifluoride dietherate (BF\textsubscript{3}·Et\textsubscript{2}O), dibutyltin dilaurate catalyst (T-12) and trifluorotoluene (TFT, 99+%)\textsubscript{3}) were from Aldrich. Butane diol (BD),
Figure 2.1. Schematic of near surface polyurethane morphology.
tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained from Acros Organics (99+%). BrOx was vacuum distilled at 85°C/5 mmHg. IPDI, BD, fluorinated alcohols, catalysts and organic solvents were used as received.

**Synthesis: Monomers:** 3-Methyl-3-(2,2,3,3,-tetrafluoropropoxymethyl)oxetane, (4F), and 3-methyl-3-(2,2,3,3,4,4,5,5-octafluoropentyloxymethyl)-oxetane, (8F), were synthesized by replacing Br in BrOx with fluorinated alcohols using phase transfer catalysis (Scheme 2.1).

As an example for 4F, 41.25 g (250 mmol) BrOx, 46.2 g (350 mmol) 2,2,3,3,-tetrafluoropropan-1-ol and TBAB (5 g, 0.0125 mmol) were heated to 60 °C in 20 ml water. KOH (15.78 g, 87%) was dissolved in 20 ml water and added dropwise over 1 hr. The mixture was held at 75 °C with stirring for 72 hr. 4F was extracted from the aqueous layer using CH₂Cl₂. The resulting solution was dried with magnesium sulfate and solvent was evaporated using a rotovap. GC-MS showed a small percentage of BrOx. Short path distillation gave 99%+ 4F monomer. 8F monomer was prepared in a similar manner.

**Poloxetane diols:** 4F diol and 8F diol were prepared by cationic ring opening polymerization as described previously (Scheme 2.2). In brief for 4F, a three necked round bottom flask was placed in a cooling bath, purged with N₂ for 45 min and CH₂Cl₂ (7 mL) was added. BD (0.23g, 2.54 mmol) then BF₃·Et₂O (0.73g, 5.13 mmol) were injected via syringe. A refrigeration system (PolyScience- Model 912) was used to cool the solution to -9 °C. 4F monomer (5.54 g, 25.65 mmol) in 7.0 ml CH₂Cl₂ was then added. After overnight stirring (13 hr), the solution was warmed to room temperature washed sequentially with 3 wt% HCl (aq) and 3% NaCl (aq) and added drop wise to MEOH:H₂O (3:1, v:v) for precipitation. The fluorous diol layer was separated and placed in a vacuum oven for solvent evaporation (40 °C, 36 hr).
Scheme 2.1. Synthesis of 4F (x=1) and 8F (x=3) monomers.

\[ 
\begin{align*}
\text{Br} & \quad \text{CF}_2\text{H(CF}_2)_x\text{CH}_2\text{OH} \\
\text{TBAB} & \quad \text{KOH in water} \\
\text{H}_2\text{O} & \quad 70^\circ\text{C, 48hrs} \\
\end{align*}
\]

\[ 
\begin{align*}
x = 1; & \quad 4F \\
x = 3; & \quad 8F \\
\end{align*}
\]
Scheme 2.2. Synthesis of P[4F] and P[8F] diols; 4F (x=1) and 8F (x=3)
A number of reactions were carried out with monomer : catalyst ratios of 10:1 and 20:1. Under these conditions $M_n$ ranged from 4.8 to 11.6 kDa. There was no systematic dependence of molecular weight on monomer to catalyst ratio. A 4F sample with $M_n$ of 10 kDa, designated P[4F-10] was chosen for polyurethane synthesis. P[8F-9.8] was prepared following the same procedure.

**Polyurethanes**: Polyurethanes were made by the conventional soft block first procedure. All polyurethanes contained 40 wt% hard block IPDI-BD. The polyurethanes are designated as U[4F-10] and U[8F-9.8].

Briefly, as one example, P[4F-10] (3.6 g, 0.36 mmole) in 1.5 ml of THF was added to a three-necked round bottom flask containing IPDI (1.75 g, 7.58 mmole). The solution was heated to 70 °C under nitrogen purge. Dibutyltin dilaurate catalyst (4 drops, 10 wt% in THF) was added. The reaction was followed using FT-IR, i.e., the growing carbonyl peak at 1716 cm$^{-1}$. After 4 hrs, the carbonyl peak remained unchanged indicating the completion of prepolymer reaction. After the prepolymerization step, 1,4-butandiol (0.65 g, 7.21 mmole) in 5.5 ml THF was added drop wise. The mixture was kept at 70 °C until complete disappearance of NCO peak at 2267 cm$^{-1}$ (~3 hr). The mixture was cooled to room temperature and added drop wise to methanol/water (1:3) to affect precipitation of the polyurethane. Residual solvent was evaporated under vacuum at 60 °C for 48 hr.

U[8F-9.8] was prepared similarly using trifluorotoluene (TFT) as solvent.

**Characterization**: Monomer purity was established using GC-MS (HP-6890 series). Monomers with less than 98% GC purity were further distilled to obtain purity > 99%. $^1$H-NMR spectra for fluorous diols and polyurethanes were obtained on a Varian Inova 400 MHz spectrometer in $d$-chloroform. Fluorous diol molecular weights ($M_n$) were determined by end group analysis. Gel Permeation Chromatography (GPC, Viscotek) was used to determine $M_n$ and $M_w$. 

22
employing polystyrene standards. A TA-Q 1000 series™ (TA instruments) Temperature Modulated Differential Scanning Calorimetry (MDSC) was used for determination of thermal transitions at a heating rate of 3 °C/min. and +/- 0.5 °C modulation every 60 sec.

**Coatings**: Specimens for contact angle measurements were prepared from THF solutions (10wt%). Cover slips (Corning, 24 x 40 x 0.5mm) were dipped in polymer solution to obtain evenly distributed, homogenous surfaces on both sides. Solvent was removed at 25 °C for 4 hrs and then at 60 °C over night under reduced pressure. Coatings for AFM studies were made by drip coating the same solutions on glass cover slips.

### 2.3 Instrumentation

**Modulated Differential Scanning Calorimetry**

M-DSC was done with a TA-Q 1000 Series instrument (TA Instruments) with modulation amplitude of ±0.5 °C and modulation period of 20 s. The sample (5−15mg) was equilibrated at −90 °C followed by a heating ramp of 15 °C/min to 180°C. Zinc, tin, and lead standards were used for energy and temperature calibration.

**X-Ray Photoelectron Spectroscopy (XPS)**

XPS was carried out with a Thermo Fisher Scientific ESCALAB 250. This instrument has monochromatized Al K α X-ray and low energy electron flood gun for charge neutralization. X-ray spot size was approximately 500 mm. Pressure in the analytical chamber during spectral acquisition was less than 2 x 10⁻⁸ Torr Pass energy for survey spectra was 150 eV. The take-off angle was 90 °. The data were analyzed with the Thermo Avantage software (v4.40).
**Atomic Force Microscopy (AFM)**

Morphological analyses of polyurethane surfaces were carried out using a Dimension-3100 (Digital Instruments, CA) atomic force microscope with a NanoScope V controller. Imaging was performed in tapping mode using a microfabricated silicon cantilever (40 N/m, Veeco, Santa Barbara, CA) in air. The tapping force was increased from soft to hard by decreasing the setpoint ratio $r_{sp}$ or $A_{exp}/A_o$, where $A_o$ is free oscillation amplitude and $A_{exp}$ is the experimental oscillation amplitude. Images were analyzed by using NanoScope v710r1 software.

**Wetting Behavior**

Dynamic contact angles (DCA) were obtained using a Cahn Model 312 Analyzer (Cerritos, CA). The surface tension of the probe liquid (Nanopure water) was checked before each measurement and was typically 72.6 ± 1 dyn/cm. Beakers used for DCA analysis were cleaned by soaking in an isopropanol/potassium hydroxide base bath for at least 24 h, rinsing with nanopure water and treated with a gas/oxygen flame.

DCA measurements were based on the Wilhelmy plate method. A coated slide is attached to the electrobalance via a clip. The stage with the beaker of water was raised and lowered with a speed of 100 μm/s. Resulting force versus distance curves (fdc’s) are used to calculate advancing ($\theta_{adv}$) and receding ($\theta_{rec}$) contact angles. The dwell time between the advancing and receding test segments was 1 sec. Five cycles (~3 min/cycle) in succession were obtained to study any change in wetting behavior on exposure to water.

**2.4 Results and Discussion**

**Monomers.** Monomers 4F and 8F, (Scheme 2.1) were prepared in high yield by the Williamson synthesis. Several ring opening polymerization reactions were carried out with varying monomer to catalyst ratios. $^1$H-NMR end group analysis (Figure 2.2) gave $M_n$ of 6.5-
11.0 kDa for P[4F] and 5.2-9.8 kDa for P[8F]. Under the reaction conditions employed there was no systematic trend of $M_n$ and monomer to catalyst ratio.

Polyoxetane diols P[4F-10] and P[8F-9.8], were selected for further study. Confirmation of structures was obtained from $^1$H-NMR spectroscopy. Figure 2.2 represents $^1$H-NMR spectra with peak assignments for monomer 4F and P[4F-10] and P[4F-10] after treatment with TFAA. Two doublets at 4.43 and 4.62 ppm were assigned to methylene protons (e) of the oxetane ring in 4F. The disappearance of those signals indicates the full conversion of monomer 4F into P[4F-10] during ring opening polymerization. Moreover, all protons in P[4F-10] shifted to higher field compared to monomer 4F. This observation is typical for the oxetane derivatives as reported previously.  

To determine the number average molecular weight $M_n$ for P[4F-10], the polymer solution in CDCl$_3$ was treated with excess of TFAA for 30 min at 40°C followed by $^1$H-NMR analysis (Scheme 2.3). Degree of polymerization was calculated from the sum of integrals of the shifted methylene protons (e) next to trifluoroacetyl end groups against the methyl proton integral. End group analysis reveals that the P[4F-10] has a number average molecular weight of 10 kDa.

**Polyurethanes.** U[4F-10], where IPDI/BD (hard block) is 40 wt% and 4F $M_n$ is 10 kDa, was prepared in THF/DMF while TFT was used for U[8F-9.8]. The two polyurethanes are designated U[4F-10] and U[8F-9.8]. GPC analysis of P[4F-10] gave $M_w = 36.1$ kDa and $M_n = 21.2$ (PDI = 1.7). P[8F-9.8] could not be characterized by GPC due to poor solubility in THF.

**Modulated differential scanning Calorimetry**

Thermal analysis by modulated DSC gave glass transition temperatures of $-46$ °C for P[4F-10] and $-56$ °C for P[8F-9.8] (Figure 3). The 10 °C lower $T_g$ for P[8F] was surprising considering
Scheme 2.3. End-group functionalization with TFA; Rf = -CH$_2$O-CH$_2$-CF$_2$-CF$_2$H.
Figure 2.2. $^1$H-NMR spectrum of 4F, P[4F-10] diol and P[4F-10]-TFA (end group peaks circled).
the longer fluorous side chain. No thermal transitions were observed at higher temperatures confirming the amorphous state of these viscous liquid diols above $T_g$.

Thermograms for the two polyurethanes along with their pure soft blocks are shown in Figure 2.3.

Compared to P[4F-10], the $T_g$ for the U[4F-10] soft block shifts upward by 21°C. The shift in $T_g$ for 8F-9.8 diol to U[8F-9.8] is 10°C. Based on the difference in $T_g$’s, the Fox equation (Eq 2.1) was used to estimate the percent soft block in the pure soft block domain (Table 2.1).

\[
(T_g)^{-1} = w_{SOFT} (T_g^{SOFT})^{-1} + w_{HARD} (T_g^{HARD})^{-1}
\]

Eq 2.1

The calculations were based on the glass transition temperature of pure hard block as 85°C. Based on % soft block in the pure soft block domain obtained from the FOX equation we can see that there is more phase mixing for U[4F-10] than for U[8F-9.8]. Less phase mixing for U[8F-9.8] is attributed to the minimal intermolecular interactions and low solubility parameter of the four –CF$_2$ groups counteracting –CF$_2$H hydrogen bonding.

**AFM.** Atomic Force Microscopy was used to analyse the surface morphology of the two polyurethanes. The 10 x 10 µm images shown in Figure 2.4 for U[4F-10] and U[8F-9.8] were taken at $r_{sp}$=0.7 which is relatively hard tapping. The setpoint ratio of 0.7 was chosen to readily observe the presence of near surface hard block for the polyurethanes.

Both polyurethanes show near surface phase separation which confirms two phase morphology of the materials. It is however observed that the size of phase separated features is different for the two polyurethanes. The U[4F-10] shows nanoscale phase separations and very low
Figure 2.3. Thermograms for polyurethanes and precursor polyols – A) Dashed line - U[4F-10], solid line – P[4F-10], B) Dashed line U[8F-9.8], solid line- P[8F-9.8].
Table 2.1. Calculation of phase mixing in U[4F-10] and U[8F-9.8]

<table>
<thead>
<tr>
<th></th>
<th>Soft block $T_g$ (polyurethane) ($^\circ$C)</th>
<th>Soft block $T_g$ (Pure) ($^\circ$C)</th>
<th>Soft block in pure soft block (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U[4F-10]</td>
<td>-25</td>
<td>-46</td>
<td>77</td>
</tr>
<tr>
<td>U[8F-9.8]</td>
<td>-46</td>
<td>-56</td>
<td>89</td>
</tr>
</tbody>
</table>
Rq of 5.7 nm. The P[8F-9.8] however shows a slightly different morphology with two scales of phase separation – (a) microscale phase separation (Figure 2.4) 2-3 µm fringed circular features and (b) nanoscale phase separation.

To further investigate the surface features, images were obtained at a scan size of 1 x 1 µm (Figure 2.5). The 1 x 1 µm images show well defined nanoscale phase separated domains for both U[4F-10] and U[8F-9.8]. This is similar to what has been observed for copolyoxetane soft blocks with trifluoroethoxymethyl and polyethylene oxide (3F-b-ME3) side chains. The model (Figure 2.6) for the self-aggregation of the 3F moieties to form Janus-like nanostructures proposed by Zhang fits the observations for U[4F-10] and U[8F-9.8]. It is however observed that for the 8F soft blocks the self-aggregation of fluorinated nanodomains is more pronounced giving rise to microscale features. This can be attributed to the fact that due to the longer chain length in the 8F soft blocks, demixing is relatively higher and is driven by the self-association of the fluorous moieties forming larger aggregated microscale features.

**XPS.** Atomic percentages of carbon, fluorine, nitrogen and oxygen was analyzed by XPS. The table given below shows that there is a higher percentage of fluorine in the U[8F-9.8] than U[4F-10] as expected.

The data also shows the presence of nitrogen on the near surface of both the polyurethanes. Since nitrogen is present only in the hard block, this confirms the observation from the Differential Scanning Calorimetry measurements that both polyurethanes have phase mixing of soft and hard blocks. The observed percentage of nitrogen is however less than the calculated bulk percentage if there was 100% phase mixing. The higher percent of nitrogen in U[4F-10] confirms higher phase mixing.
Figure 2.4. 10 x 10 µm TM-AFM images at $r_{mp}=0.7$: U[4F-10], a) 2D Height (x= 500 nm) b) phase; U[8F-9.8], c) 2D Height (x= 500 nm); d) phase.
Figure 2.5. 1 X 1 µm TM-AFM images at $r_p=0.7$: U[4F-10], a) 2D Height (x= 500 nm) b) phase; U[8F-9.8], c) 2D Height (x= 500 nm); d) phase.
Figure 2.6. Model for surface nanoscale phase separation for the fluorous soft blocks.
Table 2.2. Atomic percentages of elements in U[4F-10] and U[8F-9.8]

<table>
<thead>
<tr>
<th>Name</th>
<th>U[4F-10]</th>
<th>U[8F-9.8]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated -U</td>
<td>Calculated-soft block only</td>
</tr>
<tr>
<td>F1s</td>
<td>16.6</td>
<td>28.6</td>
</tr>
<tr>
<td>O1s</td>
<td>15.8</td>
<td>14.3</td>
</tr>
<tr>
<td>C1s</td>
<td>63.7</td>
<td>57.1</td>
</tr>
<tr>
<td>N1s</td>
<td>3.9</td>
<td>2.77</td>
</tr>
</tbody>
</table>
High resolution C1s spectra reveal signals from 5 different carbon environments of varying binding energies extending over an interval of approximately 9 eV. The peak assignments given in Table 2.3 are based on characteristic binding energies already established. Unexpectedly, a peak at 293 eV for –CF$_3$ is seen for both the polyurethanes. Fluorocarbon moieties are known to be unstable under X-ray exposure during XPS analysis. For example, poly(vinylidene fluoride) undergoes elimination of HF on being exposed to high energy electron beam. It is assumed that there is transformation of –CF$_2$H to –CF$_3$ in the X-ray chamber. The mechanism for this transformation is unclear. For the purpose of analysis, F from both -CF$_3$ and -CF$_2$ are included in atom % F.

Peak deconvolution was carried out by gaussian distribution for each peak and regression by least square method. The resulting contribution percentages obtained for each carbon bond is summarized in Table 2.4.

Dynamic contact angle (Wilhelmy plate) contact angles. Fundamental wetting behavior of -CH$_2$CF$_2$CF$_2$H and –CH$_2$(CF$_2$)$_3$CF$_2$H side chain soft blocks was sought for comparison with a –CF$_3$ analog and for comparison to previously studied longer, rigid rod analogs having –CF$_3$ and CF$_2$H termini. Dynamic contact angle (DCA) measurements (Wilhelmy plate) were used to investigate wetting behavior. As described previously, the resulting advancing (adv) and receding (rec) force distance curves (fdc’s) provide $\theta_{\text{adv}}$ and $\theta_{\text{rec}}$ via Eq 2.2.

$$ F = \frac{m}{g} = P \gamma \cos \theta \quad \text{Eq 2.2.} $$

where F is the force derived from respective mass (m) changes on immersion and emersion, g is the gravitational constant, $\gamma$ is the liquid surface tension, and $\theta$ is the contact angle.
Figure 2.7. High resolution C1s spectra for U[4F-10] and U[8F-9.8]
Table 2.3. XPS peak assignments and binding energies of U[4F-10] and U[8F-9.8]

<table>
<thead>
<tr>
<th>Structure</th>
<th>U[4F-10]</th>
<th>U[8F-9.8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CF$_3$</td>
<td>293.2</td>
<td>293.0</td>
</tr>
<tr>
<td>-CF$_2$H</td>
<td>290.7</td>
<td>290.5</td>
</tr>
<tr>
<td>-OCF$_2$CH$_2$</td>
<td>289.4</td>
<td>289.2</td>
</tr>
<tr>
<td>-C-C-O</td>
<td>286.4</td>
<td>286.1</td>
</tr>
<tr>
<td>-C-C-C</td>
<td>284.8</td>
<td>284.6</td>
</tr>
</tbody>
</table>
Table 2.4. Percent contribution of each carbon environments for U[4F-10] and U[8F-9.8].

<table>
<thead>
<tr>
<th>Structure</th>
<th>U[4F-10] (at % C)</th>
<th>U[8F-9.8] (at % C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CF₃</td>
<td>1.2</td>
<td>3.3</td>
</tr>
<tr>
<td>-CF₂H</td>
<td>12.0</td>
<td>13.3</td>
</tr>
<tr>
<td>-OCF₂CH₂</td>
<td>4.9</td>
<td>4.2</td>
</tr>
<tr>
<td>-C-C-O</td>
<td>44.9</td>
<td>50.3</td>
</tr>
<tr>
<td>-C-C-C</td>
<td>37</td>
<td>28.9</td>
</tr>
</tbody>
</table>
Extrapolating an fdc to the point of maximum (or minimum) initial mass upon immersion eliminates the need for a buoyancy correction to F.

An important step after DCA analysis comprises measuring post-test water surface tension. Any diffusion of contaminants from the coating changes water surface tension and hence contact angles. This simple test provides important information that differentiates whether contact angles as a function of repeated immersion is due to surface reorganization or water contamination.\textsuperscript{71}

Ring opening polymerization leads to the production of cyclic species. Such immiscible species diffuse into water and, due to lower density, concentrate on the water surface. Diffusion of such species is rapid for polysiloxane coatings and invariably effects both the accuracy and precision of contact angle measurements.\textsuperscript{71} For polyurethanes with P[3F-3.4], P[4F-10], and P[8F-9.8] soft blocks, the change in surface tension of the test water compared to pristine water was minimal. Thus, except for slight water contamination by U[3F-3.4], contact angles are used with confidence to assess relative wetting behavior and extent of surface reorganization during immersion.

The total immersion time after five cycles is \~15 min. For U[3F-3.4] $\theta_{\text{adv}}$ is 102°, which is in the > 100° range expected for a fluorous polymer at the air-liquid-solid interface.\textsuperscript{70} A 4° change in $\theta_{\text{adv}}$ was observed after several fdc cycles (Table S2). Part of this diminution (estimated 1-2°) is due to slight water contamination that was not eliminated despite several reprecipitations of the polyurethane. The receding contact angle ($\theta_{\text{rec}} = 46°$) and contact angle hysteresis ($\Delta_\theta = \theta_{\text{adv}} - \theta_{\text{rec}}$) for U[3F-3.4] ($\Delta_\theta = 52°$) reflect the presence of a surface soft block that rapidly undergoes enthalpically driven reorganization characteristic of “soft” surfaces.
Water hydrogen bonding to the -CH₂ group adjacent to -CF₃ was proposed previously to account for this observation.⁷²,⁷³ Hydrogen bonding to ether oxygens (side chain and main chain) may also occur.

Compared to U[3F-3.4], U[4F-10] has a 6° higher initial θ<sub>adv</sub> of 108° and a 6° lower θ<sub>rec</sub> (40°). This result stands in contrast to prior studies that compared contact angles for -CF₃ and –CF₂H terminated systems for which decreases of 10-20° for θ<sub>adv</sub> -(CF₂)₇CF₂H and similar side chain termini were found compared to –CF₃ analogs.⁵⁵-⁵⁷ Compared to U[3F-3.4], U[8F-9.8] also has a higher initial θ<sub>adv</sub> of 108° and an even lower θ<sub>rec</sub> (30°).

U[4F-10] has an initial contact angle hysteresis (Δθ) 68°. After five cycles, Δθ decreased to 62° mainly due to a decrease in θ<sub>adv</sub>. This decrease is ascribed to thermodynamically driven surface reorganization involving water adsorption driven by hydrogen bonding. After drying, the five cycles shown are once again reproduced. U[8F-9.8] had an even higher Δθ of 78°. In contrast, for block copolymers with –(CF₂)₇CF₂H and similar side chain termini Δθ of 7 to 19° were reported.⁵⁵,⁵⁷

Models are shown for wetting behavior of surfaces having long –(CF₂)₇CF₃ (Fig 1.9A) terminated side chains and analogous –(CF₂)₇CF₂H (Fig 1.9B). These models are applicable to long side chain fluorous methacrylates investigated by Katano⁴⁷ and Takahara⁴⁸ and to Ober’s poly(styrene-b-semifluorinated isoprene) block copolymers.⁵⁵ Surfaces with –(CF₂)₇CF₃ (and longer) terminated side chains are characterized by high contact angles and low Δθ (Figure 2.9A). Such systems are enthalpically stabilized by the formation of ordered phases driven by side chain crystallization.
Surfaces having \(-(\text{CF}_2)_n\text{CF}_2\text{H}\) and similar side chain termini have lower \(\theta_{\text{adv}}\) but retain low \(\Delta\theta\) in the 7 to 19\(^\circ\) range.\(^{55,57}\) The lower \(\theta_{\text{adv}}\) was ascribed to the dipolar nature of the \(-\text{CF}_2\text{H}\) end group that enhances hydrogen bonding highlighted in Figure 2.9B-i.\(^{55}\) The formation of ordered phases stabilizes the \(-\text{CF}_2\text{H}\) surface against reorganization on short time scales, but over the course of weeks contact angles decrease.\(^{55}\)

Surfaces with a single \(-\text{CF}_3\) terminal group as in U[3F-3.4] are depicted in Figure 2.9C. These surfaces have fairly high \(\theta_{\text{adv}}\) (ca. 106\(^\circ\)C), though not as high as well ordered surfaces having, longer \(-(\text{CF}_2)_n\text{CF}_3\) terminated side chains (Figure 2.9A). The amorphous nature of the soft blocks, which are too short to form ordered side chain phases results in low \(\theta_{\text{rec}}\) and high contact angle hysteresis (\(\Delta\theta = 52\^\circ\)).

Figure 2.9D shows a model for the wetting behavior of U[4F-10]. Here, hydrogen bonding of \(-(\text{CF}_2)_n\text{CF}_2\text{H}\) to the side chain ether oxygen with the formation of a five membered ring is proposed. Other dipolar interactions of \(-\text{CF}_2\text{H}\) to main chain ether oxygens may occur (not shown). Recalling that thermal analysis data suggest incomplete soft block-hard block phase separation, even \(-\text{CF}_2\text{H}\) hydrogen bonding to near surface amide carbonyl moieties may occur.
Figure 2.8. Dynamic contact angle force distance curves for A) U[3F-3.4], B) U[4F-10], C) U[8F-9.8]; solid line: 1st cycle, dashed line: 5th cycle.
Figure 2.9. Wetting models for dry (d) and immersed (i) surfaces: A rigid –CF₃ terminated side chains; B, rigid –CF₂H terminated side chains; C, 3F side chains and D, 4F side chains. H-bonding interactions are highlighted; terminal –CF₂H hydrogen bonding is highlighted and circled; 4FOx-ether H-bonding is designated with an arrow. In D-d, shaded semicircle highlights –CF₂CF₂- surface concentration driven by -CF₂H hydrogen bonding to side chain oxygen.
2.5 Conclusion

The wetting behavior of the polyurethanes described in this study with unique short chain soft blocks having –CF₂H chain ends present interesting possibilities. The presence of fluorine in these soft blocks aids in surface concentration of the soft block as is observed in the XPS results. The model for the wetting behavior also predicts the stability of these soft blocks on the surface not only in dry but also wet conditions. In their work with polyurethanes having P[AB] copolyoxetanes as soft blocks where A has a fluorinated and B has quaternary ammonium side chains respectively, Kurt has shown the excellent antimicrobial properties exhibited by these polyurethanes. Kurt’s study involved the use of fluorinated oxetanes with side chains having –CF₃ end groups. While studying the stability of positive charge on the surface of these polyurethanes, Gupta⁷⁴ has reported the rapid loss in surface accessible positive charge over a period of 80 seconds. The new polyurethanes discussed in this work opens the possibility of stabilizing the positive charge on the surface by replacing the –CF₃ end group with a –CF₂-CF₂H end group. Due to the amphiphilic nature of the –CF₂-CF₂H based side chains, they can act as a better chaperone for the quaternary ammonium side chains on the surface of a polyurethane.
Table S2.1. Monomer/initiator ratio vs molecular weight for 4F

<table>
<thead>
<tr>
<th></th>
<th>monomer/initiator ratio</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[4F]-2</td>
<td>20:01</td>
<td>5800</td>
</tr>
<tr>
<td>P[4F]-3</td>
<td>20:01</td>
<td>11600</td>
</tr>
<tr>
<td>P[4F]-4</td>
<td>20:01</td>
<td>4760</td>
</tr>
<tr>
<td>P[4F]-5</td>
<td>20:01</td>
<td>10150</td>
</tr>
<tr>
<td>P[4F]-6</td>
<td>10:01</td>
<td>6930</td>
</tr>
<tr>
<td>P[4F]-7</td>
<td>10:01</td>
<td>10000</td>
</tr>
<tr>
<td>P[4F]-8</td>
<td>10:01</td>
<td>8100</td>
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Table S2.2. Contact angles for polyurethanes (5 cycles)

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>Cycle</th>
<th>$\theta_{\text{adv}}$</th>
<th>$\theta_{\text{rec}}$</th>
</tr>
</thead>
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<tr>
<td></td>
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</tr>
</tbody>
</table>
Chapter 3.

Fluorous / quaternary polyurethane modifiers for coatings with enhanced antimicrobial stability.

3.1 Introduction

Antimicrobial coatings offer promise in helping curb the spread of infections for applications such as biomedical devices, molded articles, keyboards and touchpads.\(^1\) Development of biocidal coatings for medical devices is one of the most important areas of research because of the risk of infections related to urinary catheters, cardiovascular devices, and hip replacement devices.\(^75\) The need for advances in contact kill coatings is clear due to the growing problem of bacterial resistance to antibiotics and even biocides.\(^12, 13, 76\)

Over 30 years ago Isquith demonstrated contact kill for glass surfaces functionalized with alkylammonium moieties and concomitant absence of “zone of inhibition” characteristic of biocide release.\(^77\) The rapid kill of bacteria by covalently bound alkylammonium functionality is due to chemisorption of bacteria, which have negatively charged outer membranes. Cell death occurs due to membrane disruption. Because contact kill precludes biocide entry into the metabolic process, bacterial buildup of resistance does not occur.\(^19, 25, 78\) Contact kill promises long term antimicrobial stability.

Polycation thin films have shown contact kill on glass and silicon wafers\(^13, 19, 32, 33, 79-84\) and fibers.\(^85\) Numerous methods are known for attaching polycations or their precursors to surfaces, including chemical grafting,\(^86, 87\) layer-by-layer deposition\(^88\) and plasma polymerization.\(^89\) Development of easily utilized contact antimicrobial coatings has seen less attention.
Alkylammonium modification of a polyurethane hard blocks was reported by Cooper, but this approach compromised bulk mechanical properties and achieved only modest contact kill effectiveness. Alkylammonium coatings based on polyethylene imine (PEI) have been reported by Klibanov. PEI and a four-component PEI copolymer were alkylated and dip coated onto substrates. Optimum compositions were effective against both Gram(+) and Gram(-) bacteria.

Regardless of antimicrobial effectiveness, translation to applications is unlikely for elaborate surface functionalization methods. For coatings, accessible surface antimicrobial function is required but such functionality jeopardizes bulk properties. Cooper’s thorough investigation brought to light the compromise of bulk properties via incorporation of quaternary functionality, but bulk properties for polycation systems is not usually addressed.

A blend approach to creating desired surface functionality while preserving bulk properties is illustrated in Figure 2.1 This strategy was discussed in detail previously. In brief, this approach (1) leverages thermodynamically driven surface concentration of soft blocks in polyurethanes. (2) utilizes copolyoxetane soft blocks with A and B repeat units that generate a synergistic, functional pair, e.g., the A repeat unit may act as a “chaperone” to surface-concentrate B groups, and (3) achieves compositional economy by using the P[AB]-soft block polyurethane as a minor blend constituent such that PSM defines the surface properties and the matrix polymer defines bulk mechanical properties and adhesion to substrate.

Noting the pioneering research of Worley, a hydantoin PSM was successfully employed for oxidative –N-Cl biocidal function. A systematic increase in biocidal effectiveness was found with increasing PSM concentration up to an asymptote of ~ 1.6 wt%.
Figure 3.1. Schematic for a conventional polyurethane (soft block, solid line) modified with a PSM having a copolymer soft block (dashed line) with A and B side chains (one set shown).
Figure 3.2 Components of HMDI-BD-P[(3FOx)(C12)-86:13-Mn]
Figure 3.3. Components of matrix polyurethane- HMDI-BD(50)-PTMO(1000)
Employing the PSM model (Figure 3.1) HMDI-BD(30)-P[(3F0x)(C12)-87:13-6.5] U-1, Figure 3.2, was generated as a PSM. The selected matrix polyurethane HMDI/BD(50)-PTMO is shown in Figure 3.3.

In a spray test, 2 wt% PSM U-1 provided 100% kill against a 10^6 CFU/mL aerosol challenge of Gram(-) (P. aeruginosa, E. coli) and Gram(+) (S. aureus) bacteria during an exposure time of 30 min. Excellent contact antimicrobial kill was consistent with U-1 surface concentration by XPS analysis.

A subsequent investigation of stability for U-1 modified coatings showed a rapid loss of antimicrobial effectiveness over two weeks at ambient conditions. This loss of contact kill was correlated with a rapid decrease in streaming potentials, which scale to surface accessible charge density. Coatings with 1 wt% U-1 modifier showed a precipitous decrease (~50%) in streaming potentials in less than two minutes, that is, four measurement cycles (Figure 3.4). In summary, U-1 modified base polyurethane displayed slow loss of surface accessible quaternary charge in air, and worse, rapid loss in the presence of the 10^{-3} M NaBr used as an electrolyte for streaming potential measurement.

Changes in topography and morphology accompany loss of near surface quaternary charge are available separately. From these studies it is apparent that, phase separation is driven by incompatibility of the fluorous moieties in U-1 (Fig 3.1) Even though the overall concentration of U-1 used in these surface modification studies was low (0.5 – 2 wt%) the high ratio of 3F to C12 led to near-surface concentration and phase separation similar to that observed for fluorous modifiers alone.
Figure 3.4. Streaming potentials vs. pumping cycle (20 sec/cycle) for: A, neat P[(3F0x)(C12)] modifier and B, 2 wt% modified base polyurethane.
The unexpected temporal instability of quaternary charge led to a focus on stabilization strategies. In considering the P[AB] soft block, replacement of 3F with amphiphilic 4F seemed an attractive option based on the notion that -CF_2H hydrogen bonding would provide an enthalpic contribution (ΔH_{mix}, Eq 3.1) to the free energy of mixing so as to disfavor phase separation. Such H-bonding was proposed by Ellison to explain decreased surface tension upon replacement of terminal -CF_3 by –CF_2H.\textsuperscript{54}

\[ \Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T\Delta S_{\text{mix}} \quad \text{Eq 3.1} \]

Taking into account enthalpically driven hydrogen bonding, a model comparing –CF_3 terminated side chains with –CF_2H based side chains has been discussed. wherein the amphiphilic nature of a –CF_2H based side chain has been discussed. It was hypothesized that a –CF_2H side chain is enthalpically stabilised in dry and wet conditions and hence is less prone to surface phase separation. Keeping this hypothesis in mind, a new strategy has been explored whereby a fluorinated side chain with a –CF_2H end group instead of 3F, is utilized as a “chaperone” for the quaternary group (C12).

**Acronyms/Designations**

The P[AB] telechelic diols with 4FOx (4F) and N, N´dimethyl dodecylamine (C12) as side chains are designated as P[(4F)(C12)-x: (1-x)-M_n] with x representing the 4F percentage in the diol and (1-x) is the percentage of C12. M_n is the number average molecular weight of the telechelic obtained by \textsuperscript{1}H-NMR end group analysis. The polyurethane PSM containing 4,4´-(methylene bis(p-cyclohexylisocyanate) (H_{12}MDI) and butanediol (BD) as the hard block and P[(4F)(C12)-x: (1-x)-M_n] as the soft block is designated as U[(4F)(C12)-x: (1-x)-M_n].
3.2 Experimental Section

Materials

\(N,N'-\text{Dimethyl}dodecylamine\) (C12) was generous gift from Lonza (Allendale, NJ). 3-Bromomethyl-3-methyl oxetane (BrOx) was a gift from OMNOVA Solutions, Akron, OH. Methylene chloride (CH\(_2\)Cl\(_2\)), dimethylformamide (DMF), dimethylacetamide (DMAc) and tetrahydrofuran (THF) were obtained from Aldrich and dried by storing over 4 Å molecular sieves. Boron trifluoride dietherate (BF\(_3\)OEt\(_2\)), 4,4'-\(\text{methylene-bis}(p\text{-cyclohexyl isocyanate})\) (HMDI), dibutyltin dilaurate catalyst (T-12), tetrabutylammonium bromide (TBAB), 1,4-dibromobutane diethyl carbonate and 1,1,1-tris-(hydroxymethyl)ethane, 2,2,3,3,-tetrafluoropropan-1-ol were also obtained from Aldrich and used as received. 1,4-Butanediol (BD) and 2-(2-methoxyethoxy) ethanol were purchased from Acros Chemicals and used as received.

Synthesis of monomers

\textbf{4F:} 3-Methyl-3-(2,2,3,3,-tetrafluoropropoxymethyl)oxetane (4F), was synthesized by substituting Br in BrOx with fluorinated alcohols using phase transfer catalysis (TBAB). A typical synthesis involved combining 41.25 g (250 mmol) of BrOx with 46.2 g (350 mmol) of 2,2,3,3,-tetrafluoropropan-1-ol with in presence of TBAB (5 g, 0.0125 mmol) followed by heating to 60 °C in 20ml of water. KOH (15.78g, 87%) was dissolved in water (20 ml) and added drop wise over one-hour period. This solution was then heated to 75 °C and stirred for 72 hr. The resulting 4F oxetane is separated from the aqueous layer using CH\(_2\)Cl\(_2\). The resulting solution was dried with magnesium sulfate and solvent stripped using a rotovap. GC-MS showed a small percentage of BrOx. Short path distillation gave 99%+ 4F monomer.
**BBOx:** The precursor to BBOx (bromobutyl oxetane) is 3-(hydroxymethyl)-3 methyl oxetane (HOOx) which was prepared via the pyrolysis of diethyl carbonate and 1,1,1 tris (hydroxymethyl) ethane. BBOx was prepared from HOOx and dibromobutane via a phase transfer catalysis reaction and is also described in the literature.

**Synthesis of P[(4F)(BBOx)-x:(1-x)-Mn] copolyoxetanes**

Cationic ring opening polymerization was followed for preparation of the copolyoxetanes. Diols with two different ratios of 4F:C12 were prepared to study the effect of changing mole % of C12 on the antimicrobial properties of the surface modifier. The two diols obtained were P[(4F)(BBOx)-86:14] and P[(4F)(BBOx)-66:34].

To produce P[(4F)(BBOx)-66:34], 4F (15.12 g, 70 mmol) and BBOx (7.11 g, 30 mmol) were added to 40 mL of anhydrous CH2Cl2 in a 100 mL round bottom flask. Boron trifluoride dietherate (BF3OEt2) acts as the catalyst and 1,4-butanediol is the initiator and co-catalyst. The monomer to initiator ratio was chosen as 50:1 to obtain the desired molecular weight of 5kDa. Accordingly, 0.18g of BD was added to the reaction mixture. The reaction mixture was then delivered via a metering pump (0.138 mL/min) to a nitrogen purged reaction vessel which contained 30 mL of anhydrous CH2Cl2 and 0.56 g of BF3OEt2. The initiator to catalyst ratio was maintained at 2:1. The reaction mixture was kept at -5 °C overall for 15 hrs. The mixture was warmed to ambient temperature and quenched with 40 mL H2O. The organic layer was sequentially washed with 30 mL of 3 wt % HCl (aq), 30 mL of 3 wt % NaCl (aq) and 30 mL of deionized water. The organic layer was placed in a rotary evaporator at 60 °C for solvent removal. The product was then placed in a vacuum oven at 60 °C for 24 hr. Yield of P[(4F)(BBOx)-66:34] was 91%.
P[(4F)(BBOx)-86:14] was prepared in a similar procedure by changing the monomer mole ratio. The feed details for both diols is given in Table 3.1.

The final (4F)(BBOx) ratio in the telechelic and their molecular weights were determined using \(^1\)H-NMR analysis.\(^{46,60}\) The molecular weight of P[(4F)(BBOx)-86:14] was found to be 4.2 KDa and that of P[(4F)(BBOx)-66:34] was 6.0 KDa. These diols were then quaternized by the substitution of C-Br with N,N dimethyl dodecyl amine (C12) in acetonitrile for 18 hours. A plot of \(^1\)H-NMR spectra and calculation are provided in given in Supplemental information.

A typical reaction for the preparation of P[(4F)(C12)-66:34 involved adding 3.72 g (15% excess) of C12 to 10 g of telechelic in 50 mL of acetonitrile. \(^1\)H-NMR analysis was carried out to confirm complete substitution.

These are then used as soft blocks for making polyurethanes (Figure 3.5) U[(4F)(C12)-86:14-4.2] and U[(4F)(C12)-66:34-6] using the soft block first method, where the ratio of the hard block to soft block was 30:70 (wt/wt).\(^{46}\)

The base polyurethane was synthesized using a two-step solution polymerization, (Scheme 3.1) using PTMO (1000) as soft block and HMDI-BD as the hard block (50 wt%).\(^{35,46,94}\)

**Preparation of blends and coatings**

Taking cue from prior work\(^{90,95}\), 2, 1 and 0.5 wt% blends of the surface modifier in base polyurethane was prepared. These are designated as 2%-U[(4F)(C12)-66:34-6.0], 1%-U[(4F)(C12)-66:34-6.0] and 0.5%-U[(4F)(C12)-66:34-6.0] respectively. Unlike the 3F based polyurethane, 4F polyurethanes were insoluble in THF. DMAC (dimethyl acetamide) was used as an alternative
Table 3.1. Feed for preparation of P[(4F)(C12)-x:(1-x)-Mₙ]

<table>
<thead>
<tr>
<th>Telechelic</th>
<th>4F</th>
<th>BBOx</th>
<th>BD</th>
<th>BF₃</th>
<th>Monomer/Initiator ratio</th>
<th>Calculated Ratio</th>
<th>Observed Ratio</th>
<th>Mₙ from ¹H-NMR (Kda)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[(4F)(BBOx) -86:14</td>
<td>18.36</td>
<td>3.55</td>
<td>0.18</td>
<td>0.57</td>
<td>50:1</td>
<td>85:15</td>
<td>86:14</td>
<td>4.2</td>
</tr>
<tr>
<td>P[(4F)(BBOx) -66:34</td>
<td>15.12</td>
<td>7.11</td>
<td>0.18</td>
<td>0.57</td>
<td>50:1</td>
<td>70:30</td>
<td>66:34</td>
<td>6.0</td>
</tr>
</tbody>
</table>
Figure 3.5. Polyurethane surface modifier with P[(4F)(C12)-p:(1-p)] soft block
Scheme 3.1. Preparation of base polyurethane - HMDI-BD(50)-PTMO(1000).
solvent. Blends of the PSM in DMAC and base polyurethane in THF produced transparent coatings. Over a period of 7 days, the coatings became less transparent, which suggested phase separation of the surface modifier. This prompted the use of DMAC as a solvent for both modifier and base polyurethane. Coatings were prepared by drip coating glass slides and glass cover slips with the blend. Due to the low volatility, DMAC was removed by heating the coatings (120°C), overnight. Transparent coatings were obtained. Due to the low volatility of the solvent dip coating of cover slips for dynamic contact angle measurements was not feasible.

### 3.3 Instrumentation

**Modulated Differential Scanning Calorimetry**

M-DSC was done with a TA-Q 1000 Series instrument (TA Instruments) with modulation amplitude of ±0.5 °C and modulation period of 20 s. The sample (5–15 mg) was equilibrated at −90 °C followed by a heating ramp of 15 °C/min to 150 °C. Zinc, tin, and lead standards were used for energy and temperature calibration.

**X-Ray Photoelectron Spectroscopy**

XPS measurements were carried out on the Thermo Fisher Scientific ESCALAB 250 X-ray photoelectron spectrometer. This instrument has monochromatized Al K α X-ray and low energy electron flood gun for charge neutralization. X-ray spot size for these acquisitions was on the order of 500 mm. Pressure in the analytical chamber during spectral acquisition was less than 2 x 10⁻⁸ Torr. Pass energy for survey spectra was 150 eV. The take-off angle was 90 °. The data were analyzed with the Thermo Avantage software (v 4.40). Coatings with 2, 1 and 0.5 wt% modifier were cut and attached to the sample holder using carbon tape.
Wetting behavior

Static contact angles were obtained by using a Ramé-Hart goniometer equipped with an LCD camera. Deionised water (~18.2 MΩ) system was used as a probe liquid. A 2µL drop was placed on the coating surface and the image was captured immediately and after five minutes to evaluate contact angle stability. Captured images were analyzed to determine contact angles.

Zeta Potential

Surface analysis for zeta potential measurements was carried out using a SurPASS electrokinetic analyzer (Anton PAAR). Drip coated microscope slides were employed. The clamping cell attachment for the SurPASS analyzer was used for measurements of the Zeta Potential on coated glass slides (Figure 3.6).

The electrolyte was 1mmol NaBr (aq).

The zeta potential (ζ) is determined using the Helmholtz-Smoluchowski method (Eq 3.2)

\[
\zeta = \frac{\Delta E}{\Delta P\eta\kappa} \frac{\kappa}{\varepsilon_o}
\]

Here, \(\Delta E\) = measured streaming potential across the channel (V)

\(\Delta P\) = applied pressure

\(\kappa\) = conductivity of electrolyte solution (S/m)

\(\eta\) = viscosity (kg/mS)

\(\varepsilon\) = permittivity of the solution

\(\varepsilon_o\) = vacuum permittivity

\(\zeta\) = zeta potential (V)
Figure 3.6. Schematic for the Anton Paar clamping cell. Active sample area (25 mm × 5 mm) shown in red.
Atomic Force Microscopy:

Morphological analyses of polyurethane surfaces were carried out using a Dimension-3100 (Digital Instruments, CA) atomic force microscope with a NanoScope V controller. Imaging was performed in tapping mode using a microfabricated silicon cantilever (40 N/m, Veeco, Santa Barbara, CA) in air. Images were analysed using the Nanoscope v710 software.

Bactericidal Test

Aerosol spray testing has been used in prior studies of non-leaching biocidal materials and was the primary method of determining biocidal activity of the U[(4F)(C12)] PSM blends. Agar plates were streaked with the desired bacteria from a stock culture kept frozen at -70 °C and incubated at 37 °C for 18-24 hrs. From this plate a single colony was collected and used to inoculate 10 mL of Luria broth. This culture solution was incubated for 18-24 hrs at 37 °C. After incubation, the 1:100 dilution of the culture was prepared and incubated at 37 °C until an optical density of 0.2-0.3, which corresponds to $10^8$ CFU/ml of bacteria, was observed for 1 mL of culture. This culture is then used as the stock suspension in bacterial challenges.

A stock bacteria suspension with a concentration of $10^6$ colony forming units (CFU)/mL was prepared from the above suspension and used for the spray test. Drip coated cover slips were sprayed with this suspension for ~1sec and weighed to determine the amount of bacteria suspension deposited. Sprayed slides were then placed in a constant humidity (95%) environment. After 60 min, the slides were placed in sterile saline solution and vortexed for 2 min. Suspension aliquots (100 µL and 100 µL x 10 dilutions) were removed and spread onto agar plates and incubated at 37 °C for 18 h. After incubation, bacteria colonies were counted to obtain the percent kill.

A schematic of the spray test is given below (Scheme 3.2).
Scheme 3.2. Schematic for aerosol spray test – A. Bacterial suspension sprayed on coated glass cover slips, B. Cover slips stored incubated for 1 hr, C Cover slips vertexed in saline D. Saline solutions diluted, E. 100 µL solutions plated and CFUs counted within 18-24 hr.
3.4 Results and discussions

As discussed earlier, the goal of this study is not only to develop antimicrobial polymeric coatings without affecting the bulk properties of the polymer but also to have coatings that retain their antimicrobial properties over a period of time. To achieve these goals, 2%-U[(4F)(C12)-86:14-4.2] and 2%-U[(4F)(C12)-66:34-6.0] were prepared to obtain preliminary data for down selection of the better PSM. Zeta potential and antimicrobial tests were performed on these coatings. Zeta potential was studied against a standard polypropylene sample in the SurPASS electrokinetic analyzer and it was observed that the 2%-U[(4F)(C12)-66:34-6.0] coating had a higher zeta potential value than the 2%-U[(4F)(C12)-86:14-4.2] coating. Antimicrobial tests with *Pseudomonas aeruginosa* shows better biocidal property for 2%-U[(4F)(C12)-66:34-6.0] (Table 3.2). Hence 2%-U[(4F)(C12)-66:34-6.0] was chosen for further investigations.

**Modulated Differential Scanning Calorimetry**

Thermal analysis of the soft block as well as the surface modifier polyurethane (PSM) was carried out to analyze the extent of phase mixing of the hard and soft blocks. It was observed that the glass transition temperature for P[(4F)(C12)-66:34-6.0] was $-47.5\, ^\circ\mathrm{C}$ which is typical of short side chain P[AB] diols as observed earlier.\(^4^6\) Thermal analysis of the polyurethane showed a shift in the soft block $T_g$ to $-19.8\, ^\circ\mathrm{C}$ (Figure 3.7). For similar polyurethanes with HMDI-BD hard blocks, the pure hard block $T_g$ was $86\, ^\circ\mathrm{C}$.\(^4^6\) The Fox equation was used to calculate the extent of phase mixing. From the DSC data there is 25% hard block in the soft block domain.
Table 3.2. Zeta potentials and biocidal testing for 2 wt% U[(4F)(C12)-p:(1-p)]

<table>
<thead>
<tr>
<th>p</th>
<th>1-p</th>
<th>ζ (mV)\textsuperscript{a}</th>
<th>PA % kill\textsuperscript{b}</th>
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</thead>
<tbody>
<tr>
<td>86</td>
<td>14</td>
<td>68.2</td>
<td>90</td>
</tr>
<tr>
<td>66</td>
<td>34</td>
<td>92.1</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Zeta potential (10^{-3} \text{ M NaBr})

\textsuperscript{b} PA, \textit{Pseudomonas aeruginosa}. 
Figure 3.7. Thermal analysis of soft block and polyurethane
X-ray Photoelectron Spectroscopy

The blend coatings were studied by XPS to understand surface composition. According to the model proposed in Figure 2.1, the soft block (P[(4F)(C12)-66:34-6]) from the modifier polyurethane (U[(4F)(C12)-66:34-6]) is surface concentrated. A survey spectrum for each of the blends confirmed the presence of carbon, oxygen, fluorine and nitrogen. Table 3.3 gives comparison between the calculated and observed atom percentages of the four elements present in the surface modifier – carbon, oxygen, fluorine and nitrogen. Two scenarios were assumed for calculating elemental atom percents (at%). In the first column atom percents are for completely phase mixed soft and hard blocks and the surface modifier as a whole is present on the surface. The second column atom percentages were calculated assuming 100% soft block on the surface which is predicted by the model shown in Figure 2.1.

Table 3.3 shows that there is no significant difference in the observed nitrogen content for the three blends. The atomic percentages are higher than that for P[(4F)(C12)-66:34-6] soft block only but much less than that for U[(4F)(C12)-66:34-6].

The nitrogen peaks for each blend were studied and the N1s signals for each blend were plotted. (Figure 3.8)

The N1s peaks observed at 399.7 eV, which is typical of –CO-NH– confirms the presence of hard block on the surface. The samples were further analysed for the presence of quaternary ammonium nitrogen by carrying out 30 scans. Previously, the binding energy for quaternary nitrogen has been observed at 401.6 ± 0.3. While a quat peak was not seen for 0.5 and 1 wt% blends, the 2 wt% blend showed two distinct peaks (Figure 3.9) which suggests that increasing the percentage of surface modifier in the blend helps in increasing the availability of positive charge on the surface.
Table 3.3. Calculated vs observed atom percentages of 0.5, 1 and 2 wt % blends of surface modifier.

<table>
<thead>
<tr>
<th></th>
<th>Calculated (at %) - modifier</th>
<th>Calculated (at %) - Soft Block only</th>
<th>Observed atom %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>atom percent in blend</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>76</td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>O</td>
<td>72</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
High resolution C1s spectra reveal signals from 5 different carbon environments of varying binding energies extending over an interval of approximately 9 eV. The peak assignments according to published values of binding energies and the percentage contribution from each peak is given in Table 3.4 and the spectra is plotted in Figure 3.10.

Figure 3.10 shows the presence of –CF₃ ( B.E. 293 eV ) as observed earlier for polyurethanes having –CF₂H side chains in the soft block (Chapter 2). This is attributed to the degradation of –CF₂CF₂H by the X-ray beam. This hypothesis was tested for a representative sample by using a longer exposure time in the chamber and will be discussed in detail in the next chapter.

The percentage of fluorine observed on the surface is also much higher than what it would have been if the polyurethane as a whole was on the surface. The XPS results prove the validity of the model described previously. It also suggests that although the surface is mostly comprised of the soft block there is also phase mixing of soft and hard blocks on the surface.

From the XPS analysis it is clear that the modifier is concentrated on the surface of the blends with the 2 wt% blend having the highest amount of quarternary ammonium on the surface.

**Wetting behavior**

The three blends of U[(4F)(C12)-66:34-6.0] were investigated for their wetting behavior using the Rame-Hart Goniometer. The results show the hydrophilic nature of the surfaces which is due to the presence of the quaternary salt with a possible contribution from the chain end –CF₂H. The results however do not indicate any particular trend in wetting behavior for the different blends (Figure 3.11).
Figure 3.8. High resolution N1s peaks for modified base polyurethane. Percent modifier shown.
Figure 3.9. N1s spectra for 2 wt% blend after 30 scans
Figure 3.10. High resolution C1s spectra for modified base polyurethane – percent modifier shown.
Table 3.4. Percent contribution of each carbon environment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Binding Energy (eV)</th>
<th>Wt % of modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>CF₃ from CF₂</td>
<td>293</td>
<td>3.14</td>
</tr>
<tr>
<td>CF₂</td>
<td>290.8</td>
<td>0</td>
</tr>
<tr>
<td>O-CH₂CF₂..</td>
<td>289.1</td>
<td>6.72</td>
</tr>
<tr>
<td>C-C-O</td>
<td>286.2</td>
<td>39.71</td>
</tr>
<tr>
<td>C-C-C</td>
<td>290.8</td>
<td>50.43</td>
</tr>
</tbody>
</table>
Figure 3.11. Contact angle and sessile drop images for modifier blends. Percentage of modifier in the blend.
Zeta Potential

PSM structure/near-surface charge correlations are essential in guiding the choice of P[AB]-copolyoxetane polyurethane surface modifiers so as to optimize efficacy and stability. Thus quantification of accessible quaternary ammonium charge is of critical importance in understanding biocidal efficacy. Measurement of quaternary charge density has most often employed fluorescein dye binding and subsequent release of bound dye by an ion exchange surfactant such as dodecyl trimethyl ammonium chloride. This method was explored but could not be adapted to quantify surface charge for polyurethanes containing PSMs. This failure was attributed to nonspecific dye adsorption and slow desorption from coatings that have thicknesses of tens of micrometers.

Streaming potential measurements have long been known for quantifying surface charge. Assessing surface accessible charge by the streaming potential method was elegantly demonstrated for thin films formed by alternating polyelectrolyte deposition. Here, the alternating positive and negative potentials attest to the charge of the last polyelectrolyte deposited. Rubner and Lichter also employed streaming potentials measurements for studying antimicrobial function of polyelectrolyte multilayers comprising poly(allyl amine) hydrochloride and poly(sodium 4-styrene sulfonate).

For assessing surface accessible charge on P[AB] polyurethane coatings, a microfluidic capillary method was developed by Gupta. The capillaries were coated according to the process shown in figure 3.12. Stock polymer solutions of neat HMDI-BD(30)-P[(3F)(C12)] and 1 wt% blends with base PU were prepared coated on to fused silica capillaries using a syringe pump. The coated capillaries were air dried by pumping air through them and finally vacuum dried overnight at ambient temperature. 1 mM NaBr solution was used as the electrolyte and passed through the capillaries to measure the streaming potential. Although this method provided data that were
congruent with biocidal test results obtained for the 3F-based polyurethanes, it involved the difficult task of uniformly coating 100 µm capillaries. Hence for easier and more reliable measurements, the surface electrokinetic analyser was used for measuring the available positive charge on the surface of the blends (Figure 3.6).

Detailed zeta potential measurements were carried out for 2, 1 and 0.5 wt% blends of U[(4F)(C12)-66:34-6.0] with base polyurethane HMDI-BD(50)-PTMO(1000). The coatings prepared on glass slides were tested for stability in water for 5 consecutive runs which lasted for about 2 hrs. Each run in the SurPASS instrument consisted of two cycles and lasted for approximately 15 min. A flow check to ensure the absence of air bubbles in the channel was performed before each consecutive run. It was observed that the zeta potential values were extremely sensitive to the measurement conditions such as the pH and dielectric constant of the electrolyte. Hence, the zeta potential of base polyurethane was measured before the start of the experiment under similar operating conditions and was used as control. The zeta potential for base polyurethane was found to be -37± 4 mV at a pH of 6.8-7.0 which is the pH of the electrolyte under the operating conditions. Zeta potentials for the blends were then compared to that of base polyurethane to ascertain whether the surfaces had higher positive charge than base polyurethane. Measurements were taken within one week and 4 weeks of coating of the blends to analyse their stability in air. The results obtained for coatings no more than one week after deposition are summarized in Figure 3.13.

The results show a very clear distinction in the surface accessible positive charge for the 2, 1 and 0.5 wt% of the blends. To start with, this proves the theory discussed in the beginning that the fluorous side chain of the soft block acts as a chaperone for the quaternary ammonium side chain to be on the surface. Hence, increasing the amount of C12 increases the surface accessible positive
charge. The most important observation from the data is that there is a 16% drop in zeta potential for the 2 wt % blend in 2 hr. This is a clear step forward. Previously, Gupta observed a drop of 35% over the course of 2 mins for 2 wt% coatings with U[(3F)(C12)-86:13-5.1].

The same designate coatings were tested again after four weeks to assess stability of surface accessible positive charge. 3 consecutive runs were measured for each sample. The results are summarized in Figure 3.14.

It was observed that the zeta potential for the blends had not deteriorated over time and there is an unexpected rise in zeta potential value for 1%-U[(4F)(C12)-66:34-6]. This phenomena needs to be analysed by performing tests with larger sample size. It was also observed that there was gradual decrease in positive potential with increase in exposure time.

Results from the zeta potential measurements showed increased stability of the 4F-based surface modifiers in aqueous medium compared to the 3F based coatings.
Figure 3.12. Schematic of the Microfluidic capillary method – Polymer solution passed through capillary and air dried to obtain coated capillary.
Figure 3.13. Zeta potential of blends within 1 week of coating for 2, 1 and 0.5%-U[(4F)(C12)-66:34-6]: A. normalized against base polyurethane. Zeta potential of base polyurethane = -36.9 mV.; B. raw data (n=4)
Figure 3.14. Zeta potential of blends after 4 wk of coating for 2, 1 and 0.5\%-U[(4F)(C12)-66:34-6]: A. normalized against base polyurethane. Zeta potential of base polyurethane = -36.9 mV.; B raw data (n=4)
**Atomic force microscopy**

In his study with 3F-copolyoxetane soft block surface modifiers Brunson observed near surface microscale phase separated features and a dynamic surface with the number of phase separated features increasing with time. This phase separation driven by the incompatibility of the fluorous moieties in the polyurethane resulted in depletion of positive charge from the surface over time in air and almost instantly in water. Surface morphology of fluorinated polyoxetanes with IPDI as the hard block was discussed in Chapter 2 wherein polyurethane with P[(4F)-10] soft block showed near surface nanoscale phase separations.

Due to its amphiphilic nature, (Chapter 2) 4F was hypothesized to be a better alternative for a more stable surface. Accordingly TM-AFM images were studied for the blends of U[(4F)(C12)-66:34-6.0]. To understand the surface morphology with changing surface modifier concentration, TM-AFM images of 2%-U[(4F)(C12)-66:34-6.0], 1%-U[(4F)(C12)-66:34-6.0] and 0.5%-U[(4F)(C12)-66:34-6.0] were investigated.

Figure 3.15 shows 25 × 25 µm scans of the blends at a setpoint ratio of 0.9. The blends show near surface microscale (~1µm) phase separations similar to that reported earlier for fluorinated copolyoxetanes. The surface roughness increases with increasing surface modifier content in the blend.

The images were obtained within one week of coating of the samples.
Figure 3.15. TM-AFM images of blends with varying wt % of polymer surface modifier – within 1 wk of coating. 0.5%-U[(4F)(C12)-66:34-6]-a. 3D Height, b. Sectional height, c. Phase, 1%-U[(4F)(C12)-66:34-6]-d. 3D Height, e. Sectional height, f. Phase, 2%-U[(4F)(C12)-66:34-6]-g. 3D Height, h. Sectional height, i. Phase. Scan size: 25 ×25 µm; Setpoint ratio: 0.9.
Figure 3.16. TM-AFM images of blends with varying wt % of polymer surface modifier – after 4 wk of coating. 0.5%-U[(4F)(C12)-66:34-6]-a. 3D Height, b. Sectional height, c. Phase, 1%-U[(4F)(C12)-66:34-6]-d. 3D Height, e. Sectional height, f. Phase, 2%-U[(4F)(C12)-66:34-6]-g. 3D Height, h. Sectional height, i. Phase. Scan size: 25 ×25 µm; Setpoint ratio: 0.9.
To analyze the surface stability for the 4F-based coatings, the samples were investigated under similar conditions after 4 weeks. The images obtained (Figure 3.16) show similar phase separated microscale features but does not indicate significant change in the surface morphology over time. This is certainly a step ahead towards achieving a more stable surface compared to what had been observed for the 3F-based modifiers where micropeak like features started appearing on the surface within 2-3 days of preparation of the coatings and increased in number with time.

**Antimicrobial tests**

Several antimicrobial testing methods have been used for determination of biocidal efficacy. The aerosol spray method is a simple and efficient way of quantifying surface antimicrobial activity. Experiments to determine the percent kill of bacteria on coming in contact with these coatings helped determine potential applicability of these coatings in healthcare. The XPS and zeta potential measurements confirmed the presence of quaternary ammonium function (Scheme 2.2). Biocidal tests on these surfaces were carried out to confirm the antimicrobial nature of the surfaces.

To be useful, the coatings not only need to be bactericidal after preparation but also need to retain their bactericidal efficacy over time. Antibacterial tests were performed on three different blend concentrations (0.5, 1 and 2 wt %). The results are listed in Table 3.5. Images of individual plates are given in the supplemental information.

The results in Table 3.5 indicate very good antimicrobial stability for the base polyurethane modified with U[(4F)(C12)-66:34]. Although there is a slight loss in antimicrobial activity for the coatings after 4 weeks it is not as significantly lower than the freshly prepared coatings and is a huge improvement from the results obtained from the 3F coatings.
Table 3.5. Antimicrobial property of blends ( % kill)

<table>
<thead>
<tr>
<th>Bacterial Challenge</th>
<th>Time after coating</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 wk</td>
<td>4 wks</td>
<td>1 wk</td>
<td>4 wks</td>
<td>1 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td>100</td>
<td>99</td>
<td>100</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
<td><em>Eshcherechia coli</em></td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>97</td>
<td>100</td>
<td>99</td>
<td>NA</td>
<td>92</td>
</tr>
</tbody>
</table>
3.5 Conclusion

P[AB] copolyoxetanes, where A has a fluorinated side chain with –CF₂H end group and B consists of an alkyl ammonium side chain, are easily prepared and incorporated into an HMDI/BD polyurethane. Using dimethyl acetamide as a common solvent 0.5, 1 and 2 wt % blends were prepared. Solvent evaporation gave surface modified base polyurethane coatings. A preliminary study establishes that the antimicrobial property and zeta potentials were enhanced by increasing the alkyl ammonium content in the soft block. The concept of correlating surface active positive charge by zeta potential measurements with antimicrobial studies was validated. Both zeta potential measurement and biocidal tests identified the 2 wt%-U[(4F)(C12)-66:34-6.0] as the most suitable blend among those tested for contact antimicrobial coatings to establish temporal stability.

As a subject for future work, cytotoxicity studies to determine the biocompatibility of these coatings related to their specific applications would determine their viability for applications such as catheters.
Supplemental Information

\(^1\)H-NMR analysis

The 4F:BBOx repeat unit ratio was determined using \(^1\)H NMR spectroscopy. From the \(^1\)H NMR spectrum, the area from the peaks corresponding to the methylene groups of the BBOx side chain (Figure S1.1, peaks A and B) as well as the total methyl area (peak C) were applied to the Eq. 1 to determine the mole faction of the BBOx methyl groups (\(A_{peakA}\) = the area of the BBOx peak A, \(A_{peakB}\) = the area of BBOx peak B, \(A_{peakC}\) = peak area of total methyl groups, \(n_{BBOx,clis}\) = the mole fraction of the BBOx repeat unit).

\[
\frac{3(A_{peakA} + A_{peakB})}{4(A_{peakC})} = n_{BBOx}
\]  

(1)

From the above equation, the mole ratio of P[(4F)(C12)-66:34] is determined when

\(A_{peakA} = 3.68;\ A_{peakB} = 4.25;\ A_{peakC} = 17.63\)

The molecular weight of the telechelic is obtained by comparing peaks C and D (contribution from end-group hydrogen) and is found to be 6.0 KDa.

To ensure complete substitution of BBOx by the C12-amine, the NMR spectra for P[(4F)(C12)-66:34] was analysed. For complete substitution, the ratio of integrals for peaks F and E is expected to be 3. From the analysis of peak integrals it found that area of peak E=7.35 and that of peak F = 23. This gives a ratio of 3.1 which proves complete substitution.
Figure S3.1 $^1$H-NMR spectra for mole ratio and molecular weights calculation
Figure S3.2. $^1$H-NMR spectra for P[(4F)(C12)-66:34]
Figure S3.3. Agar plates with *Pseudomonas aeruginosa*.
**Figure S3.4.** Agar plates with *Escherichia coli*
Figure S3.5. Agar plates with *Staphylococcus aureus*.
Chapter 4.


4.1 Introduction

Chapter 2 presented a new contact antimicrobial strategy based on a polyurethane modifier containing a HMDI/BD hard block and a copolyoxetane soft block having 4F and C12 side chains (Figure 4.1). This U[(4F)(C12)-66:34] modifier was incorporated at low levels into a conventional base polyurethane (HMDI/BD(50)-PTMO. Excellent contact kill for sprayed on challenges of Gram +/- bacteria was observed for low weight percent U[(4F)(C12)-66:34] modifier. Zeta potentials, which provide a measure of surface accessible charge, showed quat charge was stable over the course of two hours in dilute NaBr.

The stability of the U[(4F)(C12)-66:34] modifier was much improved over a prior –CF3 based quat copolyoxetane. However, further improvements in stability and ease of processing are desired based on (1) zeta potentials for modified base polyurethane show a 10% decrease after 2 hr in the test medium (10^{-3} M NaBr), (2) preparation of the U[(4F)(C12)-66:34] modifier requires polyurethane synthesis, and (3) coatings required the use of DMAC solvent which has low volatility and is therefore difficult to remove from solvent cast coatings.

In their pioneering work with hybrid coatings, Saegusa and Chujo showed that terminating ring-opening polymerization by aminopropyltriethoxysilane followed by cohydrolysis with TEOS (tetraethoxysilane) led to optically transparent polymer hybrids. The present study, explores the feasibility of end capping a P[AB] diol containing a quat side chain with a siliceous isocyanate and using a cross linker to stabilize the copolyoxetane surface modifier. Guided by the fact that
surface modifier polyurethanes containing the P[4F:C12-66:34] soft block showed good antimicrobial properties, new hybrid surface modifier concept was tested with this diol. A model for the modification is given in figure 4.2.

In this four step process, (1) the P[4F:C12-66:34] diol is endcapped with isocyanatopropyl triethoxysilane, (2) Inorganic domain is increased with an alkoxysilane, (3) a solution of the end-capped diol/alkoxysilane is added to that of base polyurethane and (4) the solution is used for generating coatings (Scheme 4.1). Saegusa and Chujo used TEOS as an inorganic network precursor (sol-gel) but under water-sparse hydrolysis/condensation TEOS is volatile resulting in safety hazard and and uncertain hybrid compositions. Preliminary experiments showed that bis(triethoxysilyl)ethane (BTESE) demonstrated negligible volatilization cure (b.p. 119 °C) and good hydrolysis condensation reactivity (Scheme 4.1). Previously BTESE has been used by Volksen in the preparation of porous oxycarboxilane spin-on low dielectric thin films. In his recent study on triblock hybrid elastomers, Chakrabarty reported the addition of 10 wt% BTESE as a crosslinker for increasing the siliceous domain. Following this work, 10 wt% BTESE is added to base polyurethane and blended with the end capped diol. Following the same principles discussed for the linear polyurethane modifier in the previous chapter, the fluorinated end capped diol is expected to concentrate on the surface. The end capped diol is expected to be ‘locked’ on the surface by the nanoglass domain provided by the crosslinker as depicted in Figure 4.2.
Figure 4.1 Polyurethane surface modifier - U[(4F)(C12)-66:34]
Figure 4.2. Model for new modification strategy – the ‘bottle brush nanoglass’ concept; A=4F, B=C12
4.2 Experimental Section

**Materials:** P[4F:C12-66:34] diol was prepared according to the method discussed in the experimental section of Chapter 3. 3-isocyanatopropyltriethoxysilane (SII 6455) and bis(triethoxysilyl)ethane (SIB 1817, BTESE) were purchased from Gelest, Inc. Dibutyltindiacetate was used as a catalyst and was purchased from Aldrich. Tetrahydrofuran, 99.6%, (for analysis ACS, stabilized with BHT) was obtained from Acros.

**Preparation of Hybrid Blends.** Coatings were prepared in four steps comprising endcapping the copolyoxetane diol, adding BTESE to augment the siliceous domain, adding this combination to a solution of base polyurethane, and dip or drip coating. A specific example for utilizing 2 wt% P[(4F)(C12)-66:34], isocyanatopropyltriethoxysilane, BTESE and HMDI/BD(50)-PTMO-1000 is described below.

The preparation of P[(4F)(C12)-66:34-6] was described in Chapter 3. The reaction takes place in four steps shown in Scheme 4.1.

In step 1, the diol is end-capped with isocyanatopropyltriethoxysilane using a 1:2 molar ratio of diol to silane. A solution of 0.27 g of isocyanatopropyltriethoxysilane - 2 in 10 ml of THF and DBTDA (0.5 wt %) catalyst was prepared in a 100 ml round bottom flask. A solution of P[(4F)(C12)-66:34-6] diol (2.72 g) – 1 in 20 ml of THF was added dropwise to this solution under dry nitrogen purge. The disappearance of the isocyanate peak (Figure 4.3) was studied at one hour intervals by infrared spectroscopy to ensure 100% endcapping of the diol. The weight of the solution is 5.6 g which includes the weight of the end capped diol - 3 and THF. Complete conversion of –NCO to –NHCO takes place in 4 hrs.
Scheme 4.1. Preparation of U-4F/C12-SiO\textsubscript{1.5}
Figure 4.3. Disappearance of isocyanate peak (2270 cm\(^{-1}\)) over time: a) 30 mins after start of reaction, b) completion of reaction (4hrs)
In step 2, 0.1 g of BTESE - 4 is added to a solution containing 0.02 g of endcapped diol in THF (for a 2 wt% blend)-5. Step 3 consists of preparation of three different blends of the endcapped diols with base polyurethane. For a 2 wt% blend, 0.98 g of base polyurethane in THF is added to the above solution. Additional blends of base polyurethane with 0.5 and 1 wt % endcapped diol were prepared. 10 wt% BTESE was added in each case. Table 3.1 gives the details of feed added for making coatings.

The base polyurethane for these coatings was the same as that described in Chapter 3- HMDI-BD(50)-PTMO(1000).35,46,94

Step 4 consists of preparation of the coatings. Coatings were prepared within 15 mins of Step 3. Microscope slides and glass cover slips were drip coated for zeta potential, AFM measurements and antimicrobial tests respectively. Dip coated slides were prepared for studying wetting behavior of samples via dynamic contact angle measurements. Cure was overnight at ambient temperature followed by 100 °C for 24 hr. BTESE has been used in the preparation of porous oxycarbosilane spin-on low dielectric thin films109-111 but not as a precursor for hybrids. The choice of BTESE for increasing the wt% of the siliceous domain or chemical network (CN) was based on experiments that demonstrated negligible volatilization during cure (b.p. 119 °C) and adequate hydrolysis / condensation reactivity. For BTESE, Si$_2$(CH$_2$)$_2$O$_3$ is the nominal composition after hydrolysis / condensation (Eq 4.1).

For brevity, the hydrolysis / condensation cure composition, which includes the siliceous co-network generated by hydrolysis / condensation cure of the end capped 4F/C12 copolyoxetane, is designated “siliceous” and represented as “-SiO$_{1.5}$”.

$$\text{Si}_2(\text{CH}_2)_2(\text{OEt})_6 + 3\text{H}_2\text{O} \rightarrow \text{Si}_2(\text{CH}_2)_2\text{O}_3 + 6 \text{C}_2\text{H}_5\text{OH} \quad \text{Eq 4.1}.$$
Table 4.1. Composition of feed for hybrid coatings

<table>
<thead>
<tr>
<th>End-capped diol (wt%)</th>
<th>End-capped diol (g)</th>
<th>Base PU (g)</th>
<th>BTESE</th>
<th>Total Weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.005</td>
<td>0.995</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.99</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>0.98</td>
<td>0.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
The copolyoxetane P((4F)(C12)\text{-}66:34\text{-}6) used in this study, where 6 is the soft block $M_n$ (6 kDa), is designated 4F/C12. The modifier composition is designated 4F/C12-SiO$_{1.5}$. An example of a designation for a 2 wt% modified base polyurethane HMDI/BD(50)-PTMO-1000 (U) is U-(4F/C12-SiO$_{1.5}$)-2.

4.3 Instrumentation

\textit{Infrared Spectroscopy}. Infrared spectra in transmission were obtained with a Nicolet 400 FT-IR spectrometer to monitor the completion of end capping reaction. IR spectra was collected between 4000 and 400 cm$^{-1}$ at 4 cm$^{-1}$ resolution using an average of 32 scans.

\textit{X-ray photoelectron spectrometry (XPS)}. Measurements were carried out with a Thermo Fisher Scientific ESCALAB 250 instrument. Analysis utilized monochromatic Al K$\alpha$ X-rays and with an X-ray spot size of 500 mm and a TOA of 90°. Pass energy for survey spectra was 150 eV. Pressure in the analytical chamber during spectral acquisition was maintained at $2 \times 10^{-8}$ Torr while an argon electrostatic flood source affected charge neutralization. Cured samples were cut and attached to the surface of a silicon wafer using carbon tape. Data were analyzed with the Thermo Avantage software (v. 4.40).

\textit{Atomic Force Microscopy}: A Dimension Nanoscope V (Veeco, CA) atomic force microscope with silicon cantilevers (40 N/m) was used for morphological analysis. Unless otherwise noted, imaging was performed in tapping mode at a setpoint ratio ($r_{sp} = A_{exp}/A_o$) of 0.9 (soft tapping), where $A_o$ is free oscillation amplitude and $A_{exp}$ is the experimental oscillation amplitude.
**Zeta Potential measurements:** A SurPASS Electrokinetic Analyzer (Anton PAAR, Ashland, VA) was used to investigate the zeta potentials of the coatings. A SurPASS clamping cell attachment was used for measurements of zeta potentials of the coated glass slides. The electrolyte used was 0.1 mmol NaBr solution. The choice of electrolyte was governed by the presence of a common anion (Br\(^-\)) in the polyurethane so that secondary factors such as anion exchange would not present interference.

**Bactericidal Test:** Bacterial spray testing has been used in previous studies of non-leaching biocidal materials \(^{32, 33}\) and was the primary method of determining biocidal activity of the P[(4F)(C12)-66:34-6]Si\(_{1.5}\) PSM blend. Agar plates were streaked with the desired bacteria from a stock culture kept frozen at -70 °C and incubated at 37 °C for 18-24 hrs. From this plate a single colony was collected and used to inoculate 10 mL of Luria broth. This culture suspension was incubated for 18-24 hrs at 37 °C. After incubation, the 1:50 dilution of the culture was prepared and incubated at 37 °C until an optical density of 0.2-0.3 was observed for 1 mL of culture. Once the desired optical density has been achieved, the culture suspension was used in bacteria challenges.

A biocidal test was devised to deposit the bacterial suspension via an aerosol spray. Using a stock bacteria concentration of 10\(^6\) colony forming units (CFU)/mL, slides coated with 2 wt%, 1 wt % and 0.5 wt% blends where sprayed for 1 second and weighed to determine the amount of bacteria suspension deposited. Sprayed slides were then placed in a constant humidity (85-95%) environment. Keeping the samples at constant humidity is important because control experiments in ambient air showed irreproducible fractions of dead bacteria as a function of time which is likely to the bacteria experiencing osmotic shock.\(^{39}\) After 60 min, the slides were placed in saline solution and vortex stirred for 2 min to remove bacteria from the surface. 100 µL aliquots of this suspension
were removed and spread onto agar plates that were incubated at 37 °C for 18 h. After incubation, bacteria colonies were counted to obtain the percent kill relative to the base polyurethane control.

_Swell/ water absorption test:_ To estimate the extent of water uptake of the coatings, glass cover slips, 22 mm sq were coated with the blends and immersed in DI water. The initial weight of the slides was noted. The slides were taken out after 24 hours and droplets of water on the surface were gently shaken off. The final weight of the slides was used to estimate the percent water absorption for modified base polyurethane.

**4.4 Results and discussion:**

The main aim of this work was to develop an antimicrobial coating that is stable in the presence of water and air for longer periods of time. The stability determines suitability for demanding applications such as catheters. It was therefore important to determine stability by surface characterization techniques for different wt % surface modifier.

Previously charge stability in water of base polyurethane coatings modified with 2 wt% U[(3F)(C12)-87:13-5.1] was only a few minutes. Increasing the C12 wt % and using the amphiphilic 4F side chain improved the stability in water to at least 2 hours. In this work we sought even greater stability. With that goal in mind, analysis of the biocidal properties of the blends was carried out both within a week and also after 4 weeks of coating preparation.

**X-ray photoelectron spectroscopy:**

Elemental surface composition of the was analysed with X-Ray photoelectron spectroscopy. The aim was to study the difference between expected percentage of carbon, oxygen, fluorine, silicon and nitrogen on the surface and observed percentages. A survey scan was done at three different areas of the surface of each blend. Table 3.2 shows the surface composition based on the P[(4F)(C12)-66:34-6] soft block alone and the P[(4F)(C12)-66:34-6]Si1.5 hybrid domain.
From the above data it is seen that the observed percentage of nitrogen is quite lower than the calculated amount considering only P[(4F)(C12)-66:34] on the surface. The calculated atom % of nitrogen considering only the endcapped diol to be on the surface is 6.49. This is much higher than the observed amount of 1.73. This suggests phase separation wherein the total available nitrogen from the quaternary ammonium side chain and also from the end capping agent is not available on the surface. (This is also partly due to experimental errors of XPS wherein the nitrogen signal was not intercepted well). However, slight increase in the percentage of nitrogen with increasing surface modifier diol suggests that the availability of quaternary ammonium on the surface depends on the amount of surface modifier in the blend. To further investigate the presence of quaternary ammonium on the surface, surface scan for nitrogen was carried out for the three blends (Figure 3.4). The N1s lines for the three blends shows an interesting trend. The peak corresponding to 401 eV is that of nitrogen from the quaternary ammonium side chain of the diol. It is observed that the peak area increases with increasing concentration of the diol in the blend.

The N1s signal for each blend was obtained after 30 scans of a particular area of the sample. An interesting feature of the signal is the presence of two nitrogen peaks which corresponds to the two forms of nitrogen that are expected to be present on the surface. The peak at 399.6 eV corresponds to covalently bound nitrogen which is present in the isocyanate used for end capping as well as in the hard block of the base polyurethane. The smaller peak at a higher binding energy of 401 eV corresponds to the nitrogen from the quaternary ammonium side chain. It is clear from the N1s peaks that the presence of quaternary ammonium on the surface increases with increasing concentration of diol in the blend. It also confirms the hypothesis that the fluorinated side chain in the diol would act as a chaperone for the quaternary ammonium side chain, bringing it to the surface of the coating.
Table 4.2. Calculated vs observed atomic percentages of elements on the surface of blends

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<thead>
<tr>
<th></th>
<th>Calculated (at %)</th>
<th>Observed (at %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P[(4F)(C12)-66:34]</td>
<td>Endcapped-P[(4F)(C12)-66:34]</td>
</tr>
<tr>
<td>O</td>
<td>11.06</td>
<td>19.4</td>
</tr>
<tr>
<td>C</td>
<td>72.46</td>
<td>58.48</td>
</tr>
<tr>
<td>F</td>
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<td>7.32</td>
</tr>
<tr>
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<td>8.31</td>
</tr>
<tr>
<td>N</td>
<td>1.88</td>
<td>6.49</td>
</tr>
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</table>
Figure 4.4. High resolution N1s scan for modified base polyurethane. Percent 4F/C12-SiO$_{1.5}$ shown.
Figure 4.5. High resolution C1s scan for modified base polyurethane. Percent 4F/C12-SiO$_{1.5}$ shown.
Table 4.3. Contribution of different carbon environments for modified base polyurethane. Percent 4F/C12-SiO\textsubscript{1.5} shown.

<table>
<thead>
<tr>
<th></th>
<th>Contribution of different carbon bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>CF\textsubscript{3} from CF\textsubscript{2}</td>
<td>1.90</td>
</tr>
<tr>
<td>CF\textsubscript{2}</td>
<td>0.71</td>
</tr>
<tr>
<td>O-CH\textsubscript{2}CF\textsubscript{2}.</td>
<td>2.59</td>
</tr>
<tr>
<td>C-C-O</td>
<td>35.59</td>
</tr>
<tr>
<td>C-C-C</td>
<td>59.22</td>
</tr>
</tbody>
</table>
Table 4.4. Comparison of two consecutive runs for 1 wt % U-4F/C12-SiO$_{1.5}$

<table>
<thead>
<tr>
<th>Contribution of different carbon bonds for 1 wt % blend</th>
<th>Run 1</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF$_3$ from CF$_2$</td>
<td>1.76</td>
<td>2.35</td>
</tr>
<tr>
<td>CF$_2$</td>
<td>1.28</td>
<td>1.64</td>
</tr>
<tr>
<td>O-CH$_2$CF$_2$..</td>
<td>3.99</td>
<td>3.68</td>
</tr>
<tr>
<td>C-C-O</td>
<td>31.95</td>
<td>31.91</td>
</tr>
<tr>
<td>C-C-C</td>
<td>61.02</td>
<td>60.42</td>
</tr>
</tbody>
</table>
C1s scans for the blends with subsequent peak assignments show the presence of \(-\text{CF}_3\) bonds in all the three blends at a binding energy of approximately 293 eV (Figure 4.5).

Since the starting material does not contain any \(-\text{CF}_3\) end groups it was discussed earlier that the possible explanation for the presence of \(-\text{CF}_3\) in the polyurethane was due to reorientation of \(-\text{C}-\text{F}\) bonds in presence of high energy electron beam in the X-Ray analysis chamber. Fluorocarbon moieties are known to be unstable under X-ray exposure during XPS analysis. For example, poly(vinylidene fluoride) undergoes elimination of HF on being exposed to high energy electron beam. To test this hypothesis, the same area of a 1 wt % hybrid blend was interrogated for two consecutive runs. For the hypothesis to hold true, the second run should have higher percentage of \(-\text{CF}_3\). Table 4.4 gives the result for the study where the percentage of \(-\text{CF}_3\) is observed to be nominally higher.

Although the data is within the limits of experimental error, it gives merit to the hypothesis for the presence of \(-\text{CF}_3\) groups on the surface of the hybrid blends. However, marginal increase in \(-\text{CF}_2\) content is also observed which suggests that the mechanism for for the formation of \(\text{CF}_3\) needs more exploration. 19F-NMR studies also needs to be carried out to verify the fluorinated species in the starting materials.

**Atomic force microscopy:**

Although surface morphology of the polymer surface modifiers, studied by atomic force microscopy does not give an indication of their antimicrobial properties, a study of the topology and morphology provides an idea of the extent of phase separation, if any, of the blends and hence can provide important information about their performance over a period of time. Images were taken at a setpoint ratio \(r_{sp}\) of 0.9 which can be considered as “soft tapping” with a scan size of 25 µm x
Two sets of images were studied to observe the change in the morphology of the surfaces with time. Figure 4.6 shows the images of surfaces of the blends within 1 week of coating. Near surface phase separation is observed for all the three blends with the 1 wt % blend exhibiting maximum roughness. The size of phase separated features is observed to be increasing with the increase of the percentage of surface modifier in the blend. A recently proposed model for fluorous/PEG soft block polyurethanes discusses the tendency of fluorous groups to self-aggregate creating nanoscale building blocks for microscale phase separated features. A depletion zone surrounds the microscale domain. Similar microscale phase separation is observed for AFM images for the blends with the size of the features being smallest in the 0.5 wt % blend suggesting higher self aggregation for higher percentage of modifier.

It had been observed by Brunson in his work with antimicrobial PSMs having –CF₃ based side chains that the nature of the surface was extremely dynamic. To study the change in surface morphology for the hybrid surfaces the same slides were interrogated after 4 weeks of coating.

The atomic force microscopy images show some unique characteristics. The surface features are extremely stable over a period of one month. Although phase separated features appear for all the three blends, the surface does not undergo any morphological changes. This corroborates the model discussed in the beginning of the chapter - the crosslinking of endcapped soft block stabilizes the soft block on the surface. However the presence of phase separated microscale features also suggests that the surface is not a homogeneous distribution of soft block alone which agrees with XPS analysis.

**Zeta Potential:**

Relating surface accessible positive charge to the antimicrobial property of a blend is a unique and much less tedious process that helps assess not only the bactericidal property of a blend.
Figure 4.6. Tapping mode AFM images of blends at setpoint ratio of 0.9 within 1 week of coating; 0.5 wt%: a) 3D Height, b) Sectional, c) Phase; 1 wt%: d) 3D Height, e) Sectional, f) Phase; 2 wt%: g) 3D Height, h) Sectional, i) Phase. Scan size: 25 × 25 µm.
Figure 4.7. Tapping mode AFM images of blends at setpoint ratio of 0.9 within 1 week of coating; 0.5 wt% : a) 3D Height, b) Sectional, c) Phase ; 1 wt% : d) 3D Height, e) Sectional, f) Phase ; 2 wt% : g) 3D Height, h) Sectional, i) Phase. Scan size: 25 × 25 µm.
but also the durability of the blend. The presence of quaternary ammonium on the surface of the blends is already confirmed by XPS analysis. To quantify the surface charge, glass slides coated with 0.5, 1 and 2 wt % blends were tested in the zeta potential analyser (SurPASS). A 1mM solution of NaBr was used as the electrolyte. Since the main aim of this work was to obtain a stable, positive surface, the slides were analysed by a continuous flow of electrolyte for a period of 3 hours by performing . The results (Figure 4.8) obtained show the remarkable stability of these surfaces over a period of time. The surface charge is compared against base polyurethane and there appears to be a positive trend of zeta potential for 0.5 to 2 wt % blends. The 0.5 wt % blend shows very little difference from the base polyurethane. Although the overall zeta potential value for the 1 and 2 wt % blends appears to be negative, the surface of the blends show an overall stable positive charge that is 22 mv higher than the base polyurethane which shows a zeta potential value of -37 mv under similar measurement conditions.

The zeta potential values show excellent stability of the blends over time. For the 2 wt % blend the zeta potential remains almost constant for a period of three hours within which eight individual runs of 2 cycles each were carried out. Zeta potential increases by about 7 mV for 1 wt % blends while the 0.5 wt % blend proves to be slightly better than a control sample of base polyurethane. This validates the absence of measurable quaternary ammonium nitrogen for the 0.5 wt% blend as observed by XPS.

The samples were stored in petri dishes under normal atmospheric conditions and zeta potential for these blends were again tested after 4 weeks of coating to observe any changes in the surface active positive charge.

From the zeta potential measurements it is seen that the surface is characterized by remarkable stability over a period of time. It is also observed that under similar measurement
conditions, the surfaces of the blends have higher positive potential than the base polyurethane. This helps in establishing the presence of quaternary ammonium on the surface and the excellent stability over 3 hours confirms the hypothesis that the crosslinking stabilizes the charge on the surface. The measured value of the zeta potential for these surfaces is however observed to be lower than that of the linear modifier that has been discussed in Chapter 3. This needs to be investigated further to confirm if the siliceous phase sequesters the positive charge partially for these blends or the values were influenced by differing measurement conditions like pH of the electrolyte.

**Antimicrobial Analysis:**

A study of the antimicrobial property of the linear modifier was reported in the previous chapter. The blend with 2 wt % surface modifier (U-4F/C12-SiO$_{1.5}$-2) had the best bactericidal property exhibiting a 100% kill for the three strains of bacteria tested when the blends were tested within one week of coating. The blends showed somewhat reduced antimicrobial activity over a period of time. Since the main aim for developing the hybrid coatings is to stabilize the surface charge for the coatings, similar antimicrobial tests were carried out for the three blends within one week and four weeks of coating. The three strains of bacteria that were tested for the analysis – *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* - are common causes for hospital acquired infections.

The results obtained are summarized in Table 4.5

Images of bacterial colonies on agar plates are provided in supplemental information.

The results establish the excellent antimicrobial properties of the 1 and 2 wt % blends. Lower kill percentages of 0.5 wt % blend is observed. The biocidal data also confirms the results obtained by XPS and zeta potential measurements.
Figure 4.8. Zeta potential of blends within 1 wk of coating for 2, 1 and 0.5% - U-(4F/C12-SiO1.5): A. normalized against base polyurethane. Zeta potential of base polyurethane = -36.9 mv.; B raw data. (n=4)
Figure 4.9. Zeta potential of blends after 4 wk of coating for 2, 1 and 0.5%- U-(4F/C12-SiO$_{1.5}$): A. normalized against base polyurethane. Zeta potential of base polyurethane = -35.1 mV.; B raw data. (n=4)
Table 4.5. Summary of antimicrobial data for modified blends (% kill).

<table>
<thead>
<tr>
<th></th>
<th>Within one week after coating</th>
<th>Four weeks after coating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>74</td>
<td>84</td>
</tr>
</tbody>
</table>
Table 4.6. Percent water uptake after 24 hours.

<table>
<thead>
<tr>
<th>Blend</th>
<th>Initial Weight (mg)</th>
<th>Final Weight (mg)</th>
<th>Percent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 wt%</td>
<td>41</td>
<td>41.09</td>
<td>0.22</td>
</tr>
<tr>
<td>1 wt%</td>
<td>46.1</td>
<td>46.15</td>
<td>0.11</td>
</tr>
<tr>
<td>2 wt%</td>
<td>46.6</td>
<td>46.64</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Swell test:

Since the end application of these polymeric coatings is for medical equipment like catheters it is important to determine the extent of water absorption for these surfaces. It has been observed by Cooper\textsuperscript{35} that water absorption of hard block modified polyurethane antimicrobial surfaces lose their mechanical properties in contact with hydrated surfaces. The results obtained (Table 4.6) show extremely low water absorption on the surfaces of the blends.

4.5 Conclusion:

In the previous chapter the result of modification of the fluorinated side chain in P[AB] copoloxetane based surface modifiers was discussed. It was seen that replacing the –CF\textsubscript{3} end group of the fluorinated side chain with a –CF\textsubscript{2}CF\textsubscript{2}H side chain greatly enhanced the stability of the surface active positive charge but did not completely “lock” the charge on the surface. To achieve this, the present work described the unique method of endcapping the diol with a siliceous isocyanate and creating a “nanoglass” surface by crosslinking the diol.

This hybrid approach is a more effective method for surface modification because

(a) Separate synthesis of a P[AB]-polyurethane modifier is eliminated,

(b) Unlike 4F-based linear PSMs that are only soluble in DMAc, which has low volatility, the 4F/C12 diols permit coating solutions in THF greatly facilitated processing.

The results show a clear improvement in stability for surface accessible positive charge. No noticeable morphological change of the surface was observed even after four weeks of coating preparation. The 1 and 2 wt % blends have excellent antimicrobial property that was retained over a
period of one month. Continuous flow of electrolyte for over three hours has almost no effect on the stability of the positive charge.

The results obtained from the study establishes a new hybrid organic/inorganic procedure for surface modification. The correlation of zeta potential of the surface of coatings with antimicrobial studies can be effectively used as a method to narrow down on the different blends to be investigated for tedious antimicrobial studies.
Supplemental Section

Figure S4.1. Agar plates with *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2%</th>
<th>1%</th>
<th>0.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk old samples</td>
<td>100%</td>
<td>100%</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>4 wk old samples</td>
<td>100%</td>
<td>100%</td>
<td>83%</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5. Kill kinetics of biocides.

5.1 Introduction

The world’s smallest life forms have most often proved to be the most deadly. HAIs affect more than 2 million people every year in the US alone causing more than 90000 deaths as a result. The fight against microorganisms gave rise to a global demand for antimicrobials both in solution and on surfaces. The present work concentrates on preparation of coatings with stable and effective antimicrobial activity and studies the kill kinetics of the biocides.

Antimicrobial coatings may be broadly classified into two types: biocide release and contact kill. In biocide release systems polymeric coatings are loaded with biocides that are released in the vicinity of the microbial cells thus causing cell death. Several surface modification techniques have been studied describing antibiotic or metal ion releasing systems. Tributyltin esters of polyacrylates have been known as release kill agents due to the slow release of TBT which is biocidal. Although these coatings are highly effective as biocides certain factors like gradual surface depletion and development of biocide resistant strains and cytotoxicity towards human cells limit their application in various fields.

Contact antimicrobial function is accomplished by covalently bonding the biocide to the polymeric surface, thereby promising stability over time. The need for advances in contact kill coatings is clear due to the growing problem of bacterial resistance to antibiotics and even biocides.

Over 30 years ago Isquith demonstrated contact kill for glass surfaces functionalized with alkylammonium moieties and concomitant absence of “zone of inhibition” characteristic of biocide
Figure 5.1. Structure of P[AB]-telechelics that become soft blocks for P[AB]-polyurethanes. R: CF₃CH₂O-(3F).
Figure 5.2. Polyurethane surface modifier with \( P[(4F)(C12)\cdot p:(1-p)] \) soft block where \( p=0.66 \).
The rapid kill of bacteria by covalently bound alkylammonium functionality is due to chemisorption of bacteria, which have negatively charged outer membranes.\textsuperscript{1,18} Cell death occurs due to membrane disruption. Because contact kill precludes biocide entry into the metabolic process, bacterial buildup of resistance does not occur and hence contact kill promises long term antimicrobial stability.

Kurt has shown that concentrating quaternary groups with fluorous “chaperone” side chains yielded effective contact antimicrobial coatings.\textsuperscript{39} Kurt’s study involved development of blends of very small quantities of polymer surface modifiers (0.5-2 wt\%) with a commonly used polyurethane designated as base PU.

However, a later study showed that the 3F side chain resulted in phase separation and inactivation of biocidal effectiveness.\textsuperscript{46} Replacing the 3F moiety with amphiphilic 4F (-CF\textsubscript{2}CF\textsubscript{2}H end group) (Figure 5.2) was shown to stabilize the C12 quaternary function. Long term biocidal stability on polymer surface (Chapter 4).

The P[(4F)(C12)] copolyoxetane approach has a combination of features for polyurethane surface modification that includes (1) preferential soft block surface concentration,\textsuperscript{44,45,58,59} (2) a bottle brush-like copolyoxetane architecture, having multiple 4F and C12 side chain “bristles” that are pseudo-chain ends and are entropically surface concentrated,\textsuperscript{115} (3) differing solubility parameters for the P[(4F)(C12)] soft block that enhances phase separation and surface concentration, (4) a low P[(4F)(C12)] \( T_g \) that may enhance the C12 conjugation with bacterial target, (5) a multiplicity of short, environmentally acceptable –CH\textsubscript{2}CF\textsubscript{2}CF\textsubscript{2}H side chains that provide a “green” method for surface concentrating functionality, and (6) compositional economy by using the P[(4F)(C12)] soft block polyurethane as a minor constituent (\( \leq 2 \) wt\%) in a blend such
that the polymer modifier defines the surface properties and the base polymer defines bulk properties (Figure 1). Compositional economy via a blend approach, was thoroughly discussed by Mayes, who modified poly(vinylidene fluoride) membranes for resistance to protein adsorption via surface segregation of the amphiphilic comb polymer poly(methyl methacrylate-co-polyoxyethylene methacrylate).\textsuperscript{116}

It was discussed in Chapter 3 that although 4F based modifiers exhibited excellent surface stability, zeta potential measurements showed gradual loss of positive charge over a period of 2 hrs. Seeking to further stabilize near surface quaternary charge, the P[(4F)(C12)-66:34] soft block was endcapped with isocyanatopropyl triethoxysilane and incorporated into a hybrid network generated by sol-gel chemistry. This enhanced processability by eliminating the step of developing separate surface modifier polyurethane (Chapter 4). Coatings developed using this method were found to retain a stable positive charge for a period of month.

A probable mechanism for the bactericidal action of the surface modifiers containing a hydrophobic chain end chaperoning a quaternary ammonium chain has been discussed in Chapter 3. Contact kill mechanism assumes rapid pore formation and rupture of outer/cell membrane of bacteria when in contact with an antimicrobial surface. Bacterial kill kinetics have clear practical importance as well as it contributes to understanding of the mechanism of contact kill, but kill kinetics for polycation-functionalized coatings have not been extensively studied.

In 1991 Peyret reported killing curves for antibiotics in solution.\textsuperscript{117} Kill kinetic studies on solution antimicrobials have been reported for different cationic/ polycationic species like biguanide groups\textsuperscript{118}, ceragenins,\textsuperscript{119} copolyoxetanes,\textsuperscript{120} cationic amphiphilic polymethacrylate derivatives.\textsuperscript{121} Kill kinetic studies revealed excellent antimicrobial properties of quaternary ammonium-PEG based copolyoxetanes in solution.\textsuperscript{120}
The time taken to obtain 100% kill on antibacterial coatings, an important factor that can determine their application in various fields, has however not been studied widely. In 1978, Isquith’s pioneering work on creation of durable antimicrobial surfaces by the application of a cationic alkoxy silane, 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride on glass discussed the rate of kill of bacteria for these surfaces. In his work, treated glass surfaces were vortexed along with the bacterial culture solution and the resulting solution was tested at specific time intervals to study the rate of kill. The results had shown 99% reduction of bacteria within 15 minutes of exposure and he reported that the rate of kill against time followed an exponential function.

In his work with hydantoinyltri hydroxy siloxane, Worley has studied the biocidal efficacy of poly[3-(5,5-dimethylhydantoinylpropyl)hydroxysiloxane] functionalized silica gel surfaces, which, when chlorinated with sodium hypochlorite become biocidal due to the release of chlorine. In their study it was observed that complete kill of bacteria takes place within 30 seconds of exposure to these films.

In the present work, kinetics of contact kill has been studied on 2 wt%-U[(4F)(C12)-66:34-6]Si1.5 coatings. A series of bactericidal tests were performed at different time intervals on these coatings to determine the optimum time of exposure for the bacteria with the biocide.

It is assumed from the proposed mechanism of bactericidal action for the quaternary ammonium functionalized polyurethanes discussed previously, there is rapid kill of bacteria on contact with the surface modifier. However, the bacterial solution, which is sprayed on the coated surfaces remains on the surface as microscale hemispherical droplets (Figure 4.3). As a result the time taken for bacteria on the top surfaces of the droplets to come in contact with the antimicrobial
Figure 5.3. Bacterial suspension on the coated slide – suspension remains in form of hemispherical droplets.
surface will determine the amount of time that is required to obtain a 100% kill of bacteria on the surface.

The rationale behind this study is to optimize the exposure time for the coating that has shown highest biocidal efficacy in the previous studies.

5.2 Experimental Section

**Materials:** P[4F:C12-66:34] diol was prepared according to the method discussed in the experimental section of Chapter 2. 3-isocyanatopropyltriethoxysilane (SII 6455) and bis(triethoxysilyl)ethane (SIB 1817, BTESE) were purchased from Gelest, Inc. Dibutyltindiacetate was used as a catalyst and was purchased from Aldrich. Tetrahydrofuran, 99.6%, (for analysis ACS, stabilized with BHT) was obtained from Acros.

**Preparation of Hybrid Blends:** The 2 wt% hybrid blend was prepared in a two-step process as described in Chapter 3.

**Preparation of Coatings:** Coatings were prepared within 15 mins of preparation of the hybrid blend to avoid excessive crosslinking. Drip coated cover slips 2.5 x 2.5 mm in dimension were used as samples for antimicrobial tests. Cure was overnight at ambient temperature followed by 100 °C for 24 hr.

The bactericidal properties of these coatings were compared against unmodified base polyurethane.

**Bacterial solution:** Previous antimicrobial studies with these hybrid coatings have not shown significant differentiation in biocidal activity of modified polyurethane coatings for the three strains of bacteria tested – *Pseudomonas aeruginosa, Eschericia coli* (gram negative) and
Figure 5.4. *Pseudomonas aeruginosa* colonies on Agar Plate
*Staphylococcus aureus* (gram positive). Hence *Pseudomonas aeruginosa*, which is one of the most commonly found bacteria that primarily affects immunocompromised individuals and can cause a host of infections including those of the respiratory tract, eye, bone, skin, joint and urinary tract was chosen as the subject of study.

*Pseudomonas aeruginosa* is a Gram-negative, aerobic rod-like bacteria belonging to the bacterial family Pseudomonadaceae. Like other members of the genus, *Pseudomonas* is a free-living bacterium commonly found in soil and water and is a pathogen often found in nosocomial environments.122

According to the CDC, the overall incidence of *P. aeruginosa* infections in U.S. hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.

### 5.3 Test Procedure:

Bacterial spray testing has been used in other studies of non-leaching biocidal materials32, 33, 39 and was the primary method of determining biocidal activity of the U-(4F/C12-SiO_{1.5})-2 coatings.

Agar plates were streaked with the desired bacteria from a stock culture kept frozen at -70 °C and incubated at 37 °C for 18-24 hrs. From this plate a single colony was collected and used to inoculate 10 mL of Luria broth. This culture suspension was incubated for 18-24 hrs at 37 °C. After incubation, the 1:100 dilution of the culture was prepared and incubated at 37 °C until an optical density of 0.2-0.3 was observed for 1 mL of culture. Once the desired optical density has been achieved, the culture suspension is used in bacteria challenges.
A biocidal test was devised to deposit the bacterial suspension via an aerosol spray. A schematic of the spray test has been given in Chapter 3 (Figure 3.2). A stock suspension with bacteria concentration of $10^6$ colony forming units (CFU / mL) was sprayed on coated slides for $1 \pm 0.1$ seconds and weighed to determine the amount of bacteria solution deposited. Sprayed slides were then placed in a constant humidity (95%) chamber. Keeping the samples at constant humidity is important because control experiments in ambient air showed irreproducible fractions of dead bacteria as a function of time which is likely to the bacteria experiencing osmotic shock. After the desired exposure times, the slides were placed in 10 mL saline solution and vortex stirred for 2 min to leach out bacteria adsorbed on the slides. One hundred microliter aliquots of this solution were removed and spread onto agar plates that were incubated at 37 °C for 18 h. After incubation, bacteria colonies were counted to obtain the percent kill.

**Exposure times:** Initial studies with 2wt% coatings at 30 and 60 mins exposure times had shown that the latter was more effective. Exposure times of 15, 30, 60 and 120 mins have been chosen for the present work.

Base polyurethane coatings were used as a positive control and were subjected to exposure times of 15 mins and 120 mins to ensure that the bacterial spray solution used had live bacteria.

### 5.4 Results and Discussion

The results of the bactericidal tests show time dependence for bacterial kill. It was observed that there was an average of 15 bacterial colonies on the agar plate that had aliquots from the saline solutions containing slides subjected to 15 mins of exposure to the bacterial spray. Although 30 mins of exposure was capable of killing 91% of the bacteria, 100% kill was observed within an hour. Figure 4.5 shows the images of representative plates after 24 hours of plating. The base
polyurethane had an average of 138 and 130 colonies after 15 mins and 120 mins of exposure which shows that the condition in the humid chamber was conducive for bacterial growth.

### 5.5 Conclusion:

Various approaches have been researched to develop non-leaching antimicrobial coatings based on common modified or synthesized quaternized polymers. Research on kill kinetics is useful to understand the mechanism of contact kill. However a thorough investigation on kill kinetics will also require studies on cellular adhesion\(^{123}\) since a layer of dead cells or protein can quickly deactivate contact antimicrobial activity. Thus a relationship between cationic/amphiphilic surfaces, cellular adhesion and antimicrobial activity needs to be explored alongside kill kinetic studies.

The present work provided an insight into the average time taken for complete kill of bacteria when a 0.6mg of suspension containing \(10^6\) CFU/ml of *Pseudomonas aeruginosa* is sprayed on the antimicrobial surface. It confirms the viability of application of 2 wt% hybrid coatings for medical applications and also gives an indication on the optimum exposure time required for samples undergoing spray testing. The work is however a preliminary study on kill kinetics and does not shed light on the mechanism of contact kill of bacteria on surfaces. A testing method involving continuous contact of bacterial solution containing live bacteria on the antimicrobial surface and simultaneous removal of dead bacteria from the surface is being investigated.
Figure 5.5. Agar plates after 18 hrs of incubation. Exposure times for - U-(4F/C12-SiO\textsubscript{1.5})-2: a) 15mins, b) 30mins, c) 60 mins, d) 120 mins; Base PU- e) 15mins, f) 120mins
A plot of percent kill vs time is given below. (Figure 5.6)

Figure 5.6. Percent kill vs time for U-(4F/C12-SiO$_{1.5}$)-2.
Chapter 6.

Conclusion and future research

Development of thermally, environmentally, and temporally stable antimicrobial coatings that are suitable for biomedical applications is a challenging topic of contemporary research. One path to soft surface modification that was started by Kurt is a combination of both fluorous and biocidal moieties so that the fluorous group, via its low surface energy, enables processing compatible migration to the surface and acts as a “chaperone” for the biocidal group.\(^{39}\)

Prior work demonstrated that polymer surfaces modified with polyurethanes containing P[(3F):(C12)-m:n] polyoxetane soft blocks (where 3F is a trifluoroethoxy side chain and C12 is the quaternary ammonium containing side chain) have non-leeching contact kill.\(^{39}\) However, research by Brunson, showed that these fluorous/ cationic surface modifiers slowly loose biocidal effectiveness over time.\(^{74}\)

The aim of this project was to identify a suitable fluorine based constituent for the quaternary ammonium containing oxetane that will not only act as a suitable chaperone for the quaternary ammonium side chain but also stabilize the charge on the surface for long term durability. In the present research polyurethanes with short chain –CF\(_2\)H terminated soft blocks were studied for their wetting behavior, near surface morphology and surface composition. A model explaining the amphiphilic nature of the –CF\(_2\)H fluoropolymers was proposed. It was hypothesized that the amphiphilic nature of 4F (-CF\(_2\)CF\(_2\)H) would prevent phase separation in aqueous medium and hence, introduction of 4F as the hydrophobic fluorous moiety to act as the chaperone for the C12 group in P[AB] soft blocks for polyurethanes would thermodynamically favor a more stable surface in the air as well as in water.
Results obtained for blends of U[(4F)(C12)-66:34-5.7]-linear polyurethane (Chapter 3) as well as for U-(4F/C12-SiO₁.₅) – hybrid modification (Chapter 4) established 4F as a useful chaperone for C12 side chains in the modifiers. Apart from identifying a better and more stable surface antimicrobial it was also the aim of this research to establish a correlation between surface accessible positive charge from zeta potential measurements and antimicrobial effectiveness. This has been successfully done as zeta potential measurements have been found to be directly correlated to antimicrobial properties. This correlation will be extremely useful in future research and development to quickly identify the effectiveness of a coating before embarking on tedious antimicrobial studies.

Kinetics of biocide kill showed that 91% of bacteria (Pseudomonas aeruginosa) were killed within the first half hour of exposure to the biocidal coatings (Chapter 4) and 100% kill was observed within 1 hr. The present work therefore identified a the suitable fluorine based chaperone for surface concentration of quaternary nitrogen on a commonly used polyurethane to impart antimicrobial properties. However, further exploration is required. Some of the future goals for this project are highlighted below.

**Zeta potential studies:** As mentioned, the unique concept of correlating zeta potential with antimicrobial properties of coatings has been explored in the present work. The key goal of this study was to (i) analyse the stability of the surfaces in aqueous medium and (ii) to look for observable trends if any with varying concentrations of the surface modifiers in the blends. Although these objectives were achieved, the zeta potential analysis opens the door to broader possibilities that need to be explored. The sampling size for this analyses reported herein was limited. Analysing more samples where preparation and storage conditions was systematically varied would help in firming up statistics. Specifics that need to be addressed include – 1. does zeta
potential actually increase on immersion in water as was observed in certain cases, 2. does this correlate to biocidal activity and 3. the recovery of zeta potential in air.

**Commercial base polyurethane:** The present study involved surface modification of base polyurethane prepared in the lab. Future work with commercial polyurethane having similar composition would help in understanding the viability of these surface modifications in real life situations and would also be more cost effective.

**Different microorganisms:** The present work was limited to analyzing the interaction of common bacteria that are responsible for healthcare associated infections with the antimicrobial blends. Quaternary ammonium compounds in derivatized polyethyleneimines have been shown to be virucidal. Further exploration to analyse the effect of modified blends on viruses is important for their future applicability as coatings for medical equipment.

**Bacterial resistance to quats:** While the use of quats as effective antimicrobial agents has been accepted widely, parallel research to study bacterial resistance to quats has also been performed. In 1952, a study by Chaplin on *Serratia marcescens*, showed the possibility of development of resistance in Gram negative bacteria by the process of elaboration of a lipid which is retained on the cell surface and is capable of withstanding the disruptive surface force of the quaternary ammonium containing disinfectant. Resistance development to quats in solution by *Pseudomonas aeruginosa* has been attributed to its ability to change the cell surface hydrophobicity by lipopolysaccharide modification. Several studies in solution antimicrobials have reported the gradual development of bacterial resistance to quats and various mechanisms have been suggested. Development of bacterial resistance to quats on surfaces however, has not been widely explored. A study of increased bacterial resistance to the polycationic surface modifiers will be an important topic for future exploration.
**New testing method:** A major concern for the efficacy of the aerosol spray test method on contact kill surfaces is the possibility of adherence of a layer of dead bacteria on the surface resulting in deactivation of the surface. To counter this issue, new methods of surface modification have been explored by various researchers. Antimicrobial contact active surfaces have been developed by Chen by attaching poly(N,N-dimethyl-N-ethoxycarbonylmethyl)-N-[2′-(methacryloyloxy)ethyl]-ammonium bromide) via atom transfer radical polymerization. The constant contact with the aqueous environment results in hydrolytic cleaving of the ethyl ester next to the quarternary ammonium head group. The resulting zwitterionic head group is no longer biocidal, but effectively repels all attached microbial cells, dead or alive. Though this effect has not been reported as reversible, it is an interesting method of counteracting the issue discussed above. More recently surfaces with alternative killing and repelling properties achieved by a change of temperature has been reported. For the present study, a new test method involving immersion of the polymeric blend in the bacterial solution under constant agitation and removal of aliquots of the solution at set time intervals to evaluate percent kill is being explored. Constant agitation of the solution is expected to prevent the adherence of dead bacteria on the surface of the modified blend.

**Mechanism of contact kill:** The mechanism by which surfaces interact with pathogenic organisms for contact kill has been discussed extensively. For contact kill, analogies have been drawn to cell wall disruption proposed for solution kill by polycations. However, elucidating a more detailed mechanism for rapid contact kill at the bacteria-solid surface interface remains an important research goal.

**Cytotoxicity and hemolytic activity:** Antimicrobial coatings for medical applications should not only possess excellent biocidal properties but also be biocompatible based on their area of application. Several studies, both for solution antimicrobials as well as biocidal coatings have
attempted to understand the factors that control toxicity or selectivity of antimicrobials.\textsuperscript{135-137} Klibanov’s study on the effect of bactericidal N-hexyl,methyl-PEI surface coatings on mammalian cells using live/ dead fluorescence assay shows no appreciable detrimental effects of these coatings on mammalian cells. This has been attributed to the much larger size of mammalian cells.\textsuperscript{19} However, there is still no complete understanding of all the factors that are responsible for toxicity and control selectivity. In solution, cytotoxicity and hemolytic activity studies on quaternary ammonium based P[AB] copolyoxetanes have showed relatively low cytotoxicity for HFF and HDF cell lines and for RBCs.\textsuperscript{120} Thus cytocompatibility studies with human cells for P[AB] copolyoxetane based coatings will address the question of whether the favorable results from solution will translate to surfaces. This will be a major focus area of future research for these copolyoxetane based antimicrobial coatings and will determine their usage in medical equipment like catheters.
References:


Vita

Asima Chakravorty was born in Kolkata, India on August 15, 1973. She graduated from Calcutta University, India, with a Bachelor of Science (Chemistry) in 1996 and Bachelor of Technology (Chemical Technology) in 1999. Prior to joining the Graduate School in VCU, she has worked for eight years in different areas related to Chemical Engineering. A major part of her professional career was spent in General Electric’s polymer research lab in Bangalore, India, where she has been responsible for research on Environmental Stress Cracking of Plastics. Apart from earning her Six Sigma Green Belt, she has also been recognized for new method developments in the lab.

Asima has worked towards developing antimicrobial coatings as part of her research studies in VCU. She has been awarded the prestigious Hibbs Waller Scholarship and Graduate School Thesis Dissertation Assistantship from VCU in 2012.