September 2013

Functionalized Polyisobutylene: From Synthesis to Properties and Applications

Solmaz Karamdoust
The University of Western Ontario

Supervisor
Dr. E. R. Gillies
The University of Western Ontario

Graduate Program in Chemistry

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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FUNCTIONALIZED POLYISOBUTYLENE: FROM SYNTHESIS TO PROPERTIES AND APPLICATIONS

(Thesis format: Integrated Article)

by

Solmaz Karamdoust

Graduate Program in Chemistry

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

Polyisobutylene (PIB) is a polymer well known for its desirable properties such as high chemical stability, impermeability to gases, and biocompatibility in certain applications. However in some applications it is limited by properties such as high hydrophobicity, lack of chemical functionalities, and the adsorption of proteins and organisms to its surface. Butyl rubber (IIR) is a copolymer of isobutylene (IB) with small percentages of isoprene (IP). Typically these IP units serve as sites for the covalent cross-linking of the IIR, in addition can serve as sites for further functionalization of IIR. These modifications can expand the potential applications of IIR. This thesis describes the development of different approaches to obtain both the attractive properties of the starting material in addition to new properties arising from its further modification. For example, antibacterial IIR surfaces were prepared using hyperthermal hydrogen induced cross-linking (HHIC) as a means to covalently attach antibacterial polymers to the IIR surface. The antibacterial properties of the modified surfaces were investigated against Gram-positive and Gram-negative bacteria. In addition, using a synthetic method allowing for the clean modification of the double bonds of IIR and the grafting of poly(ethylene oxide) (PEO) chains along the polymer backbone, a library of linear PIB-PEO graft copolymers (lin-PIB-g-PEO) was successfully generated containing up to 83 wt% PEO content. The properties of the resulting graft copolymers were studied both on surfaces and in solution. These studies were further extended to generate arborescent PIB-PEO graft copolymers (arb-PIB-g-PEO) with the aim of comparing the effect of architectures on the properties of the resulting PIB-PEO graft copolymers. Finally, a UV-cross-linkable IIR was synthesized by chemical modification of IIR backbones with cinnamate moieties. The physical and mechanical properties of the resulting UV-cured IIR polymers were studied.

Keywords
Butyl rubber, hyperthermal hydrogen induced cross-linking, poly(ethylene oxide), antibacterial, arborescent, UV-curing
Co-Authorship Statement

The work described in this thesis contains contributions from the author as well as coworkers from Western, Surface Science Western (SSW), LANXESS Inc. and supervisor Dr. Elizabeth Gillies. The exact contributions of each are described below.

Chapter 1 was written by the author and was reviewed by supervisor Dr. Elizabeth Gillies.

The work described in Chapter 2 was a collaboration between the author, other members of the Gillies group, SSW, and LANXESS Inc. All the experimental work corresponding to the synthesis and preparation of the polymeric surfaces were carried out by the author under the supervision and guidance of Dr. Gillies and Dr. Lau. The evaluation of the antimicrobial properties was primarily carried out by Binyu Yu, a Ph.D. student under the supervision of Dr. Jun Yang (Western Engineering). Later, the author repeated the antimicrobial experiments. Yu Liu, a Ph.D. student under the supervision of Dr. Jun Yang, helped the author with the AFM studies and Dr. Colin Bonduelle a postdoctoral fellow in the Gillies group provided general project guidance. Dr. Greg Davidson (LANXESS Inc.) performed preliminary experiments on the project while a postdoctoral fellow in the Gillies group. Dr. Goran Stojcevic from LANXESS Inc. provided general feedback on the project. The Manuscript was prepared by the author and Dr. Gillies.

Chapter 3 describes a project proposed by Dr. Gillies and the author. All the chemistry corresponding to the synthesis and characterization of the graft copolymers were carried out by the author under the supervision and guidance of Dr. Gillies. Ryan Amos, an MESc student supervised by Dr. Gillies, primarily did the cell culture experiments. After his graduation, Bethany Turowec, an MESc student under the supervision of Dr. Gillies completed the cell culture experiments. Sharon Guo and Lorenzo Ferrari from LANXESS Inc. provided general project guidance and feedback. Manuscript preparation was carried out by the author and Dr. Gillies.

For Chapter 4, all the described experimental work was performed by the author under the supervision of Dr. Gillies. The starting polymer was synthesized by Dr. Patrick Crewdson, a postdoctoral fellow at LANXESS Inc. Also, Lorenzo Ferrari from LANXESS Inc. provided general project direction and guidance. A draft of a manuscript
was written by the author with Dr. Gillies providing assistance with editing and final preparation.

Chapter 5 describes a project proposed by Dr. Wei Wu, a postdoctoral fellow under the supervision of Dr. Gillies. Initial polymer synthesis and characterization was developed by Dr. Wu, and he performed the kinetic studies. After his departure, the experimental work was mostly repeated by the author in addition to further characterization of the polymers. Bethany Turowec performed cell culture work. A draft of a manuscript was prepared by Dr. Wu and the author, and was edited by Dr. Gillies.
Acknowledgments

Apart from my own efforts, the successful completion of this thesis depends largely on the encouragement and guidance of many others. I take this opportunity to express my gratitude to all those people who have made this dissertation possible, and because of whom my graduate experience has been one that I will cherish forever.

Above all, I thank god for giving me the desire to carry out this work. Next, I would like to show my greatest appreciation to my supervisor, Dr. Elizabeth Gillies, for her years of immense support, inspiration and excellent guidance. She taught me how to question thoughts and express ideas and helped me overcome the challenges throughout my graduate studies.

I would like to express my gratitude to LANXESS Inc. for providing the financial support that made this research possible. Special thanks go to Lorenzo Ferrari, Dr. Patrick Crewdson, Dr. Greg Davidson and Dr. Mark Ingratta for providing useful comments, remarks and engagement throughout the progress of this thesis.

I would like to acknowledge all of the past and current members of the Gillies group for their encouragement, support and insightful comments throughout the entire process. From the Gillies group, special thanks to Andrew Wong, who was always willing to help and give his best suggestions, and for carefully reading and commenting on my thesis. Also, I would like to thank Dr. Colin Bonduelle for his guidance at the beginning of my research, Ryan Amos and Bethany Turowec for helping with the cell culture experiments, and our lab technician, Aneta Borecki for collecting SEC data.

Furthermore, my sincere gratitude and respect goes to my thesis examiners Dr. Martin, Dr. Whitney, Dr. Workentin and Dr. Zhang for taking the time to read my thesis.

Many thanks to the support staff here at Western: Dr. Willans in the NMR room, Dr. Ragogna and his group members for allowing the use of his instruments, all of the Chemstores staff, as well as our graduate secretary, Darlene McDonald.

From Surface Science Western, I would like to thank: Dr. Nie for training on the AFM, Mary Jane Walzak for training in FTIR and Mark Biesinger for his assistance in XPS.

I would like to thank my husband, Saman Maleki for his constant support. He was always there cheering me up, and stood by me through the good times and bad.
Last, but by no means least, I owe my deepest gratitude to my parents and sisters to whom this dissertation is dedicated; they have been a constant source of love, concern, support and strength all these years, and without them I could not have made it here. I love you and I will always be grateful for your love.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>arb</td>
<td>arborescent</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>BIIR</td>
<td>brominated butyl rubber</td>
</tr>
<tr>
<td>BR</td>
<td>polybutadiene rubber</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>BOPP</td>
<td>biaxially oriented polypropylene</td>
</tr>
<tr>
<td>CA</td>
<td>cyanoacrylate</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming units</td>
</tr>
<tr>
<td>CIIR</td>
<td>chlorinated butyl rubber</td>
</tr>
<tr>
<td>CMC</td>
<td>critical micelle concentration</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMEM</td>
<td>dulbecco’s modified eagle medium</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FDA</td>
<td>food and drug administration</td>
</tr>
<tr>
<td>HDCE</td>
<td>hindered dicumyl ether</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IB</td>
<td>isobutylene</td>
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<tr>
<td>IIR</td>
<td>isobutylene-co-isoprene rubber</td>
</tr>
<tr>
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<td>inimer</td>
</tr>
<tr>
<td>IP</td>
<td>isoprene</td>
</tr>
<tr>
<td>IR</td>
<td>infrared spectroscopy</td>
</tr>
<tr>
<td>HHIC</td>
<td>hyperthermal hydrogen induced cross-linking</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>MAAn</td>
<td>maleic anhydride</td>
</tr>
<tr>
<td>m-CPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>$M_n$</td>
<td>number average molecular weight</td>
</tr>
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</table>
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
$M_w$ weight average molecular weight
MW molecular weight
NMR nuclear magnetic resonance
NR natural rubber
ODTS octadecyltrimethoxysilane
PBS phosphate buffered saline
PDI polydispersity index
PDMAAM poly($N,N$-dimethylacrylamide)
PDMAEMA poly(2-(dimethylamino)ethyl methacrylate)
PEO poly(ethylene oxide)
PIB poly(isobutylene)
PIP polyisoprene
PMMA poly(methyl methacrylate)
PPH$_3$ triphenylphosphine
PS Polystyrene
PVA poly(vinyl alcohol)
PVAc poly(vinyl acetate)
s singlet
SBR styrene butadiene rubber
SCVP self-condensing vinyl polymerization
SEC size exclusion chromatography
SIBS poly(styrene-$b$-isobutylene-$b$-styrene)
TEM transmission electron microscopy
$T_g$ glass transition temperature
THF tetrahydrofuran
TPE thermoplastic elastomers
UV ultraviolet
wt% weight percentage
XPS x-ray photoelectron spectroscopy
Chapter 1

1 Polyisobutylene-based Materials: Properties and Applications

1.1 General Introduction

Rubbers are natural or synthetic polymeric materials, that exhibit elastic behavior at and around room temperature. Their high and reversible extensibility and damping properties distinguish them from other types of materials.¹ Rubbers or elastomers are known to generally exhibit impermeability to water and air, high resistance to attack by other chemicals and they dissolve slowly in appropriate solvents to form solutions that have high viscosities, even at low concentrations. The penetration of small solvent molecules between tangled polymer chains is temperature and time dependent. Rubbers are also capable of adhering to metals and textile fibers. By combining rubbers with metals, the tensile strength is enhanced considerably while a reduction in extendibility is observed. This use in composites increases the range of application of rubbers.¹²

Depending on the type and amount of rubber chemicals and additives in a compound, in addition to the degree of vulcanization, different properties with respect to hardness, strength, and elasticity can be achieved. Vulcanization is a process that changes the chemical structure and improves the quality of rubber. The chemical reactivity of the polymers depends on whether the main chain is saturated or not. The presence of a double bond enables vulcanization, however it also makes the material susceptible to oxidation and ozone attack. All unsaturated rubbers such as natural rubber (NR), styrene butadiene rubber (SBR), polybutadiene rubber (BR), butyl rubber (IIR) are generally vulcanizable by sulfur. However, they react in different ways due to the difference in their basic structure, degree of unsaturation, and location of the double bond.²³

More than half of the global production of natural and synthetic rubber is used in tires, and the reminder for a wide variety of industrial and consumer products.³ For
instance, rubbers are highly utilized in insulation applications where their flexibility is needed and is combined with high resistance to the passage of an electric current.

1.2 Different Types of Rubbers: Properties and Applications

1.2.1 Natural Rubber (NR)

\[
\begin{align*}
\text{CH}_2 & \quad \text{H}_2\text{C} \\
\text{C} & \quad \text{C} \\
\text{H}_3 & \quad \text{H}
\end{align*}
\]

Scheme 1.1. Chemical structure of cis-1,4-polyisoprene (NR).

NR is an elastomeric hydrocarbon polymer originally derived from the latex sap of trees (especially trees of the genera Hevea and Ficus). It has the chemical composition of polyisoprene (PIP), and it is reported that NR is mainly composed of IP units in a cis-1,4 monomer configuration, therefore its true chemical name is cis-1,4-PIP. The singular repeat units structure out of the many possible isomers (Scheme 1.2) accounts for many of the special properties of NR. NR is insoluble in a range of organic solvents, however it will swell when immersed in organic solvents and it is very flexible and extremely waterproof. With the exception of butadiene rubber, NR has the highest elasticity of all rubber types. In addition, it has very good resistance to abrasion and fatigue.

\[
\begin{align*}
\text{1.1} & \quad \text{1.2} \\
\text{1.3} & \quad \text{1.4}
\end{align*}
\]

Scheme 1.2. Possible isomers of PIP chain.

NR is often vulcanized, a process in which the rubber is heated and sulfur, peroxide or bisphenol is added to prevent it from perishing and to improve its resistance and elasticity. Carbon black is often used as an additive to improve its strength, especially in vehicle tires. The main utility of NR is in the production of heavy-duty tires and vibration dampers. It is also used in hoses, seals, conveyor belts, coated fabrics and other products.
1.2.2 Polybutadiene Rubbers (BR)

Scheme 1.3. Chemical structure of BR.

BR is a synthetic rubber that was first produced in Europe in 1930s. The synthesis of commercial polybutadienes is mostly accomplished via solution polymerization of 1,3-butadiene monomers in the presence of organometallic catalysts. Depending on the type of the catalyst used in the polymerization process, different types of polybutadienes can be synthesized. High-cis-1,4-polybutadiene is synthesized using a Ziegler-Natta type catalyst, consisting of either cobalt or nickel salt, or organic compounds of these metals, with an alkylaluminium halide. The properties vary slightly depending on the metal used. The low-cis-1,4-polybutadiene, is polymerized using an alkyl lithium initiator such as butyllithium. It typically contains 36% cis, 54% trans and 10% vinyl derivative. Due to its high glass transition temperature ($T_g$), low-cis polybutadiene is not used in tire manufacturing. However, it can be advantageously used as an additive in plastics because of its low gel content.

High-trans-polybutadiene containing more than 90% trans isomer is produced using catalysts containing neodymium, lanthanum, or nickel. This material is a semicrystalline plastic (not an elastomer), which melts at about 80 °C. It was formerly used for the outer layer of golf balls. High-vinyl-polybutadiene (over 70%) is produced with an alkyl lithium initiator. This derivative can be advantageously used in combination with high-cis in tires. Polybutadiene with 90% vinyl, has been shown to exhibit elastomeric thermoplastic properties.

The properties of the isomeric forms of BR differ. For instance, high-cis-polybutadiene has high elasticity and is widely used, whereas the high-trans-polybutadiene is a crystalline plastic with few useful applications. BR offers good abrasion resistance and is therefore frequently used the in treads, sidewalls, and
some casing components of tires. Moreover, it can be blended with NR in order to enhance fatigue properties.\(^9\)

**1.2.3 Styrene-Butadiene Rubbers (SBR)**

![Chemical structure of SBR](image)

**Scheme 1.4.** Chemical structure of SBR.

SBR is a replacement for NR and is the most widely used synthetic rubber, which was originally developed prior to World War II in Germany. However, its industrial manufacturing began during World War II.\(^1,10\) SBR is a copolymer of styrene and butadiene. These two monomers can be polymerized in solution or via emulsion polymerization.\(^1,4,11\) With the exception of some special grades, the styrene content is around 24 wt\% (a molecular proportion in the chain of one styrene to about six or seven butadienes).\(^4\) The monomers are randomly distributed along the polymer chain, but the butadiene portion is mostly in the *trans* configuration (Table 1.1). The ratio of styrene/butadiene in the copolymer influences the properties of the polymer. With high styrene content, the copolymers are harder and less rubbery.\(^11\)

**Table 1.1.** Chemical properties of SBR.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrangement of monomers</td>
<td>Random</td>
</tr>
<tr>
<td><em>cis</em>-1,4-butadiene (wt%)</td>
<td>9</td>
</tr>
<tr>
<td><em>trans</em>-1,4-butadiene (wt%)</td>
<td>76</td>
</tr>
<tr>
<td>1,2-butadiene (wt%)</td>
<td>15</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>0.94</td>
</tr>
</tbody>
</table>
SBR is widely used in pneumatic tires, shoe heels and soles, gaskets and even chewing gum. Latex (emulsion) SBR is extensively used in coated papers, being one of the most cost-effective resins to bind pigmented coatings. It is also used in building applications.

1.3 PIB-Based Rubbers

The discovery of PIB produced via cationic polymerization of isobutylene (IB) using a Lewis acid initiating system was first reported in 1873.\textsuperscript{12-14} This discovery generated worldwide interest in academic and industrial laboratories due to its many desirable properties such as impermeability to water and gas, high elasticity, and chemical stability.\textsuperscript{15} Moreover, PIB is known to possess good biocompatibility and noninflammatory properties as many segmented polymers based on PIB have been studied as potential biomaterials.\textsuperscript{16-19} For instance, many articles have reported the combination of PIB with materials such as polyacrylates, polymethacrylates,\textsuperscript{20-23} polyurethanes,\textsuperscript{24} poly(ethylene) oxide,\textsuperscript{25,26} and poly(vinyl alcohol).\textsuperscript{27}

1.3.1 Butyl Rubber (IIR)

\[
\begin{align*}
\text{H}_2 & \quad \text{CH}_3 \\
\text{C} & \quad \text{C} & \quad \text{CH}_3 \\
\text{C} & \quad \text{H} & \quad \text{H}_2 \\
& \quad \text{H}_2 \\
& \quad \text{C} & \quad \text{C} & \quad \text{CH}_3 \\
1 & \quad 7 & \quad \text{m}
\end{align*}
\]

Scheme 1.5. Chemical structure of IIR.

IIR, also known, as isobutylene-\textit{co}-isoprene rubber is an elastomeric material synthesized via copolymerization of IB with small amounts of IP (0.5 – 4 mol%). According to ISO 1629 of 1987, it is abbreviated IIR (isobutylene isoprene rubber). IIR has been valued due to its attractive properties\textsuperscript{2} such as chemical inertness, gas/water impermeability,\textsuperscript{28} oxidative stability,\textsuperscript{29} excellent mechanical properties and biocompatibility\textsuperscript{30} resulting from low levels of unsaturation between long PIB segments. In addition, the incorporation of IP units creates double bonds allowing vulcanization with sulfur and other agents. Vulcanization results in enhanced mechanical properties as well as abrasion resistance. PIB and IIR have been employed in a variety of commercial applications.
products such as the inner linings of automobile tires, pharmaceutical stoppers, sealants, bladders, adhesives, and in the food industry for chewing gum.\textsuperscript{2,17}

IIR was first investigated by researchers Gorianov and Butlerov (1870) and Otto (1927), who found that in the presence of boron trifluoride, oily homopolymers of IB were successfully produced.\textsuperscript{31} Subsequently, in the 1930s, the I. G. Farben Company of Germany synthesized high molecular weight (MW) PIBs with rubber-like properties.\textsuperscript{1} However the resulting PIBs were not curable due to the absence of unsaturation. Non-curable homopolymers of PIB were commercialized from Badischer of Germany and Exxon Chemical Company as PANOL\textsuperscript{®} and VISTANEX\textsuperscript{®}, respectively for applications in caulks, sealants, adhesives and chewing gum.

IIR was synthesized by Standard Oil Development Company (Exxon Research and Engineering Company) in 1937 by copolymerizing IB with 1,3-butadiene in the presence of an aluminum chloride catalyst. Using this procedure, they obtained a white mass of rubber-like polymer, which was insoluble in carbon tetrachloride or other solvents. Further experiments were designed to define conditions for the preparation of soluble, curable polymers. Research by Sparks and Thomas led to the development of the first curable IB-based elastomers, by introduction of small amounts of randomly distributed diolefin into the polymer backbone.\textsuperscript{32-35} Thomas and Sparks realized that IP was a better comonomer in comparison to 1,3-butadiene due to its higher copolymerization reactivity and it yielded a more readily curable rubber.

The first commercial IIR plant was operated in Baton Rouge (Louisiana). IIR was officially introduced and commercialized in 1942. The development of halogenated IIRs started in the 1950s. The synthesis of halogenated derivatives broadened the usefulness of IIR due to its increased polarity and faster curing rates.\textsuperscript{1}

1.3.2 Synthesis of IIR

Commercial IIR is synthesized by copolymerization of highly pure monomers via cationic polymerization at low temperature (\(-100\,^\circ\text{C}\)) using an alkyl halide initiator (e.g. CH\(_3\)Cl) with a Lewis acid activator (typically AlCl\(_3\)) in chlorinated solvents (e.g. CH\(_3\)Cl
can also be used as the solvent) (Scheme 1.6 and Scheme 1.7).\textsuperscript{36,37} In order to achieve desired MWs, very pure monomers are required. The catalyst system consists of a coinitiator and an initiator. Examples of common Lewis acid coinitiators are aluminum trichloride, alkylaluminum dichloride, boron trifluoride, tin tetrachloride, and titanium tetrachloride. Typical initiators are Brønsted acids such as hydrochloric acid, water, organic acids, or alkyl halides. The most commonly used polymerization process utilizes methyl chloride as the reaction diluent and by boiling liquid ethylene the heat of reaction is eliminated, retaining the required low temperature.

![Scheme 1.6. Cationic polymerization of IIR.](image)

![Scheme 1.7. Simplified route for IIR synthesis.](image)

The polymerization mechanism involves complex cationic reactions (Scheme 1.8). In the initiation step, IB monomer reacts with the Lewis acid catalyst to form a carbenium ion. Then, during the propagation step monomer and comonomer units are added to the carbenium until chain transfer or termination occurs. The propagation of this exothermic reaction is affected by different parameters such as temperature, solvent polarity, and the presence of counterions.
Scheme 1.8. Mechanism of IIR synthesis: a) initiation, b) propagation, and c) termination.
In the chain transfer step, the carbenium ion reacts with IB or IP monomers or other species (e.g. solvents or counter ion). This terminates the growth of the macromolecule and produces a new propagating chain. Performing the polymerization reaction in lower temperatures reduces the possibility of chain transfer, resulting in IIR polymer of higher MW. Therefore, the polymerization temperature is usually between -90 °C and -100 °C. The chain transfer issue is more apparent upon synthesizing IIR with higher IP content. This is due to the comonomers’ own tendency to undergo chain transfer.\textsuperscript{38}

Termination can occur from the irreversible destruction of the propagating carbenium ion in different ways such as by the collapse of the ion pair, hydrogen abstraction from the comonomer, the formation of stable allylic carbenium ions, or reaction with nucleophilic species. The final MW of IIR is initially determined by controlling the rates of the initiation and chain transfer reactions.

Unreacted monomers and methyl chloride are removed by the addition of steam and hot water. Then they are dried and purified in preparation for recycling to the reactor. In order to eliminate agglomeration during the polymerization process, zinc or calcium stearate and antioxidants (0.4 – 1\%) are added to the hot water-polymer slurry. Sodium hydroxide is added to neutralize the aluminum catalyst, and this is eliminated as sodium chloride along with the water. It should be noted that most of the aluminum remains in the rubber as hydroxide, stearate or oxide. Stabilizers are added to the reaction, then the rubber is dried and compressed into bales which are wrapped in polyethylene or ethylene-vinyl acetate film.\textsuperscript{2,39}

1.3.3 Chemical and Physical Properties of IIR

In IIR, the IP is polymerized in a head-to-tail arrangement leading to a \textit{trans}-1,4 configuration (90 – 95\%). Chemical analysis has revealed little evidence for the presence of 1,2 and 3,4 modes of entry.\textsuperscript{40} Based on the grade of the commercial IIR, the unsaturation is between 0.5 – 4 mol\%, although it can be increased to 7 mol\%. A random distribution of unsaturation is obtained due to the low IP content and similar reactivity ratios between IP and IB.\textsuperscript{1} IIR has a $T_g$ of approximately -65 °C.\textsuperscript{36} Polydispersity indices
(PDIs), defined as the weight average molecular weight (M_w) divided by the number average molecular weight (M_n), range from 3 – 5, indicating a wide molecular mass distribution for the resulting copolymers. IIR and halogenated IIR derivatives are readily soluble in nonpolar solvents. Cyclohexane is an excellent solvent, benzene is a moderately good solvent, while pyridine and dioxane are nonsolvents.

In terms of stability, IIR has been shown to exhibit the chemical resistance expected for saturated hydrocarbons due to the very hydrophobic nature of the polymer and low level of unsaturation. It is therefore chemically inert towards acids, bases, ozone and oxidizing agents. However the unsaturation sites of IIRs can be slowly attacked by atmospheric ozone over extended periods of time, leading to degradation. This issue can be prevented by the addition of antioxidants.

PIB-based elastomers are remarkable for their low permeability to small-molecule diffusants such as N_2, O_2, H_2, He and CO_2 as a result of their efficient intermolecular packing, which leads to a relatively high density of the PIB portion in the copolymer chain. This property allows IIR to be an excellent candidate for the inner tubing of tires. Table 1.2 summarizes the diffusivity of several gases in IIR and NR.

<table>
<thead>
<tr>
<th>Gas</th>
<th>Diffusivity (cm^2/s) x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IIR</td>
</tr>
<tr>
<td>He</td>
<td>5.93</td>
</tr>
<tr>
<td>H_2</td>
<td>1.52</td>
</tr>
<tr>
<td>O_2</td>
<td>0.081</td>
</tr>
<tr>
<td>N_2</td>
<td>0.045</td>
</tr>
<tr>
<td>CO_2</td>
<td>0.058</td>
</tr>
</tbody>
</table>

The diffusivity of gases in NR relative to IIR arises from the differences between the structures of IIR and NR. As mentioned before, NR is a polymer of cis-1,4-PIP, so it
cannot pack as efficiently as IIR due to the lack of flexibility in the polymer backbone. In terms of air retention within tires, IIR was demonstrated to be at least 8 times better than NR (Table 1.3).  

**Table 1.3.** Air loss after automobile driving tests.

<table>
<thead>
<tr>
<th>Inner Tube</th>
<th>Original Pressure (psi)</th>
<th>1 Week</th>
<th>2 Weeks</th>
<th>3 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>28</td>
<td>4.0</td>
<td>8.0</td>
<td>16.5</td>
</tr>
<tr>
<td>IIR</td>
<td>28</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

IIR is known to be biocompatible and biostable for long-term applications due to its hydrolytic, oxidative and enzymatic resistance. This bioinertness can be attributed to the absence of polar bonds in the polymer main chain; other non-polar polyolefins such as polyethylene (PE) also have excellent biostability. The presence of alternating quaternary and secondary carbon atoms in the main chain makes oxidation difficult or impossible. It should be emphasized again that IIR has an order of magnitude lower permeability than any other cross-linked elastomer. This property can be useful in the prevention or delay of enzymatic degradation, combined with its excellent chemical and oxidative stability of PIB. However in certain biomedical applications the utility of IIR has faced limitations.

### 1.3.4 Halogenated IIR and Its Derivatives

IIR has been converted to a more reactive and faster curing derivative by the introduction of bromine or chlorine. Goodrich first reported the concept of halogenation of IIR in 1954-56. Brominated IIR (BIIR) was prepared from a bulk-batch halogenation, and was commercialized in 1954, but it was withdrawn in 1969. In 1961, Exxon Chemical Company commercially introduced chlorinated IIR (CIIR). Then in 1971, BIIR was once again commercialized by Polysar Limited of Canada and by Exxon Chemical Company in 1981. The incorporation of chlorine or bromine in approximately 1:1 molar ratio of halogen per IP unit of IIR, resulted in elastomers exhibiting the attractive properties of IIR as well as enhanced vulcanization rates and improved compatibility with
other highly unsaturated elastomers. Synthesis of halogenated IIR derivatives involves the “dark” reaction of a solution of IIR in hexane with elemental halogens at approximately 40 – 60 °C. During the halogenation step, hydrogen chloride or hydrogen bromide is generated, which must be neutralized. After neutralization, the aqueous phase is separated, the halogenated product is stabilized, and antioxidant is added to protect it during the recovery and finishing step. Scheme 1.9 summarizes the halogenation process to yield three potential isomers.

Scheme 1.9. Synthetic pathway for the halogenation of IIR.

The reaction of IIR with bromine or chlorine results predominantly in the substituted allylic halide structures (exo-methylene structure or product II of Scheme 1.9). This product is the most kinetically favored, and is found in the highest concentration as a result of steric constraints from the dimethyl-substituted carbon. However, under thermal conditions, this product can rearrange to the isomerized form (endo-bromomethyl product) due to the low strength of the carbon-halogen single bond (product III Scheme 1.9). Finally, dehydrobromination can result in the formation of conjugated dienes (Scheme 1.10).

Scheme 1.10. Formation of the conjugated diene derivative through dehydrobromination of the endo-bromomethyl product.
The chemical modification of halogenated derivatives by nucleophilic substitution provides a means of preparing functional polymers that cannot be synthesized via standard polymerization methods. Numerous examples of the displacement of halogen by oxygen,\textsuperscript{47} nitrogen,\textsuperscript{48,49} and phosphorus\textsuperscript{50} nucleophiles have been reported, resulting in the formation of useful derivatives for a wide range of applications. Moreover, the manipulation of the halogenated IIR through substitution reactions with amines,\textsuperscript{51} thiolates,\textsuperscript{52} sulfur,\textsuperscript{53} ethers,\textsuperscript{54} esters,\textsuperscript{46,55,56} and acids\textsuperscript{57} have been successfully developed with the aim of preserving the desirable properties of the parent material while enhancing its range of uses. The preparation of IB rich ionomers through the nucleophilic displacement of halide from BIIR has been of great interest due to their potential use in a diverse range of applications.\textsuperscript{50,58-61} The displacement of bromide from the BIIR by triarylphosphine (eg. triphenylphosphine) and trialkylamine nucleophiles (eg. N,N-dimethyloctylamine) have led to the introduction of a new class of elastomeric ionomers (Scheme 1.11). The allylic halide site allows for the reaction with and attachment of a nucleophile to the halogenated IIR polymer. Suitable nucleophiles are those having at least one neutral nitrogen or phosphorus center possessing a lone pair of electrons that are electronically and sterically accessible for participation in nucleophilic substitution reactions.

**Scheme 1.11.** Formation of cationic ionomers and conjugated dienes through modification of BIIR.

The corresponding ammonium/phosphonium bromide ionomers have shown interesting properties. For instance, IB-based ionomer composites have been derived
from a phosphonium bromide ionomer derivative (IIR-PP\textsubscript{3}Br), which resulted in the preparation of reinforced elastomer nanocomposites with improved properties.\textsuperscript{58}

Another promising application is the use of IIR ionomers in reducing populations of and/or preventing the accumulation of different organisms (such as bacteria, algae, fungi, mollusca or arthropoda).\textsuperscript{61} Over the past few decades there has been significant interest in designing polymers exhibiting antibacterial, antifungal and/or antialgal properties by impregnation with an antibacterial, antifungal or antialgal agent. However, these impregnated agents are commonly low MW compounds such as antibiotics, phenols, quaternary ammonium compounds or heavy metals such as silver, tin and mercury.\textsuperscript{61} The utility of these agents may be attractive, although they provide limited protection. This is due to the difficulty in controlling the rate of diffusion of the additive out of the polymer matrix.\textsuperscript{61} Thus, the leaching effect could eventually lead to a potential environmental risk due to the fact that it creates the possibility for the interaction of the leached material with other substances as well as enhances the microbial resistance to the agent. In other polymeric systems where the antibacterial, antifungal and/or antialgal agent is attached to the polymer, the incorporation of the active material is often part of the polymerization process, which can result in process problems and/or loss of polymer properties.

The great advantage of the IIR ionomer invention is that the ionic group is covalently attached to the polymer backbone, eliminating the leaching issue, as well as potentially increasing the antimicrobial efficacy, selectivity and handling safety of the polymer. The ionomers not only retain the properties of the original polymer, but also show enhanced physical properties, such as improved filler interaction, adhesion, and green strength. These properties are valuable in the formation of shaped articles and adhesively applied coatings. It is believed that the ionic feature of the ionomer imparts antibacterial, antifungal and/or antialgal properties.
1.4 Synthesis of Copolymers of PIB/IIR and Their Applications

1.4.1 Copolymers with Polystyrene (PS)

Linear triblock poly(styrene-\textit{b}-isobutylene-\textit{b}-styrene) (SIBS) copolymers synthesized via living cationic polymerization were introduced in the 1990s.\textsuperscript{62-64} These copolymers are known to be biostable thermoplastic elastomers (TPEs) with physical properties that overlap with those of medical-grade silicon rubber and polyurethanes.\textsuperscript{18,65} The TPE property allows SIBS to behave like a cross-linked rubber at room temperature, whereas at elevated temperature, it can be processed as a plastic. In other words, SIBS is a self-assembled physically cross-linked PIB, which is thermo- and solution formable. It is soluble in various non-polar solvents and can be spray coated or solvent cast to introduce a soft, strong, and coherent film. Moreover, it displays IIR-like properties such as excellent chemical, environmental and oxidative stability. The phase-separated structure introduces physical strength to the rubber without the need for chemical cross-linking. Figure 1.1, depicts the synthesis and resulting architecture of the SIBS. As shown, the rubbery PIB chains are held together by hard glassy PS domains.\textsuperscript{18}

\textbf{Figure 1.1.} Schematic of the synthesis of SIBS including ionization, initiation, propagation, and block formation. (\textit{Biomaterials}, 2008, 29, 448-460, Reproduced with permission from Elsevier).
The synthesis of SIBS initiates from a bifunctional initiator such as 5-tert-butyl-1,3-bis(1-methoxy-1-methylethyl)-benzene, referred to as “hindered dicumyl ether” (HDCE). This initiator becomes part of the polymer. The preparation of SIBS involves two steps in one pot. In the first step, IB is polymerized by a HDCE/TiCl₄ initiating system in hexane/methyl chloride solvent system at -80 °C under nitrogen. When the PIB reaches the desired MW, styrene is added to the reaction. Then, the polymerization is allowed to proceed until the outer PS blocks reach the desired length. The polymerization is terminated by the addition of methanol. The synthetic pathway is summarized in Scheme 1.12. SIBS has been shown to be biocompatible in vitro and in vivo. Since the Food and Drug Administration (FDA) approval of the TAXUS® drug eluting coronary stent in 2004, SIBS is used as the drug eluting coating on this stent.


1.4.2 Copolymers with PEO

In addition to block copolymers with PS, PEO has also been the subject of great interest due to its nontoxic nature and biocompatibility. The incorporation of PEO has proven to be one of the most effective means of achieving desired non-fouling properties. Amphiphilic di-, tri-, multi-, and star block copolymers of PIB and PEO were synthesized by hydrosilation of appropriately terminated PEOs with SiH-terminated PIB. The limitation of this approach is the requirement of Karstadt’s catalyst, extended reaction times, and multiple steps. Nevertheless, these copolymers have exhibited interesting properties such as phase-separated morphologies including a continuous
smooth matrix structure of PIB with dispersed PEO domains. In addition, it was shown that the swelling behavior of the copolymers in water and hexanes was a function of the hydrophobic/hydrophilic block ratio and copolymers containing 50 – 70 wt% PIB formed hydrogels. In water, the swollen PEO segments are held together by physical cross-links produced via hydrophobic forces acting between PIB domains.

Graft copolymer derivatives of IIR have been prepared from BIIR through several methods. For example, IIR-PEO graft copolymers were synthesized via modification of halogenated IIR derivatives through the reaction of halogen and the potassium salt of PEO monomethylether.\(^{47,73}\) The PEO MW varied from 750 g/mol, 2000 g/mol, and 5000 g/mol. Using PEO of 750 g/mol, a PEO content of 11 wt% was obtained. However upon reaction of PEO of 2000 g/mol and 5000 g/mol, 24 wt% and 11 wt% PEO contents were detected, respectively. The reactions were performed from 80 – 110 °C and the reaction time ranged from 5 – 30 hours.

Later, Whitney and Parent and coworkers described the synthesis of IIR-PEO graft copolymers by reaction of BIIR with potassium salts of PEO with different MWs.\(^{54}\) The resulting graft copolymers contained 10 – 30 wt% PEO content depending on the MW of the PEO. In this study, limitations imposed by the MW of PEO were mentioned and the purified graft copolymers contained substantial amounts of conjugated diene due to the fact that the reaction was performed at high temperature (115 °C) and in presence of several equivalents of potassium hydroxide.

Another method for obtaining IIR-PEO graft copolymers has involved the selective dehydrohalogenation of the allylic bromide functionality to yield an exo-conjugated diene. The resulting diene can undergo Diels-Alder cycloaddition with maleic anhydride (MAAn).\(^{57}\) In addition, the cyclo adduct can react with an alcohol to afford an acid and ester derivative (Scheme 1.13). However the reaction proceeds slowly under the conditions required to support copolymer formation, which in produced in low yields. A more efficient method involves direct displacement of bromide from BIIR using salts of maleate half-esters under phase transfer catalyzed conditions (Scheme 1.14).\(^{74}\) Adb Rabo Moustafa and Gillies later reported an improved approach to PEO grafting using Diels-
Alder chemistry by preparing clean exo-diene and also by optimizing the maleic anhydride opening with hydroxyl terminated PEO.\textsuperscript{75}

**Scheme 1.13.** Synthesis of acid and ester functionalized IIR. R=PE.

**Scheme 1.14.** Copolymer synthesis by carboxylate displacement of bromide under phase-transfer catalyzed conditions. R = PE, PEO.

While previous approaches to PIB graft copolymers allowed for the preparation and evaluation of these interesting materials,\textsuperscript{47,54,73,76-78} there have been challenges associated with these methodologies because the halide substitution and elimination
reactions are accomplished under harsh conditions with the formation of byproducts and/or with low yields.

Recently, Gillies and coworkers developed a clean and mild synthetic pathway for the preparation of linear PIB-PEO graft copolymers (Scheme 1.15). In this approach the double bond of the IP moiety is cleanly converted to an epoxide ring upon treatment with \( m \)-chloroperoxybenzoic acid (\( m \)-CPBA) at room temperature. This reaction is complete in 1 hr with high conversion to the epoxidized product (>99%). Then, the epoxide ring is opened in the presence of HCl, generating an allylic alcohol derivative. In contrast to Zaitsev’s law, the less-substituted alkene is cleanly generated, likely a result of kinetic control. The alcohol derivative is then activated with 4-nitrophénylchloroformate (4-NPC) and reacted with either hydroxyl (PEO-OH) or amine terminated PEO (PEO-NH\(_2\)) to afford linear PIB-g-PEO graft copolymers (Scheme 1.15).

![Scheme 1.15. Synthesis of linear PIB-g-PEO graft copolymers.](image)

Using this chemistry, linear PIB-PEO graft copolymers were successfully synthesized and contained different PEO contents. It should be noted that the PEO content of the resulting graft copolymers can be controlled in a reproducible manner by tuning different parameters such as the MW of PEO, IP content of the starting IIR, and the equivalents of PEO-NH\(_2\). As shown in Table 1.4, starting from IIR containing 2.2 mol\% IP content, a small library of copolymers with PEO content ranging from 2 wt\% to 65 wt\% PEO content was successfully synthesized. The resistance to protein adsorption of the resulting graft copolymers was investigated via fluorescence confocal...
Table 1.4. Characterization of a library of linear PIB-PEO graft copolymers.

<table>
<thead>
<tr>
<th>PEO-NH₂ MW (g/mol)</th>
<th>PEO-NH₂ equiv.</th>
<th>Functionalized IP units (%)</th>
<th>PEO content (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>0.05</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2000</td>
<td>0.1</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>2000</td>
<td>0.2</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>2000</td>
<td>0.4</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>2000</td>
<td>0.8</td>
<td>75</td>
<td>24</td>
</tr>
<tr>
<td>2000</td>
<td>1.2</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>750</td>
<td>1.2</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>5000</td>
<td>1.2</td>
<td>100</td>
<td>65</td>
</tr>
</tbody>
</table>

As shown in Figure 1.2, the graft copolymers exhibited interesting micrometer scale patterning upon the adsorption of fluorescently-labeled proteins at low PEO content. Importantly, surfaces coated with graft copolymers containing higher PEO content of 24 and 34 wt%, revealed no fluorescence suggesting that they exhibited resistance to protein adsorption (Figure 1.2 e and f).

1.5 Surface Functionalization of IIR/PIB

The introduction of chemical functionalities through surface modification has been of great interest as it enables the desirable properties of a bulk material to be retained, while tuning the properties of the surface for a specific application. For example, the functionalization of surfaces with PEO can result in properties such as resistance to protein adsorption and the attachment and growth of cells that are critical for many biomedical applications.
Figure 1.2. Fluorescence confocal microscopy images (543 nm) of thin films (spin-cast at 20 mg/mL from CH₂Cl₂) following adsorption of a rhodamine-fibrinogen conjugate. Images represent different wt% of PEO grafted on IIR backbone: a) 2%, b) 4%, c) 6%, d) 12%, e) 24%, and f) 34%. (*Macromolecules*, 2011, 44, 6405-6415, Reproduced with permission from the American Chemical Society).

Several approaches have been applied with the aim of functionalizing surfaces with PEO. Among all of them, a simple and effective one-step method was developed recently for the attachment of PEO onto non-functional polymer surfaces. This approach, developed by Lau and coworkers is known as hyperthermal hydrogen induced cross-linking (HHIC).\(^{81}\)

As an attractive alternative to conventional plasma treatments,\(^ {82}\) HHIC is an alternative way of cross-linking polymers to surfaces using the concept of collision kinematics. HHIC is a fast and efficient process for selectively cleaving the C-H bonds of organic compounds while preserving other chemical functionalities of the molecules on the surface. In this process, H\(_2\) is used as a light-mass projectile to collide with H of a
C-H bond of an organic molecule on a surface; this collision very effectively knocks off hydrogen atoms from the molecule but leaves the molecule otherwise intact. The, carbon radicals produced can then recombine and the precursor organic molecules are cross-linked to each other and to any surface that contains C-H bonds. This C-H cleavage method has low energy-consumption and no reliance on any other chemical reagents. The development of HHIC has led to tailor-made surface modifications such as grafting molecules with specific functional groups onto different surfaces. A practical reactor providing a high flux of such hyperthermal hydrogen for complete surface grafting in a very short time has recently been developed (Figure 1.3).

Gillies and coworkers have demonstrated the utility of HHIC in the preparation of IIR-based surfaces exhibiting non-fouling properties. In this work, IIR and epoxidized IIR were spin cast and cross-linked via HHIC. Then, the cross-linked surfaces were spin coated with PEO followed by cross-linking by HHIC. The resulting surfaces were immersed in a fluorescently labeled fibrinogen. After washing off the nonadsorbed protein, the protein adsorption and cell growth were evaluated on the surfaces. As shown in Figure 1.4a, IIR was a good substrate for cell growth. On the other hand, upon coating IIR first with epoxidized IIR as an interfacial layer, followed by PEO and HHIC, a
significant reduction in the number of cells was observed. This was attributed to the resistance of the PEO coated surfaces to protein adsorption, which is thought to be the first step in the adhesion of cells to a surface. Figure 1.4b shows the relative fluorescence values obtained by confocal microscopy. It can be concluded that HHIC is a promising technique for the preparation of protein- and cell-resistant properties from a diverse array of unreactive hydrophilic and hydrophobic surfaces containing C-H bonds.

![Graph](image)

**Figure 1.4.** a) Evaluation of cell growth on surfaces: 1a) IIR, 1b) epoxidized IIR coated with PEO, 1c) control surface of silane-functionalized PEO grafted on glass, 1d) PEO-coated silicon wafer following HHIC. b) Relative fluorescence obtained by confocal microscopy; 2a) IIR, 2b) epoxidized IIR, 2c) epoxidized IIR coated with PEO, 2d) PEO on clean silicon wafer, 2e) control surface of silane functionalized PEO grafted on glass (0.01 μg/cm²). Error bars represent the standard deviation of 10 measurements on each of 3 samples.\(^ 84\) (*Applied Materials & Interfaces*, 2011, 3, 1740-1748, Reproduced with permission from the American Chemical Society).

Functional polymer surfaces composed of polypropylene, IIR, and poly(vinyl acetate) (PVAc) were also prepared using HHIC.\(^ 85\) While many properties of IIR are advantageous, it would be useful to reduce its hydrophobicity for some applications. Biaxially oriented polypropylene (BOPP) was used as a model substrate. Then, IIR was chosen as the next layer and PVAc was selected as the third layer as it can subsequently be hydrolyzed to poly(vinyl alcohol) (PVA). Furthermore, the choice of PVAc enables for the evaluation of the functional group tolerance of HHIC. Upon cross-linking each
layer, the ester functionalities were hydrolyzed to convert the surface from hydrophobic to hydrophilic (Figure 1.5). This process demonstrated that multiple layers of cross-linked materials could be added, creating polymer laminates with each layer introducing new functionalities and properties.

![Figure 1.5. a) Preparation of laminates using HHIC, b) chemical transformations of the PVAc layer.](Langmuir, 2011, 27, 14820-14827, Reproduced with permission from the American Chemical Society)

### 1.6 Approaches to the Cross-linking/Curing of IIR

As described earlier, the presence of low levels of unsaturation in IIR, provides sites for further modification of the polymer backbone as well as for chemically cross-linking the rubber. Chemical cross-linking, also referred to as vulcanization, leads to improved mechanical properties. Cross-linked networks are formed through the reaction of IIR with suitable vulcanizing agents. Vulcanization transforms the material into a strong elastic product. Moreover, the vulcanized rubber becomes insoluble in solvents and more resistant to deterioration caused by light, heat, and aging processes.

Three common methods of vulcanizing IIR are accelerated sulfur vulcanization, dioxime cross-linking, and resin curing.¹ Lightly vulcanized rubber exhibits good elastic properties, whereas highly vulcanized rubber is a hard and rigid material. The modulus and $T_g$ are increased due to the high restriction of the macromolecular chain mobility.
brought on by cross-linking. Goodyear discovered sulfur vulcanization of NR in 1839 and developed new applications for rubber in industry.\textsuperscript{64}

Sulfur vulcanization is the most popular system owing to its low cost, easy availability, good processing and physical properties, and its adaptability to diverse methods, heating media and compounding ingredients. However, the process of vulcanization by sulfur alone is a slow and inefficient process due to the high activation energy (270 kJ/mol) of sulfur ring opening, which leads to prolonged curing times at high temperatures (~ 6 hours at 140 °C). In addition, large amounts of sulfur are needed and not all of the sulfur forms true cross-links. Therefore, the resulting vulcanized product is prone to oxidative aging and has poor mechanical properties. By using accelerators, the efficiency of the rubber-sulfur reaction can be improved. The commonly utilized accelerators are sulfenamides. The basic action of accelerators is to split the S\textsubscript{8} rings into smaller fragments, which will then react with rubber. Different sulfur-based compounds such as thiazoles, thiurams and dithiocarbamates have been used in the vulcanization process. An example of a sulfur based cross-linking approach is shown in Scheme 1.16a. Another example is shown in Scheme 1.16b where an oxidizing agent oxidizes \textit{p}-quinone dioxime, leading to an active cross-linking agent, which can rapidly vulcanize the IIR at room temperature.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Scheme1.16.png}
\caption{Vulcanization of IIR: a) sulfur-based cross-linking, b) dioxime curing.}
\end{figure}

Another approach for chemically cross-linking IIR is the use of CIIR and BIIR derivatives\textsuperscript{1}. The main difference between these two halogenated derivatives results from the higher reactivity of C-Br bond compared to that of C-Cl. Therefore BIIR exhibits a
higher versatility in vulcanization. It requires lower levels of curing agent, cures faster and has a greater affinity for co-vulcanization with other highly unsaturated elastomers. Depending on the application, BIIR can be cured using different curing systems. For instance, zinc free materials are needed in some special pharmaceutical closure applications. In this case, BIIR is capable of being vulcanized without zinc oxide or any other zinc salts. Recommended curing agents are diamines such as hexamethylene diamine carbamate. Zinc oxide, preferably in presence of some stearic acid, can be used as a sole curing agent for halogenated IIR. A proposed mechanism by Baldwin is based on the formation of stable C-C cross-links through a cationic polymerization process.\(^1\)

After vulcanization, the majority of the halogen originally present in the polymer can be extracted as zinc halide. When zinc oxide is used as the curing agent, the necessary initiating amounts of zinc halide are likely produced as a result of thermal dissociation of some of the allylic halide to yield hydrogen halide. Then subsequent reaction of the hydrogen halide with zinc oxide provides a zinc halide catalyst.\(^1\)

In addition, BIIR can be cured with sulfur as a sole curing agent. Both derivatives can be cross-linked by zinc oxide and peroxides. CIIR can be cured through bis-alkylation reactions or heat reactive phenolic resin curing systems. The above-mentioned cross-linking systems require the addition of a curing agent. Therefore, they are not suitable for biomedical applications due to the possibility of leaching toxic additives from the materials. So far there is no example of chemically cross-linking IIR derivatives without the need for some form of a chemical curing agent.

1.7 Arborescent PIB (arb-PIB)

The development of various controlled/living polymerization techniques during recent years has provided the possibility to synthesize a wide variety of architectures including star-like structures,\(^{16}\) dendritic (hyperbranched and arborescent) structures,\(^ {86,87}\) self-assembling block copolymers,\(^ {88}\) and others. In particular, dendritic structures have been of particular interest over the past couple of decades. For example, dendrimers display spherical symmetry comprising a central core surrounded by a regular branching pattern.\(^ {89}\) They were first introduced by Vögtle in 1978.\(^ {90}\) These highly regular structures with short branches can be prepared by two different approaches, convergent and
divergent syntheses. In the convergent method, the synthesis starts from the outer part of the molecule, connecting the branches in successive steps and finally forming a core. On the other hand, in the divergent method the synthesis starts from the core, successively growing the branches. Among all the existing branched polymers, dendrimers appeared to be highly interesting because of their spherical symmetry. They exhibit a controlled and symmetric structure and very narrow MW distribution. However, the complicated and time-consuming synthetic routes associated with this class of branched polymers present a major drawback in commercial applications.

Hyperbranched polymers are less symmetric than dendrimers. The first polymers with hyperbranched structures were prepared by condensation polymerization, as outlined by Flory in 1941. The resulting polymers had short branches and imperfect architectures. A novel approach for the synthesis of hyperbranched polymers termed as “Self-Condensing Vinyl Polymerization” (SCVP) was developed in the early 1990s by Fréchet and coworkers. This method allows the synthesis of hyperbranched structures using vinyl monomers that also contain an initiating group. Since such species combine the functions of a monomer and an initiator, they were called “inimers” (IM, initiator-monomer) (Scheme 1.17).

![Scheme 1.17](image)

**Scheme 1.17.** Self-condensing vinyl polymerization mechanism.

Gauthier and Möller introduced arborescent (arb) (tree-like) polymers in 1991. Arborescent polymers combine the characteristics of dendrimers and hyperbranched polymers, with longer polymer chains between the branching points. These were
prepared by successive grafting of polymeric building blocks (graft-on-graft) leading to well-defined structures and narrow MW distributions. High MW \textit{arb}-PSs and \textit{arb}-PIPs were prepared successfully by this method, but the synthetic routes were rather laborious and lengthy.

Subsequently, an alternative and commercially feasible approach was developed by Puskas and coworkers in which a small amount of a suitable \textit{inimer} was copolymerized with an olefin.\textsuperscript{40,81,82} \textit{arb}-PIBs with high MWs were successfully synthesized using 4-(2-hydroxyisopropyl)styrene and 4-(2-methoxyisopropyl)styrene as \textit{inimer} in a one-pot living-type polymerization process.\textsuperscript{41,95} In order to reach high MWs, the polymerization should be living, meaning that chain transfer, irreversible termination and other side reactions should be absent. Moreover, the \textit{inimer} should contain both initiating and propagating active sites with comparable reactivities.

Biomacromolecular engineering allows for the design and development of PIB-based biomaterials with diverse architectures. Figure 1.6 summarizes the various block copolymer architectures that exhibit TPE properties.\textsuperscript{96} Structurally, they all consist of a rubbery (soft) inner segment (core) bound to two or more glassy (hard) outer segments (corona). While macroscopically homogeneous, these polymers phase separate on a microscopic scale. The discontinuous plastic phases embedded in the continuous elastomeric phase leads to the formation of “physical cross-links”. The TPE properties are controlled by the spatial arrangement of the two-rubbery/glassy segments (eg. linear, star, \textit{arb}), the molecular characteristics of each segment (eg. MW, $T_g$, ratio of rubbery/glassy components, degree of segmental incompatibility), and the microphase morphology of the constructs.\textsuperscript{96} The linear triblock SIBS was the first generation of the PIB-based TPEs.\textsuperscript{97}

The second generation of PIB-based biorubbers were star-branched TPEs with a PIB core and PS end blocks.\textsuperscript{16} Star block copolymers are attractive alternatives to linear block copolymers due to their superior combination of physical and processing properties, including higher moduli combined with lower viscosities at similar MWs.\textsuperscript{96}
The third generation materials had an \textit{arb} core and 3-20 end blocks of PS or PS derivatives such as poly(\textit{p}-methylstyrene).\textsuperscript{98} The fourth generation has been developed with a high MW \textit{arb}-PIB core and 2 – 30 short end blocks composed of copolymers of IB with IP, \textit{p}-methylstyrene, or cyclopentadiene.\textsuperscript{86} Interestingly, block copolymers with IIR end blocks containing <5 wt\% IP content have shown phase separation and TPE behavior. This is the first example of a block-type TPE copolymer where both blocks are elastomers.

![Diagram of block architectures produced via carbocationic techniques: a) linear, b) triarm-star, c) multiarm-star, and d) \textit{arb}.](image)

**Figure 1.6.** Block architectures produced via carbocationic techniques: a) linear, b) triarm-star, c) multiarm-star, and d) \textit{arb}.

Preliminary studies have shown that \textit{arb}-PIB-based TPEs could be excellent alternatives to the linear SIBS material in biomedical applications.\textsuperscript{99} In addition, \textit{arb}-PIB-\textit{b}-PS has a favorable combination of properties such as improved dynamic fatigue and creep in comparison to the linear SIBS. This is due to a “double network” structure with a covalently branched core embedded into a self-assembling thermolabile network.\textsuperscript{97,100,101} PIB-based TPEs exhibit a unique combination of properties, including good thermal, environmental and chemical resistance, coupled with processability, excellent barrier properties as well as outstanding biostability and biocompatibility.\textsuperscript{101,102} Encrustation of \textit{arb}-PIB-\textit{b}-PS in a rabbit model was found to be comparable to or better than the medical-grade silicon rubber.\textsuperscript{99} Moreover, hemolysis and 30-and 180-day implantation studies showed excellent biocompatibility of this emerging biomaterial.\textsuperscript{99}
1.8 Scope and Objectives of This Thesis

The objective of this thesis was to explore new methods for the modification of PIB-based materials in order address the limitations of the previous approaches and to impart new properties and functions. To this end, the modification of PIB surfaces was explored and new methods were developed for the chemical functionalization of both linear and \textit{arb}-PIB. These approaches were used to provide antibacterial and antifouling properties to PIB and to enable the suspension of PIB in aqueous solution in the form of nanometer-sized aggregates.

Chapter 2 describes the application of the HHIC cross-linking approach to prepare cross-linked quaternized poly(2-(dimethylamino)ethyl methacrylate) thin films exhibiting antibacterial properties. The antibacterial properties were investigated against both Gram-positive and Gram-negative bacteria. Moreover, the process was successfully applied to prepare antibacterial IIR surfaces revealing the versatility of the HHIC approach for grafting onto unfunctionalized hydrocarbon surfaces.

Building on the synthesis of PIB-PEO graft copolymers from IIR containing 2 mol\% IP, Chapter 3 describes an extension of the synthetic methods to IIR containing 7 mol\% IP in order to obtain high PEO content PIB-PEO graft copolymers. The properties of the resulting graft copolymers, along with some of the higher PEO content materials from the previous work were studied both on surfaces and in solution.

Chapter 4 describes the synthesis of \textit{arb}-PIB-g-PEO graft copolymers. The properties of these materials, including their microphase separation, mechanical properties, and self-assembly in aqueous solution were studied and compared with the linear PIB-g-PEO graft copolymer analogues in order to elucidate the effects of macromolecular architecture on these properties.

Chapter 5 describes the synthesis and characterization of UV-curable cinnamate functionalized IIR derivatives starting from both low (2 mol\%) and high (7 mol\%) IP content. The cross-linking was performed under UV irradiation and the kinetics of the cross-linking was studied. Polymer films were prepared using different methods and their
properties, including the swelling ratio, gel content, and toxicity were fully studied. The approach was also applied to obtain cross-linked films of IIR-PEO graft copolymers.

Chapter 6 concludes and summarizes the main achievements of the chapters, and includes some suggestions for future directions.
1.9 References


2 Preparation of Antibacterial Surfaces by Hyperthermal Hydrogen Induced Cross-linking of Polymer Thin Films

2.1 Introduction

The immobilization of polymers on surfaces is of interest for a wide variety of applications ranging from electronic devices to biomaterials. It is of particular interest for the development of medical devices where coatings with specific properties or functions such as protein resistance, antimicrobial activity, and controlled drug release are often desired. The immobilization of polymers on surfaces has been achieved through various processes. For example, polymers with the appropriate chemical functionalities can be grafted onto surfaces by their reaction with complementary functionalities on the surface. Alternatively, through the conjugation of an initiator moiety to the surface, polymers can be grown from the surface. While these approaches lead to well-defined coatings, they typically involve multi-step covalent modifications of the surfaces in order to graft the polymers or initiators, which may be an obstacle to the scaling up of these coating processes. In addition, they require the presence of reactive functionalities on the surface. In order to address this limitation, surfaces have also been generated by simple coating or painting methodologies using water insoluble polymers. Layer by layer assembly processes using polyelectrolytes, as well as physisorption and chemisorption have also been investigated. However, as the polymers are not covalently immobilized using these methods there may be problems associated with delamination and their long-term stability in the presence of biological fluids should be further investigated.

It is also possible to generate functional surfaces by plasma or radiation induced grafting processes. For example, surfaces have been functionalized with PEO for

protein resistance or polyamines\textsuperscript{24,25} for antimicrobial properties. While careful tuning of such processes can provide well defined chemical functionalities in some cases,\textsuperscript{26,27} many examples result in considerable heterogeneity in chemical functionalities at the surface, making them non-optimal for biomedical applications.\textsuperscript{19,21,28} A method that could combine the efficiency and simplicity of plasma methods with the well defined chemical functionalities and polymer lengths afforded by the chemical immobilization approaches would provide a significant advancement.

We have recently developed a new and special plasma-based approach to synthesize molecular layers with tailor-made functionalities using the concept of collision kinematics.\textsuperscript{29-32} This approach involves the treatment of surfaces with H\textsubscript{2} projectiles having appropriately elevated kinetic energy to selectively cleave C-H bonds. The treatment, therein referred to as hyperthermal hydrogen induced cross-linking (HHIC), can thus be used to covalently graft function-specific molecules to a polymer surface. In layman’s terms, HHIC can be illustrated with the hard sphere approximation. According to this approximation, the maximum energy transfer between two colliding species is determined by the two masses with the formula $4M_1M_2/(M_1+M_2)^2$. This simple model suggests that for a projectile of H\textsubscript{2}, in the head-on collision with H of a C-H bond, the maximum kinetic energy transfer is 89\%, while if the target is C, the maximum kinetic energy transfer is 49\%. By considering this, as well as known bond dissociation energies, it is possible to tune the kinetic energies of the H\textsubscript{2} projectiles in order to afford the selective cleavage of C-H bonds on a surface. The radicals generated from the C-H bond cleavages can then combine to effectively cross-link molecular films on the surface while preserving other chemical functionalities. As a diverse array of surfaces and function-specific molecules contain C-H bonds, the HHIC method should be widely applicable.

We have recently demonstrated the use of HHIC for the preparation of PEO coated surfaces that resist the adsorption of proteins and the growth of cells\textsuperscript{33} and for the preparation of polymer laminates based on polypropylene, IIR, and poly(vinyl acetate).\textsuperscript{34} The preparation of antibacterial surfaces using HHIC represents an ideal application, due to the diversity of chemical functionalities present in antibacterial molecules and the requirement of well-defined surfaces for biomedical applications. The development of
effective antimicrobial surfaces is of significant interest for a wide range of applications. For example, the decontamination of simple objects such as doorknobs, elevator buttons, and food packaging may prevent the spread of infections in everyday life, while the use of antimicrobial medical devices such as catheters and implants may lower the rate of hospital acquired infections. The covalent immobilization of the biocide is particularly important in these applications as physical immobilization methods may lead to a gradual leaching of the antimicrobial agent from the surface, resulting in contamination of the environment, sub-inhibitory concentrations that facilitate the development of resistance, and eventually a depleted supply of biocide on the surface.\textsuperscript{35-37} While the various grafting techniques have been used to attach antibacterial poly(quaternary ammonium) compounds to surfaces including glass,\textsuperscript{6,8,38-40} metal,\textsuperscript{7,41,42} silicon,\textsuperscript{5,40} and paper,\textsuperscript{6} these approaches are still limited by the challenges described above, and no ideal method has been developed.\textsuperscript{2}

Thus, we describe here the use of HHIC to prepare cross-linked thin films of quaternized poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), a known antibacterial polymer\textsuperscript{5,6,40,43} on both a model alkane modified silicon surface as well as on IIR, a common high performance elastomer. The films were extensively characterized at each step using atomic force microscopy (AFM), x-ray photoelectron spectroscopy (XPS), and contact angle measurements. The results demonstrate the effectiveness of the HHIC method in covalently immobilizing the polymer, while retaining its chemical functionality. The films are demonstrated to exhibit antimicrobial activity against both Gram-negative and Gram-positive bacteria.

2.2 Results and Discussion

2.2.1 Preparation and Characterization of Functionalized Silicon Wafers

The steps required for the preparation of antibacterial surfaces by the HHIC process are outlined in Figure 2.1. Briefly, the PDMAEMA was first spin coated onto the surface, and then was cross-linked by HHIC. The surface was washed extensively to
remove any non-immobilized polymer, and then the cross-linked overlayer was quaternized by reaction with an alkyl halide. In addition to its simplicity, an advantage of this approach is that the polymer can be prepared and characterized in solution prior to its grafting onto the surface. PDMAEMA was synthesized using a previously reported method, resulting in a polymer with a $M_n$ of 14100 g/mol and a PDI of 1.46 as determined by size exclusion chromatography (SEC) in $N,N$-dimethylformamide (DMF) with calibration relative to polystyrene standards. The MW based on end group analysis by $^1$H NMR spectroscopy was approximately 12000 g/mol.

A silicon wafer modified with octadecyltrimethoxysilane (ODTS) was selected as a model surface for the initial series of experiments. This surface is atomically flat, thus facilitating the characterization of the polymer films by techniques such as AFM. The ODTS provides C-H bonds for the covalent cross-linking of the polymers to the surface by HHIC. In addition, it converts the highly hydrophilic silicon wafer to a hydrophobic surface, which is a better model of the hydrophobic polymer surfaces that are targeted for functionalization with antibacterial polymers. These surfaces were prepared by first cleaning the silicon wafers with a Piranha solution, then reacting them with ODTS in toluene in the presence of catalytic octylamine. The resulting surfaces had a contact angle

**Figure 2.1.** Steps involved in the preparation of antibacterial surfaces using the HHIC approach.
of $(84 \pm 5)^\circ$ in comparison with $(15 \pm 3)^\circ$ for the cleaned silicon surface (Table 2.1). In addition, as shown in Table 2.2, there was a significant decrease in the silicon content and an increase in the carbon content of the surface as measured by XPS.

**Table 2.1.** Water contact angle data for surfaces (a minimum of 5 measurements were performed on each surface).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water Contact Angle (static)</th>
</tr>
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<tbody>
<tr>
<td>Clean silicon wafer</td>
<td>$(15 \pm 3)^\circ$</td>
</tr>
<tr>
<td>ODTS/silicon wafer</td>
<td>$(84 \pm 5)^\circ$</td>
</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer</td>
<td>Not determined (H$_2$O soluble)</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>$(58 \pm 2)^\circ$</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)</td>
<td>$(64 \pm 5)^\circ$</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer – washed (30 s)</td>
<td>$(57 \pm 4)^\circ$</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer – washed (180 s)</td>
<td>$(62 \pm 4)^\circ$</td>
</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer – washed</td>
<td>$(85 \pm 5)^\circ$</td>
</tr>
<tr>
<td>Quaternized PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>$(9 \pm 6)^\circ$</td>
</tr>
<tr>
<td>Quaternized PDMAEMA/ODTS/silicon wafer (180 s)</td>
<td>$(11 \pm 4)^\circ$</td>
</tr>
</tbody>
</table>

Solutions of PDMAEMA at concentrations of 1 or 5 mg/mL in dichloromethane were then spin coated onto the modified silicon wafers. In early studies it was found that using concentrations of 1 mg/mL resulted in incomplete surface coverage. Therefore, all subsequent studies were carried out at 5 mg/mL. Following spin coating, uniform films of polymer with thicknesses from 15 – 25 nm were obtained, as measured by AFM (see appendix). These thicknesses are in the range appropriate for the estimated depth limit of HHIC.$^{29-31}$

The contact angle for this surface could not be determined as the non-cross-linked polymer was soluble in water. However, as shown in Table 2.2, XPS data revealed the
presence of the PDMAEMA on the surface by the disappearance of the peak corresponding to silicon and corresponding increases in the carbon, oxygen, and nitrogen peaks from the PDMAEMA. In addition, high resolution XPS data for the C 1s region were very close to the values expected based on the chemical structure of PDMAEMA (Table 2.3, Figure 2.2).45,46

Table 2.2. XPS survey scans of surfaces.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>O</th>
<th>N</th>
<th>Si</th>
<th>Other</th>
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<tbody>
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<td>Clean silicon wafer</td>
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<td>33</td>
<td>----</td>
<td>57</td>
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</tr>
<tr>
<td>ODTS/silicon wafer</td>
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<td>17</td>
<td>----</td>
<td>20</td>
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</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer</td>
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<td>8</td>
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<td>----</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>79</td>
<td>15</td>
<td>6</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)</td>
<td>78</td>
<td>15</td>
<td>7</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (30 s) – washed</td>
<td>83</td>
<td>12</td>
<td>5</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (180 s) – washed</td>
<td>78</td>
<td>16</td>
<td>6</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer – washed</td>
<td>62</td>
<td>15</td>
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<tr>
<td>Quaternized PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>78</td>
<td>15</td>
<td>5</td>
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<td>2 (Br)</td>
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<tr>
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<td>11</td>
<td>4</td>
<td>----</td>
<td>2 (Br)</td>
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</tbody>
</table>

The PDMAEMA coated surfaces were then cross-linked by HHIC. Treatment times of 30 and 180 s were selected in order to investigate the effect of treatment time on the structure of the PDMAEMA.
Table 2.3. High resolution XPS data.

<table>
<thead>
<tr>
<th></th>
<th>% Composition of the C 1s peak</th>
<th>% Composition of the N 1s peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-C</td>
<td>C-N</td>
</tr>
<tr>
<td>ODTS/silicon wafer</td>
<td>97</td>
<td>-----</td>
</tr>
<tr>
<td>PDMAEMA theoretical</td>
<td>37.5</td>
<td>37.5</td>
</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (30 s) – washed</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>HHIC treated PDMAEMA/ODTS/silicon wafer (180 s) – washed</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>PDMAEMA/ODTS/silicon wafer – washed</td>
<td>99</td>
<td>-----</td>
</tr>
</tbody>
</table>

Following HHIC, no changes in the polymer film were observed by AFM for either the 30 or 180 s treatment time (see appendix). The contact angles of the films were measured to be $(58 \pm 2)^\circ$ and $(64 \pm 5)^\circ$ for the samples treated for 30 s and 180 s respectively, consistent with the previously reported contact angles of PDMAEMA functionalized surfaces.⁵
Figure 2.2. High resolution XPS spectra of the C 1s region of a) PDMAEMA/ODTS/silicon wafer, b) HHIC treated PDMAEMA/ODTS/silicon wafer (30 s), c) HHIC treated PDMAEMA/ODTS/silicon wafer (180 s), and the N 1s region of d) HHIC treated PDMAEMA/ODTS/silicon wafer (180 s) and e) quaternized PDMAEMA/ODTS/silicon wafer (180 s).

XPS survey scans did not reveal any significant changes in the elemental composition of either the 30 s or 180 s samples relative to the nontreated samples. In addition, analysis of the high resolution C 1s spectra (Table 2.3, Figure 2.2) revealed that the chemical functionalities of the PDMAEMA were not significantly modified by the HHIC process.\textsuperscript{45,46} The cross-linked films could be washed by immersion and sonication in CH\textsubscript{2}Cl\textsubscript{2}/NEt\textsubscript{3} (95/5) and the films remained intact as supported by AFM images, contact angle measurements (Table 2.1) and XPS (Tables 2.2 and 2.3). In contrast, when an uncross-linked film was washed under the same conditions, the AFM image showed complete removal of the material from the surface (see appendix) and the contact angle measurements and XPS results resembled those of the ODTS coated surface. Overall, these results indicate that the HHIC process was effective in covalently linking the film to the surface while retaining its chemical functionalities.

The final step for generation of the proposed antibacterial surfaces was to quaternize the tertiary amines in the PDMAEMA. This was accomplished by immersion
of the surfaces in a solution of ethyl bromide in acetonitrile. Following this treatment, the contact angles dropped to \( (9 \pm 6)^\circ \) and \( (11 \pm 4)^\circ \) for the 30 s and 180 s samples respectively. These results were consistent with the expected increases in wettability of the surfaces due to the introduction of charged amine groups. The most notable changes in the XPS results were observed in the high resolution N 1s spectra. While only \( 10 – 20\% \) of the N 1s peak corresponded to \( \text{N}^+ \) on the unquaternized surfaces, likely due to some degree of amine protonation, this increased to 80\% following quaternization and washing (Figure 2.2). The fact that a peak corresponding to unquaternized nitrogen was observed for both the 30 s and 180 s samples indicates that the reaction between the amine and ethyl bromide did not reach 100\% completion. This can likely be attributed to the inaccessibility of some amines within the polymer film following the cross-linking. Nevertheless, it would be expected that the amines at the surface of the film would be most readily quaternized in each case and these would also be most important for the antibacterial activity. To further evaluate the surface charge, the concentration of accessible quaternary ammonium groups on the surface was also quantified by a colorimetric method based on fluorescein complexation.

Assuming a 1:1 electrostatic binding between fluorescein and surface quaternary ammoniums, the surface charge density can be calculated. Using this assay, it was determined that there were \( 4.4 \times 10^{15} \) charges/cm\(^2\) on the surface. As this assay was performed in aqueous solution, the possible contribution of unquaternized but protonated primary amines to this value cannot be excluded but based on XPS analyses this should not contribute more than about 20\% of this value. In comparison with the values obtained in previous studies, this should be sufficient for antimicrobial activity.

### 2.2.2 Evaluation of the Antibacterial Properties of Coated Silicon Wafers

With quaternized cross-linked films in hand, the next step was to evaluate their antibacterial properties. This was accomplished using the antibacterial “drop test”
Both Gram-positive *S. aureus* and Gram-negative *E. coli* were tested and the cationic surfaces were compared to clean silicon wafer controls.

As shown in Table 2.4, based on this assay, the clean silicon wafer appeared to kill or inactivate 59% of *S. aureus* and 9% of *E. coli*. The result for *S. aureus* was initially surprising but can be explained by an artifact of the assay involving the adhesion and growth of live bacteria on the surface as will be described in further detail below. The quaternized surface that was treated with HHIC for 30 s killed or inactivated 99% of *S. aureus* and 90% of *E. coli*. Increasing the cross-linking time to 180 s did not significantly change the antibacterial activities for either strain of bacteria (p > 0.05). Therefore, making the reasonable assumption that a greater number of cross-links are present on the surface that was treated with HHIC for 180 s, these results indicate that the expected corresponding decrease in chain mobility does not adversely affect the antibacterial activity. This is in agreement with the results of Ye et al. on polymer coatings prepared by vapor cross-linking.

In addition, it is consistent with the results of Russell and coworkers, who have found that for quaternized PDMAEMA-coated surfaces, the primary determinant in the antibacterial efficacy is the density of positive charges on the surface rather than polymer length or other properties.

The activities of the surfaces towards the Gram-positive *S. aureus* relative to Gram-negative *E. coli* were not significantly (p > 0.05) different in the case of 180 s of HHIC treatment but the activity towards *S. aureus* was significantly (p < 0.001) greater for the sample treated for only 30 s. Greater activity towards *S. aureus* in comparison to *E. coli* has also been observed for other films comprising cross-linked quaternary ammonium salts and thus may be related either to the particular susceptibility of the *E. coli* strain or to the mechanism of action of these surfaces in relation with the different membrane structures of Gram-positive and Gram-negative bacteria.
Table 2.4. Antibacterial activities of quaternized PDMAEMA/ODTS/silicon wafer.

<table>
<thead>
<tr>
<th>Surface</th>
<th>% Bacteria Killed/Inactivated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus (Gram-positive)</td>
</tr>
<tr>
<td>Clean silicon wafer</td>
<td>(59 ± 1)</td>
</tr>
<tr>
<td>Quaternized HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)</td>
<td>(99 ± 0.1)</td>
</tr>
<tr>
<td>Quaternized HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)</td>
<td>(98 ± 2)</td>
</tr>
</tbody>
</table>

Different mechanisms of action have been proposed for cationic surfaces including the mobilization of structurally important metal cations in the membrane as well as the adhesion of cationic surfaces with the anionic phospholipids resulting in disruption of the bacterial cell membrane. The mechanism may depend on the particular surface and class of bacteria under investigation.

In order to investigate whether the apparent antibacterial activities of surfaces could be attributed to the adhesion and growth of bacteria on the surfaces, a LIVE/DEAD® BacLight Bacterial viability kit was used to visualize and differentiate between live and dead bacteria on the surface. In this assay, live bacteria appear green due to the uptake of the dye SYTO 9, which permeates all bacterial membranes, while dead bacteria appear red due to the uptake of propidium iodide which permeates only damaged bacterial membranes and dominates over the fluorescence of SYTO 9. After incubation of a clean silicon wafer with *S. aureus*, followed by rinsing of the surfaces, many live bacteria and only a very small number of dead bacteria were observed (Figure 2.3). These living bacteria would be counted as killed or inactivated bacteria in the antimicrobial drop test assay, as they would not be recovered from the surface for subsequent colony growth and counting. In contrast, essentially no bacteria were detected on the quaternized PDMAEMA-coated surfaces, thus confirming the true antibacterial activity.
Figure 2.3. LIVE/DEAD® analysis following incubation of surfaces with *S. aureus*. Live bacteria appear green in this assay, while dead bacteria appear red. a) a clean silicon wafer with many live bacteria bound, b) quaternized PDMAEMA-coated silicon surface with essentially no bound bacteria, c) cleaned IIR with many live bacteria, d) quaternized PDMAEMA-coated IIR with no bound bacteria detected. Note that the resolution of the IIR images appears lower due to the inherent non-uniformity of the surface.

2.2.3 Application to Polymer Surfaces

Having demonstrated that the HHIC method is an effective means of immobilizing antibacterial polymers on surfaces while retaining their activities, it was of interest to apply the method to a polymer surface. Cured IIR was selected as the polymer surface for several reasons. First, commercial IIR consists almost entirely of unactivated C-C and C-H bonds, making it very challenging or impossible to functionalize the surface by traditional chemical means. In contrast, the C-H bonds make it an ideal substrate for HHIC. Furthermore, IIR has recently been of interest for biomedical applications. For example, copolymers of IB and styrene have been used as coatings for vascular stents, and its use in breast implants has also been proposed due to its bioinertness and high
impermeability.\textsuperscript{53-55} In order to expand the applications of IIR in medical devices it may be useful to impart antibacterial properties to its surface.

Following the protocol described above for the ODTS modified silicon wafers, PDMAEMA was spin coated onto a cured sheet of IIR at a concentration of 5 mg/mL. Although the low Young’s modulus and non-uniformity of the surface at the nanoscale made the measurement of film thickness by AFM impossible, the C=O stretch of the carbonyl group in PDMAEMA was readily detected on the surface using attenuated total reflectance infrared spectroscopy (ATR-IR) as shown in Figure 2.4. This film was cross-linked by HHIC for 30 seconds. Due to the non-uniformity of the surface, in order to ensure complete surface coverage, this spin coating and cross-linking procedure was performed twice. Following cross-linking, the same carbonyl peak was observed by ATR-IR and the contact angle was measured to be (63 ± 6)° in comparison with (82 ± 2)° for the initial cleaned IIR surface. This contact angle is the same as that measured for cross-linked PDMAEMA on the silicon wafer described above. Neither the ATR-IR spectrum nor the contact angle changed following washing of the cross-linked film by immersion and sonication in CH\textsubscript{2}Cl\textsubscript{2}/NEt\textsubscript{3} (95/5), indicating that the PDMAEMA was effectively immobilized. The cross-linked film was quaternized under the same conditions described above for the silicon wafers. Although no visible changes were expected or detected in the ATR-IR spectrum, a reduction in contact angle to (12 ± 6)° was measured, consistent with the presence of quaternary amines on the surface. The concentration of quaternary ammonium groups on the surface was also quantified by the fluorescein assay described above, providing a value of 5.2 \times 10^{15} \text{charges/cm}^2 on these surfaces, similar to the value of 4.4 \times 10^{15} \text{charges/cm}^2 obtained on the quaternized silicon wafers.
Figure 2.4. FTR-IR spectra of a) IIR surface, b) PDMAEMA/IIR, c) HHIC treated PDMAEMA/IIR, d) HHIC treated PDMAEMA/IIR washed, e) quaternized HHIC treated PDMAEMA/IIR.

The antibacterial activities of the quaternized surfaces were measured using the same drop-test assay described above (Table 2.5). IIR itself was found to exhibit moderate antimicrobial activity against both *S. aureus* and *E. coli*. However, a LIVE/DEAD® BacLight Bacterial assay (Figure 2.3c) revealed that this apparent activity may be due to the presence of live bacteria that adhered to the surface, as was observed for the silicon wafers. Some antibacterial activity may also be attributed to the leaching of rubber additives such as zinc oxide during the assay. Upon functionalization of the surface with quaternized PDMAEMA, 93% of Gram-positive bacteria and greater than 99% of Gram-negative bacteria were killed or inactivated. No bacteria were detected on the surface using the LIVE/DEAD® assay (Figure 2.3d). The results for *S. aureus* and *E. coli* are significantly (p < 0.0001) different, though it is not obvious why these surfaces were capable of killing or inactivating *E. coli* more effectively than the *S. aureus* while the capabilities of the coated silicon wafers were the opposite. Perhaps this points to the complexity and multiple mechanisms involved in the action of these surfaces. Nevertheless, the activities of the surfaces against Gram-negative bacteria are noteworthy and provide further evidence of the effectiveness of HHIC as a means of preparing antibacterial surfaces, even from polymer surfaces lacking reactive groups for functionalization.
Table 2.5. Antibacterial activities of quaternized PDMAEMA/IIR.

<table>
<thead>
<tr>
<th>Surface</th>
<th>% Bacteria Killed/Inactivated</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIR</td>
<td>( (49 \pm 4) )</td>
</tr>
<tr>
<td>Quaternized HHIC treated PDMAEMA/IIR</td>
<td>( (93 \pm 0.1) )</td>
</tr>
<tr>
<td>( E. coli ) (Gram-negative)</td>
<td>( &gt; 99 )</td>
</tr>
</tbody>
</table>

2.3 Conclusions

The use of HHIC as a means of cross-linking antibacterial polymers to surfaces was investigated. It was found that HHIC could be applied to ODTs modified silicon wafers coated with PDMAEMA without significant destruction of the surface or the chemical functionalities of the polymer, as evidenced by AFM, XPS, and contact angle measurements. The PDMAEMA could subsequently be quaternized to provide surfaces with high antibacterial activities against both Gram-negative \( S. aureus \) and Gram-positive \( E. coli \). To demonstrate the full utility of the technique, HHIC was applied for the preparation of antibacterial IIR surfaces. The successful application to IIR, which is both nonfunctional and hydrophobic indicates that this process is likely to be very versatile and useful for the functionalization of a wide range of polymer surfaces with functional materials.

2.4 Experimental Section

**General Methods and Materials:**

PDMAEMA was prepared as previously reported.\textsuperscript{44} SEC was carried out in DMF with 0.1 M LiBr and 1% (v/v) NEt\(_3\) at 85 °C using a Waters 2695 separations module equipped with a 2414 differential refractometer and two PLgel 5 µm mixed-D (300 mm × 7.5 mm) columns from Polymer Laboratories. The calibration was performed using polystyrene standards with molecular weights ranging from 580 to 170800 g/mol. All
other chemicals were purchased from commercial suppliers and used as received. Silicon wafers were purchased from Solar Wafer. The grade of hydrogen gas was 99.99%. Cured IIR sheets were provided by LANXESS Inc. They were cut into 1 cm × 1 cm pieces then immersed in each of ethanol, deionized water, and dichloromethane for 2 hours. They were then sonicated in a 1:1 mixture of deionized water:ethanol for 30 minutes. After that they were rinsed with 1:1 water:ethanol, acetone, spun dry, and then left under vacuum at 40 °C overnight.

**Modification of Silicon Wafers with ODTS**

Silicon wafers were cut into small pieces (approximately 1.5 cm × 1.5 cm), then immersed in a 1:2 mixture of H$_2$SO$_4$:H$_2$O$_2$ (volume: volume) (Piranha solution; attention – strong acid, strong oxidizer!) to clean organic residues off the surface. This was followed by a thorough rinse in water and then ethanol. The wafers were then spun dry by air. A solution of toluene (25 mL), octylamine (1 drop) and octadecyltrimethoxysilane (0.5 mL) was prepared. The dried surfaces were immersed in this solution for 12 hours. Finally the surfaces were washed with water and then ethanol and spun dry.

**Spin Coating**

A 5 mg/mL solution of PDMAEMA in dichloromethane was prepared and was filtered through a 0.2 μm filter. It was then added dropwise onto the surface until it was completely covered with the solution. The surface was then spun at 4000 rpm for 15 seconds.

**HHIC$^{32}$**

The surfaces were treated with hyperthermal hydrogen for 30 s or 180 s. For the IIR surfaces, the spin coating and cross-linking steps were carried out twice. The conditions were: (a) the hydrogen plasma was maintained with 200 W of microwave energy, and 87.5 mT in magnetic field for increasing the plasma density; (b) protons were extracted by a grid electrode at -96 V, into the draft tube of 50 cm at 0.80 mTorr of gaseous hydrogen; and (c) ions and electrons were screened in front of the specimen with a pair of grid-electrodes biased to +60 V and -40 V. Under this set of conditions, a high flux of
hyperthermal neutral hydrogen projectiles, with appropriate kinetic energy to break C-H bonds but not other bonds undesirably, was delivered to the specimen surface. The surfaces were washed by immersion in a solution of CH₂Cl₂/NEt₃ 95/5 (v/v) overnight and then in an ultrasonic bath (Fisher Scientific Ultrasonicator, model FS20H) for 30 minutes. Finally they were rinsed with acetone, and then ethanol, and then spun dry.

**Quaternization of the PDMAEMA Surfaces**

The cross-linked PDMAEMA surface was placed in acetonitrile (1 mL). Excess bromoethane (0.3 mL) was added and the surfaces were agitated at a rate of 20 rpm using a GyroTwister (Labnet International Inc.) overnight at room temperature. The surfaces were then rinsed well with acetonitrile, acetone, spun dry, and then left under vacuum at 40 °C overnight.

**Surface Analyses**

The surface topography and PDMAEMA coverage on the modified silicon wafers were analysed by AFM using a Dimension V equipped with a Nanoscope V controller from Veeco Inc. In order to determine the film thickness, a small scratch was made on the PDMAEMA down to the Si surface. AFM measurements were carried out in tapping mode using a silicon nitride cantilever tip having radius of curvature of 10 nm and a spring constant of 40 N/m under ambient conditions. XPS analyses were carried out using a Kratos Axis Ultra spectrometer using a monochromatic Al K(α) source (15mA, 14kV) with charge neutralization. Sample data was collected at a take-off angle of 30°. CasaXPS version 2.3.14 was used for the analysis of all the XPS spectra. All spectra were referenced to the aliphatic C–H bond of 285 eV and fitting was accomplished using a Gaussian and Lorentzian ratio of 40. The binding energies at 286.9, 285.9, 286.1, 289.0 eV are attributed respectively to C-O, C-N, C-N⁺, and O=C-O species of PDMAEMA. Peak FWHW was constrained to fall within 0.9 – 1.4. All other components were allowed to freely refine. The static water contact angle of the surfaces was measured using a NRL Contact-Angle Goniometer Model 100.00. A minimum of 5 measurements were taken for each surface. Fourier transform infrared (FTIR) spectra were obtained on a Bruker IR ScopII with a micro-attenuated total reflectance (micro-ATR) attachment.
equipped with a germanium crystal. Areas of 80 – 100 µm in diameter and a depth of 1 – 2 µm were analyzed.

Evaluation of Antibacterial Properties

The antibacterial activities of the quaternized surfaces against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus* ATCC3307) and Gram-negative bacteria *Escherichia coli* (*E. coli* ATCC 29425) were studied using the antibacterial drop-test.47,48 *E. coli* or *S. aureus*, precultured in 15 mL of nutrient broth (Difco™ BD) at 37 °C for 24 h, were washed by centrifuging at 4000 rpm for 10 min. After removing the supernatant, the cells were washed with phosphate buffered saline (PBS) twice and re-suspended and diluted to approximately 3 × 10^5 colony forming units (CFU)/mL in PBS solution. The samples were placed in sterilized glass Petri dishes and sterilized by heating at 100 °C for 30 minutes. 100 µL of PBS solution with bacteria was added drop-wise onto the surface of each sample and completely covered the sample surface. The petri dishes were sealed and placed in an incubator at 37 °C with 46% humidity. After 3 h, the bacteria were washed from the surface of the sample by using 10 mL PBS in the sterilized Petri dish. From this solution, 100 µL was spread onto solid plate count agar (Difco™ BD). After incubation for 24 h at 37 °C, the number of surviving bacterial colonies on the Petri dishes were counted. The results after multiplication with the dilution factor were expressed as CFU per mL. The above experiments were carried out in triplicate for each sample. The percentage of killed/inactivated bacteria was calculated as [(CFU of initial bacterial suspension – CFUs following surface contact)/CFU of initial bacterial suspension] × 100.

Results represent mean ± SD of triplicates from three separate experiments. Statistical analyses were performed using the software Prism. When comparing two data sets, unpaired, two-tailed T tests were used. When the data involved greater than two data sets an ANOVA test followed by Tukey’s test was used.

**LIVE/DEAD® Assay**

100 µL of *S. aureus* bacterial suspension (approximately 2 × 10^6 CFU/mL) was added dropwise onto either a clean silicon wafer, quaternized PDMAEMA-coated silicon wafer,
clean IIR, or quaternized PDMAEMA-coated IIR. After 3 h of incubation (at 37 °C with 46% humidity), the surfaces were rinsed with 10 mL of PBS and 10 mL deionized water. The adherent bacteria on the surfaces were immediately stained using the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen, USA). The two BacLight stains, SYTO 9 and propidium iodide were dissolved in 0.5 mL filter-sterilized deionized water then 5 µL of each dye was diluted in 100 mL of filter-sterilized deionized water. A total of 200 µL (100 µL + 100 µL) of the dye suspension were mixed together and pipetted onto the prepared surfaces then incubated in the dark at room temperature for 15 min. Finally, the surfaces were rinsed with filter-sterilized deionized water and the fluorescence was imaged using an LSM 510 multichannel point scanning confocal microscope (Laser 488 nm for the SYTO 9 with a pass filter of 505 – 530 nm and a laser at 543 nm for the propidium iodide with a pass filter of 615 nm, magnification 63×). All the images were obtained and refined with the ZEN software.

**Determination of Surface Accessible Quaternary Amine Groups**

The surface density of quaternary ammonium groups on the quaternized PDMAEMA-coated rubber surface was measured by UV-vis spectroscopy, as previously described. Briefly, the surface (1 × 1 cm²) was dipped in 10 mL of a 1 wt% solution of fluorescein (sodium salt) in distilled water for 10 min. The surfaces were then rinsed extensively with distilled water, placed in 3 mL of a 0.1 wt% solution of cetyltrimethylammonium chloride in distilled water, and shaken for 20 min at 300 rpm to desorb the dye. The absorbance of the resulting aqueous solution was measured at 501 nm after adding 10% v/v of 100 mM phosphate buffer (pH 8.0). The concentration of the fluorescein was calculated using an extinction coefficient of 77 mM⁻¹cm⁻¹. The conversion of the dye concentration to surface charge density was determined assuming that one surface quaternary ammonium group complexes with one dye molecule.
2.5 References


Chapter 3

3 Synthesis and Properties of IIR–PEO Graft Copolymers with High PEO Content*

3.1 Introduction

The development of new copolymers is a highly versatile strategy for obtaining materials with novel properties and functions. The vast array of available monomers, combined with numerous possible synthetic approaches has opened doors to a nearly limitless number of potential molecules. Often these materials can possess the desirable properties of the individual monomers as well as new properties of the combination. Block copolymers are of particular interest as they frequently assemble in a controlled manner in the bulk, on surfaces, or in solution.1-3 These assemblies have generated significant interest for fundamental studies and for numerous applications from catalysis4 to drug delivery5 and microelectronics.6 Of the various classes of block copolymers, comb-like or graft copolymers are particularly interesting as it is possible to finely tune their architectures by adjusting both the relative chain lengths and the grafting densities.7,8 While the assemblies of these polymers in solution and on surfaces is generally less well understood than for their linear diblock and triblock counterparts, there are now examples of interesting spherical, hexagonal, cylindrical, and flower morphologies on surfaces9-12 as well as micelles,13-15 capsules,16 and polymersomes17-19 in solution.

In recent years, significant interest has emerged in the development of PIB-based materials for biomedical applications.20-23 This has arisen from their mechanical properties, which are very similar to certain soft tissues which compose the human body, as well as other favourable properties such as high chemical and biological stability. Currently, a PIB-polystyrene triblock copolymer is used as the drug eluting coating on

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the TAXUS® vascular stent. In addition, similar copolymers have been investigated as synthetic aortic valves and as corneal shunts for the treatment of glaucoma. Membranes composed of copolymers of telechelic PIB and hydrophilic polymers such as poly(N,N-dimethylacrylamide) (PDMAAm) or PEO were prepared and were shown to encapsulate cells while allowing for the exchange of oxygen, nutrients, and proteins across the membrane. PIB-poly(methyl methacrylate) (PMMA) composites have been shown to have enhanced properties relative to commercial bone cements due to the incorporation of the elastomeric PIB into the glassy PMMA material. Furthermore, multiarm PIB-cyanoacrylate (CA) copolymers have been reported as promising materials for intervertebral disk replacement due to the combination of CA chemistry and the viscoelastic properties of PIB. Despite the promise of the above materials, the further development of new PIB-based biomaterials with improved properties and functions is continually underway.

The incorporation of PEO into copolymers with PIB is of significant interest as it can impart amphiphilicity to the copolymer, as well as other desirable properties such as increased mechanical strength, thermoplasticity, and resistance to the adsorption of proteins. To this end, several syntheses of linear PIB-PEO block copolymers have been reported. Starting from IIR, a commercially available copolymer of IB and small amounts of IP (< 3 mol%), our group has recently reported a synthetic approach for derivatizing the double bond moieties of the IP units to graft PEO chains along the polymer backbone. Relative to previously reported approaches to IIR-PEO graft copolymers, the reactions were high yielding and clean, providing a high degree of control over the PEO content of the resulting materials. This allowed for the preparation of a library of graft copolymers ranging from 2 wt% to 65 wt% PEO, the highest PEO content reported to date for such graft copolymers. It was found that thin films of copolymers with low PEO content (< 18 wt%) adsorbed fluorescently labeled proteins in complex micron scale patterns, an effect that was attributed to a combination of phase separation and kinetic factors involved in the spin coating/solvent evaporation process. However, at higher PEO content (≥ 18 wt%) the films resisted the adsorption of proteins. As this property is desirable for numerous applications such as medical
implants, drug delivery vehicles, or protein production and purification equipment there is substantial interest in the further study of these materials.

Here we describe the extension of our synthetic approach for the grafting of PEO onto the backbone of a IIR containing a much higher IP content of 7 mol%. Using PEO with different molecular weights, materials ranging from 40 to 83 wt% PEO were prepared. Properties of films composed of these polymers along with selected high PEO content graft copolymers from our previous report are also described. These studies demonstrate the beneficial effect of high PEO content in providing stable films that are capable of encapsulating and releasing payloads, while at the same time resisting the growth of cells on their surfaces. Such films have the potential to serve as coatings for various medical devices.

Furthermore, the high PEO content of these amphiphilic graft copolymers facilitates for the first time the preparation of stable IIR-based nanoscale assemblies in water. The high stability, lack of toxicity, and capability of these assemblies to encapsulate hydrophobic molecules suggests their utility in various encapsulation and pharmaceutical delivery applications.

3.2 Results and Discussion

3.2.1 Copolymer Synthesis and Characterization

IIR-PEO graft copolymers 3.1, 3.2, and 3.3 (Table 3.1) were synthesized as previously reported from IIR containing 2 mol% IP and amine terminated PEO (PEO-NH₂) with MWs of 750 g/mol, 2000 g/mol or 5000 g/mol respectively. As demonstrated with these copolymers, one approach for increasing the PEO content is to increase the MW of the grafted PEO chain. An alternative approach is to introduce additional chains along the IIR backbone. As the grafting is performed through the functionalization of the IP units of the rubber, this requires the introduction of additional IP into the rubber. As such, IIR containing 7 mol% IP (high IP IIR) was explored as a starting material for the polymers prepared in this work.
Table 3.1. Structure and chemical properties of IIR-PEO graft copolymers.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>IP content in starting rubber (mol%)</th>
<th>Grafted PEO-NH₂ MW (g/mol)</th>
<th>PEO content of graft copolymer wt %</th>
<th>Mₘ b (kg/mol)</th>
<th>Tₘ c (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>2</td>
<td>750</td>
<td>18</td>
<td>850 ± 34</td>
<td>12</td>
</tr>
<tr>
<td>3.2</td>
<td>2</td>
<td>2000</td>
<td>34</td>
<td>970 ± 36</td>
<td>39</td>
</tr>
<tr>
<td>3.3</td>
<td>2</td>
<td>5000</td>
<td>65</td>
<td>1550 ± 380</td>
<td>59</td>
</tr>
<tr>
<td>3.8</td>
<td>7</td>
<td>750</td>
<td>40</td>
<td>741 ± 53</td>
<td>20</td>
</tr>
<tr>
<td>3.9</td>
<td>7</td>
<td>2000</td>
<td>60</td>
<td>1740 ± 20</td>
<td>44</td>
</tr>
<tr>
<td>3.10</td>
<td>7</td>
<td>5000</td>
<td>83</td>
<td>5040 ± 670</td>
<td>60</td>
</tr>
</tbody>
</table>

aFrom ¹H NMR, based on the relative integrations of the signals at 3.66 ppm and at 1.43 ppm corresponding to the PEO and IB units respectively.
bFrom light scattering. cFrom DSC analysis.

As shown in Scheme 3.1, high IP-IIR 3.4 was first epoxidized by treatment with m-CPBA. The epoxide 3.5 was then opened using aqueous HCl in toluene. In previous work, this was demonstrated to cleanly yield an allylic alcohol 3.6. In the case of the high IP-IIR, it was found that although this alcohol functionalized polymer remained soluble throughout the reaction, if the solvent was removed it could not be redissolved for characterization or further reaction. This was attributed to polymer cross-linking via hydrogen bonding due to the high density of backbone hydroxyls, although the possibility of a covalent cross-linking mechanism in the solid state cannot be fully excluded. On the other hand, if 4-NPC and pyridine were added directly to the above reaction mixture without workup, then polymer 3.7 could be isolated by precipitation in acetone and was fully soluble in solvents such as toluene, hexanes, chloroform, and THF in which the starting rubber is soluble. The use of this one-pot procedure reduces the number of overall steps and increases the overall efficiency of the synthetic method. The evolution of functional groups from polymers 3.4 to polymers 3.5 and 3.7 is shown in Figure 3.1(a-
c). In the last synthetic step, the activated polymer 3.7 was reacted with PEO-NH$_2$ of MWs of 750 g/mol, 2000 g/mol or 5000 g/mol to provide polymers 3.8, 3.9, and 3.10 respectively. The polymers were purified by aqueous washing and precipitation in water or diethyl ether. The yields of the graft copolymers were approximately 70% after this purification procedure.


Polymers 3.8 – 3.10 were characterized by $^1$H NMR spectroscopy, light scattering and differential scanning calorimetry (DSC). Following conjugation of the PEO-NH$_2$ and thus conversion of the activated carbonates to carbamates, the $^1$H NMR peaks corresponding to the exo-alkene and the C-H in the $\alpha$-position to the activated carbonate in the region from 4.5 to 5.5 ppm were observed to shift significantly as shown in Figure 3.1c and d. This allowed for the reaction conversion to be followed and for each of polymers 3.8, 3.9, and 3.10 it was found to be quantitative within the detection limits of NMR spectroscopy. As previously reported,$^{43,44}$ the $^1$H NMR integrations of the peaks corresponding to the PEO at 3.65 ppm and the PIB units at 1.42 ppm were compared to estimate the PEO content. Using this method, the PEO contents of polymers 3.8, 3.9, and 3.10 were 40 wt%, 60 wt%, and 83 wt% in comparison with the expected values of 50 wt%, 73 wt% and 87 wt% respectively. Thus the resulting PEO content was relatively
close to the expected value for each polymer, with discrepancies possibly resulting from the removal of higher PEO content material during the aqueous purification or perhaps from very low levels of side reactions during the reaction sequence. For example, trace peaks from 2.5 – 2.7 ppm are believed to result from aldehyde and ketone products, which are well known to result from epoxide rearrangement processes under certain reaction conditions.

![Figure 3.1](image-url)  
**Figure 3.1.** $^1$H NMR spectra (CDCl$_3$, 400 MHz) in the region of 2.5 to 5.5 ppm of: a) IIR 3.4, b) epoxidized IIR 3.5, c) activated IIR 3.7 and d) copolymer 3.9.

In our previous work, it was found that at lower PEO content the IIR-PEO graft copolymers exhibited $T_m$s that were significantly lower than those of the grafted homopolymer PEO chains (32 °C for 750 g/mol; 52 °C for 2000 g/mol; 59 °C for 5000 g/mol$^{15}$). As the PEO content increased, the $T_m$s approached those of the corresponding PEO homopolymer. This was again observed in the current work, with polymers 3.8, 3.9, and 3.10 having $T_m$s of 20 °C, 44 °C, and 60 °C suggesting that the phase separated domains of PEO are larger for the longer grafted PEO chains and also for the high IP
rubber graft copolymers due to the higher densities of PEO chains. For polymers 3.8 and 3.9, the T\textsubscript{g}s were approximately -60 °C, similar to that of the starting rubber. No T\textsubscript{g} was detected for copolymer 3.10, likely due to the low content of IIR backbone in these samples making detection challenging (see appendix).

As previously observed, the size exclusion chromatographic analysis of these polymers was problematic\cite{43,44}. Therefore, the time averaged light scattering intensities for each polymer were measured in batch mode as a function of concentration and their molecular weights were determined using Debye plots (see appendix). It was previously found that as the PEO content of the IIR-PEO graft copolymers increased, the dn/dc values for the polymers decreased from approximately 0.1 mL/g, similar to that of pure PIB, plateauing at approximately 0.055 mL/g similar to that of pure PEO at PEO content greater than 30 wt\%.\cite{43} Therefore a dn/dc value of 0.055 mL/g was used in the current calculations. In comparison with the starting IIR 3.4, for which the M\textsubscript{w} was measured to be 570 ± 18 kg/mol, the M\textsubscript{w}s of polymers 3.8, 3.9, and 3.10 were found to be 741 ± 53 kg/mol, 1740 ± 20 kg/mol, and 5040 ± 670 kg/mol. However, it should be noted that as light scattering was measured at a single angle for each polymer, and high molecular weight polymers are expected to exhibit an angular dependence, these measured M\textsubscript{w} values are only approximations and are provided mainly to demonstrate the expected trend.

3.2.2 Properties of Copolymer Films

While the adsorption of proteins to films of IIR-PEO graft copolymers with varying PEO content was extensively investigated in previous work\cite{43}, it was of interest here to explore a number of different properties of films from high PEO content graft copolymers. First, due to the very high aqueous solubility of PEO, it was thought that films of copolymers containing high PEO content might exhibit limited stabilities in aqueous solution. To investigate this, copolymers 3.1, 3.2, 3.3 and 3.10 ranging from 18 – 83 wt\% PEO were melt pressed to a thickness of approximately 0.2 mm, and disks weighing approximately 10 mg each were punched from these films. The disks were
immersed in water at 25 °C over a period of 28 days. Each week 3 films of each copolymer were removed, washed, dried and weighed to measure the average mass loss. As shown in Figure 3.2, minimal weight loss occurred over this time period. The mass loss increased with PEO content, with polymer 3.1 exhibiting negligible mass loss and polymer 3.10 exhibiting approximately 10% mass loss over this time period. Considering the PEO content of 83 wt% in polymer 3.10, the very slow dissolution/degradation of the copolymer into water was somewhat unexpected and suggests that even the low content of hydrophobic IIR backbone is sufficient to aggregate the hydrophobic domains of the copolymers, resulting in a physical cross-linking that helps maintain intact films. This result is consistent with the swellable, physically cross-linked networks obtained with linear and star-shaped PIB-PEO block copolymers.40

Figure 3.2. Mass loss from films of copolymers 3.1, 3.2, 3.3, and 3.10 upon incubation in water at 25 °C. Data points represent the average value for 3 different films and the error bars represent the standard deviations on these measurements.

Given the high stabilities of the above copolymer films, it was of interest to further explore their properties by studying the encapsulation and release of a probe molecule. In this case, the copolymer films were prepared by drop casting a solution containing the copolymer and the dye rhodamine B onto glass cover slips. Rhodamine B was selected because it is soluble in organic solvents for the film preparation, but is also
soluble in water, allowing it to be released into aqueous solution. Prior to drop casting of the copolymer, a layer of unfunctionalized IIR was first drop cast onto the surface. Without this layer, the films of copolymers 3.3 and 3.10 rapidly delaminated from the surface upon incubation in the buffer, likely due to the high hydrophilicity of both the PEO and the glass, which allowed for extensive water penetration. The deposition of IIR acted as a “primer”. Films of IIR or copolymers 3.1, 3.2, 3.3, or 3.10, containing rhodamine B were incubated at 25 °C in 100 mM, pH 7.4 phosphate buffer for 17 days. At periodic time points, the buffer was removed, its absorbance at 550 nm was measured by UV-visible spectroscopy to quantify the amount of released rhodamine B, and then the buffer solution was returned to the same films.

As shown in Figure 3.3, for each film there was a burst release of dye over the first 24 hours. The extent of this burst release was highly dependent on the PEO content of the copolymers with IIR and copolymer 3.1 exhibiting only about 5%, copolymers 3.2 and 3.3 about 25%, and copolymer 3.10 about 40% dye release over this time period. This result suggests that the dye was likely released rapidly from the surface and perhaps the PEO domains of the copolymer, which would both be readily accessible to water. Following this 24 hr period, there was no further release of dye from the IIR or copolymer 3.1 films. Due to the high hydrophobicity of the IIR component of these films it is likely that the remaining dye was completely inaccessible to the buffer solution and thus remained in the film. On the other hand the percentage of dye released actually decreased for films of copolymers 3.2, 3.3, and 3.10 over the time period from 1 to 10 days and then plateaued. This can be attributed to a gradual swelling of these films over this time period, and a concomitant equilibration of the dye between the solution and the films as the experiments were not performed under sink conditions. To confirm this, the release experiment was performed for copolymer 3.2 under the same conditions except that the incubation buffer was replaced with fresh buffer at each time point, providing sink conditions. In this case, the percentage of released dye did not decrease and nearly 80% of the dye was released over 10 days (see appendix). Overall, these results suggest that the copolymer films can swell and can encapsulate and release molecules in a manner that is dependent on the PEO content, but the burst release effect must be taken into consideration in applications.
As the adsorption of proteins to a surface is believed to be the first step in the adhesion and growth of cells on surfaces, and we have shown that films of IIR-PEO copolymers with high PEO content ($\geq 18$ wt%) resist the adsorption of proteins, it was also of interest to explore the growth of cells on films of the various copolymers. Films of copolymers 3.1, 3.2, 3.3, and 3.10 were prepared by drop casting them on glass slides having a IIR “primer” layer. The surfaces were sterilized and then seeded with C2C12 mouse myoblast cells and incubated in cell culture medium. After 2 days, the cells were fixed, washed and their nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI), while their cytoskeletons were stained with Alexa Fluor 568 phalloidin. As shown in Figure 3.4, the number of cells counted on films of copolymer 3.1 was not significantly different than on the control glass or IIR coated glass surfaces and their cytoskeletons appeared similar. However, significantly fewer cells were found on films of copolymers 3.2, 3.3, and 3.10. The cells that were detected exhibited changes in their cytoskeletons and tended to grow in clusters and on top of one another (Figure 3.5). This suggests that at these higher PEO contents, the surface becomes an unfavorable environment for the
attachment and growth of cells. This finding is consistent with previous reports on PEO-functionalized rubber surfaces, but here the surfaces were obtained from PEO block copolymers rather than via the functionalization of the polymer surface with PEO.47

Figure 3.4. Number of adhered cells per mm$^2$ for control surfaces and surfaces of copolymers 3.1, 3.2, 3.3, and 3.10 (*P <0.05 by one-way ANOVA test followed by Tukey’s test).

3.2.3 Copolymer Assembly in Aqueous Solution

Thus far, there are only a limited number of reports involving the formation of nanosized assemblies from amphiphilic PIB block copolymers in aqueous solution.41,48-50 PIB-PEO linear block copolymers have been shown to form polymersomes,41 and IIR-PEO graft copolymers have been shown to exhibit emulsifying capabilities35,44 but to the best of our knowledge there are no reports on the aqueous assembly of PIB-PEO or other amphiphilic PIB graft copolymers.
Figure 3.5. Confocal microscopy images of C2C12 cells adhered to control and copolymer surfaces: a) glass (control), b) IIR (control), c) copolymer 3.1, d) copolymer 3.2, e) copolymer 3.3, f) copolymer 3.10. The cell nuclei are stained with DAPI and cytoskeletons are stained with Alexa Fluor 568 phalloidin. Each image represents an area of 0.22 mm × 0.22 mm.

Despite the resistance of copolymer films with as high as 83 wt% PEO to direct dissolution in water, it was proposed that due to their amphiphilic character, it should be possible to obtain stable aqueous assemblies of these copolymers. Other than thin film hydration, another common approach for the preparation of polymer assemblies in water involves solvent exchange. This requires the block copolymer to be soluble in an organic solvent that is miscible with water. For the range of IIR-PEO graft copolymers described here, THF was a suitable solvent. Dissolution of copolymers 3.1 – 3.3 and 3.8 – 3.10 in THF at a concentration of 10 mg/mL, followed by dialysis against pure water led to assemblies on the order of 300 – 500 nm in diameter for copolymers 3.1 – 3.3 and approximately 200 nm for copolymers 3.8 – 3.10 as measured by dynamic light scattering (DLS). The smaller sizes of the assemblies from copolymers 3.8 – 3.10 in general relative to 3.1 – 3.3, even at similar PEO content, might result from the higher densities of grafted
PEO chains owing to the higher IP content. For copolymers \( 3.1 \) – \( 3.3 \), the assembly size decreased with increasing PEO length, which was expected, due to the increased ability of the longer PEO chains to stabilize smaller assemblies in water. However, for the series of copolymers \( 3.8 \) – \( 3.10 \), the assembly size decreased as expected for \( 3.9 \) versus \( 3.8 \), but then increased for \( 3.10 \). This can perhaps be attributed to the larger size of the 5000 g/mol PEO and thus larger hydrodynamic diameters of the resulting assemblies. This aspect would play a larger role for the aggregates as they approach unimolecular, rather than intermolecular, assemblies (Figure 3.6).

![Graph](image)

**Figure 3.6.** Z-average diameters for aqueous assemblies as a function of the amount of water added rapidly to a THF solution of copolymer prior to dialysis against water: a) copolymers \( 3.1 \) – \( 3.3 \), b) copolymers \( 3.8 \) – \( 3.10 \). Error bars represent the standard deviations on measurements from 3 separately prepared samples of the assemblies.

Instead, the control of size likely results from a kinetic effect where the gradual addition of water through a dialysis process provides the time required for copolymer chains to aggregate. On the other hand, when water is added rapidly the hydrophobic backbones of the copolymers must rapidly collapse, resulting in the formation of smaller aggregates or even unimolecular micelles. Representative DLS size distributions are shown in the appendix. It was also shown by DLS that the assemblies were stable over time with no substantial changes in size observed over a period of 6 months (see appendix). In all cases with increasing water content prior to dialysis, the assemblies
formed from copolymers containing 5000 g/mol PEO were larger than those containing 2000 g/mol PEO, supporting the above hypothesis that as the hydrodynamic diameters of the assemblies decreases the length of the PEO chains may play a larger role in determining the assembly size.

The assemblies were also imaged by transmission electron microscopy (TEM) (Figure 3.7). In these experiments the double bonds on the IIR backbone were stained with OsO₄. TEM images suggested that the diameters of the OsO₄-stained hydrophobic cores of the assemblies prepared via dialysis of copolymers from 30/70 THF/water were less than 50 nm. This is in general agreement with the DLS data considering the significant differences between these techniques (Table 3.2). In addition, the solid, spherical nature of the structures observed in TEM images for the entire series of copolymers suggested that despite the wide range of PEO content and thus varying hydrophilic/hydrophobic volume fractions, the assemblies were spherical micelles rather than cylindrical micelles or vesicles that are often observed for linear diblock copolymers with hydrophilic/hydrophobic volume fractions < 0.5.

Table 3.2. Comparison of DLS and TEM characterization of assemblies prepared by dialysis of copolymers from 30/70 THF/water.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Assembly Z-average diameter by DLS (nm) (± std devb), PDI</th>
<th>Assembly mean diameter by TEM (nm) (± std devb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>(69 ± 5), 0.3</td>
<td>(31 ± 5)</td>
</tr>
<tr>
<td>3.2</td>
<td>(27 ± 5), 0.3</td>
<td>(21 ± 4)</td>
</tr>
<tr>
<td>3.3</td>
<td>(38 ± 5), 0.2</td>
<td>(22 ± 6)</td>
</tr>
<tr>
<td>3.8</td>
<td>(56 ± 4), 0.2</td>
<td>(31 ± 6)</td>
</tr>
<tr>
<td>3.9</td>
<td>(39 ± 6), 0.2</td>
<td>(32 ± 5)</td>
</tr>
<tr>
<td>3.10</td>
<td>(58 ± 3), 0.3</td>
<td>(36 ± 6)</td>
</tr>
</tbody>
</table>

aStandard deviations on DLS measurements correspond to three separate preparations of the assemblies.
bStandard deviations on TEM measurements correspond to the diameter measurements of all assemblies observed within a given TEM image.
Figure 3.7. TEM images of assemblies formed from copolymers a) 3.1, b) 3.2, c) 3.3, d) 3.8, e) 3.9, f) 3.10. There was 70% water in the THF/water solution prior to dialysis in all cases. Staining was performed with OsO$_4$ vapor, allowing selective visualization of the rubber cores. The scale bars correspond to 100 nm.

In order to further demonstrate the presence of a hydrophobic core and the ability of the assemblies to encapsulate hydrophobic molecules, nile red was encapsulated in assemblies formed from copolymer 3.1. Nile red is known to exhibit negligible fluorescence in aqueous solution due to its extremely low solubility and extensive aggregation. However, its fluorescence increases significantly upon incorporation into hydrophobic environments such as membranes or the cores of micelles.$^{51-53}$ Unlike in previous examples, it was not possible to measure a critical aggregation concentration for the IIR-PEO copolymers using nile red, perhaps because of the capabilities of these graft copolymers to form unimolecular as well as intermolecular aggregates. Nevertheless, as shown in Figure 3.8, the incorporation of nile red into the assemblies led to greatly enhanced fluorescence in comparison with nile red in solution alone. This suggests that the assemblies formed by IIR-PEO graft copolymers have the potential to carry hydrophobic payloads, which is of interest for a variety of applications ranging from biomedical devices to cosmetics or coatings.
Figure 3.8. Fluorescence emission spectrum of nile red showing enhanced fluorescence intensity when encapsulated into assemblies formed from copolymer 3.1 in comparison with nile red in pure water.

Finally, the toxicity of new materials is a critical aspect that determines their utility in biomedical applications as well as in other applications as it is undesirable to release or dispose of toxic materials in the environment. The ability to form aqueous assemblies of IIR-PEO graft copolymers allows for the direct evaluation of their toxicities by standard cell viability assays. To accomplish this, the assemblies were prepared, diluted into cell culture medium, and then added to C2C12 mouse myoblasts at concentrations ranging from 2.0 mg/mL to 3.9 µg/mL. After 48 h, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to assess the cell viabilities in comparison with control cells that were not exposed to the materials.54 No significant toxicity was observed for any of the copolymers at any concentration evaluated (Figure 3.9). This result is promising for future applications of these polymers. It also confirms that the decreased cell growth on films of copolymers 3.2, 3.3, and 3.10 did indeed likely result from surface adhesion effects rather than the leaching of toxic materials from the films resulting in cell death.
Figure 3.9. Viability of C2C12 mouse myoblast cells in the presence of copolymers 3.1 – 3.3 and 3.10, as measured by an MTT assay.

3.3 Conclusions

In conclusion, starting from high IP IIR, it was possible to prepare new IIR-PEO graft copolymers with high PEO content. By varying the IP content of the IIR as well as the MW of the grafted PEO chains, different graft polymer architectures with PEO contents ranging from 18 to 83 wt% have been prepared. Even at very high PEO content it was possible to prepare copolymer films that were stable and underwent only gradual mass loss into water. These films released encapsulated dyes into solution and inhibited the adhesion and growth of cells on the surface in a manner that was strongly dependent on the PEO content. Despite the resistance of polymer films to direct dissolution into water, it was possible to prepare aqueous assemblies of the copolymers by a THF-water solvent exchange method. This method could be tuned to control the sizes of the assemblies. Based on TEM imaging, the assemblies resembled spherical micelles regardless of their PEO content. The encapsulation of a nile red into the assemblies
demonstrated their potential to carry a hydrophobic payload. All of the above properties, combined with their demonstrated lack of *in vitro* toxicity suggest that IIR-PEO graft copolymers exhibit potential for a variety of applications as coatings or aqueous dispersions.

### 3.4 Experimental Section

**General Procedures and Materials:**

IIR-PEO graft copolymers 3.1 – 3.3 were prepared as previously reported\(^4^3\). IIR 3.4 containing 7 mol% IP (\(M_w = 570 \pm 18\) kg/mol based on light scattering and 399 kg/mol, PDI = 1.9 based on SEC relative to polystyrene standards) was provided by LANXESS Inc.\(^4^6\). Solvents were purchased from Caledon (Georgetown, Canada) and all other chemicals were purchased from Sigma Aldrich and were used without further purification unless otherwise noted. 4-(Dimethylamino)pyridine (DMAP) was purified by recrystallization in toluene before use. \(m\)-CPBA was dissolved in toluene and dried with MgSO\(_4\) before use. Pyridine was distilled over CaH\(_2\) before use. Dry toluene was obtained from an Innovative Technology (Newburyport, USA) solvent purification system based on aluminium oxide columns. \(^1\)H NMR spectra were obtained in CDCl\(_3\) at 400 or 600 MHz using Varian Inova spectrometers. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals of CDCl\(_3\) (\(\delta\) 7.26). The PEO content in wt\% was determined from \(^1\)H NMR, based on the relative integrations of the signals at 3.65 ppm and at 1.42 ppm corresponding to the PEO and IB units respectively.

Thermogravimetric analyses were performed under a nitrogen atmosphere on a Q600 SDT TA Instrument at a heating rate of 10 °C/min. DSC was performed under a nitrogen atmosphere on a Q20 DSC TA instrument at a 150 heating/cooling rate of 10 °C/min from -150 to +200 °C. The \(T_g\) and \(T_m\) were obtained from the second heating cycle.

**MW Determination**

SEC was performed in THF using a Waters 2695 separations module equipped with a Wyatt REx differential refractometer and two PolyPore (300 mm × 7.5 mm) columns
from Agilent. The calibration was performed using polystyrene standards. For
determination of $M_w$ by light scattering, time averaged light scattering intensities were
measured for each polymer at a series of concentrations from 0.2 mg/mL to 1.1 mg/mL in
THF using a Malvern Zetasizer Nano-S instrument. Toluene was used as a standard.
Using this data, the $M_w$ for each polymer was determined from the Rayleigh equation:
\[ KC/R_\theta = \frac{1}{M_w} + 2A_2C \] using a Debye plot: \( KC/R_\theta \) versus \( C \), allowing \( 1/M_w \) to be
determined as the y-intercept. \( C \) = polymer concentration; \( R_\theta \) = excess Rayleigh ratio - the
ratio of scattered and incident light intensity; \( A_2 \) = second virial coefficient which is a
measure of solute-solvent interactions; \( P(\theta) \) = scattering function which relates the
angular variation in scattering intensity to the mean square radius of the particle; \( K = \)
\[ 4\pi^2/\lambda_0^4N_A[n_o(dn/dc)]^2 \] where \( \lambda_0 \) = vacuum wavelength of incident light; \( N_A \) = Avogadro’s
number; \( n_o \) = solvent refractive index. The measured \( dn/dc \) of 0.13 mL/g was used for
polymer 3.4, while a \( dn/dc \) value of 0.055 mL/g was used for polymers 3.8 – 3.10 based
on previous measurements for similar polymers where at PEO content greater than 30
wt% the \( dn/dc \) value was found to plateau at this value which is essentially the \( dn/dc \) of
PEO.\textsuperscript{43} It should be noted that as a single angle was evaluated for each polymer, and high
MW polymers are expected to exhibit an angular dependence to their scattering intensity,
the $M_w$ values provided from light scattering data are given only as approximations to
demonstrate trends.

**Synthesis of Epoxidized IIR 3.5**

IIR 3.4 (0.20 g, 0.25 mmol of IP units, $M_w = 399$ kg/mol, PDI = 1.9) was dissolved in dry
toluene (10 mL). In a separate flask, a solution of \textit{m}-CPBA (0.21 g, 1.25 mmol) in dry
toluene (13 mL) was dried over MgSO\textsubscript{4}, and then added to the solution of 3.4. The
reaction mixture was stirred overnight at room temperature. The solvent was then
reduced \textit{in vacuo} and the epoxidized IIR 3.5 was purified by precipitation from toluene (4
mL) into acetone (8 mL) twice. Yield: 0.20 g, 92\%.\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, \( \delta \), ppm):
2.71 (br s, 1H), 1.42 (s, CH\textsubscript{2} PIB, 26H), 1.11 (s, CH\textsubscript{3} PIB, 78H). $M_w = 378$ kg/mol, PDI =
2.8.
Synthesis of 4-NPC Activated IIR 3.7

Epoxidized rubber 3.5 (0.17 g, 0.21 mmol of epoxidized units) was dissolved in dry toluene (6 mL). 37% aqueous HCl (20 µL, 0.22 mmol) was added and the reaction mixture was stirred for 1 hr at room temperature to form hydroxylated rubber 3.6. 4-NPC (0.62 g, 3.1 mmol) was then added, followed by pyridine (0.30 mL, 3.6 mmol) dropwise. The reaction mixture was stirred for 2 h at room temperature. Pyridine salts were then removed by filtration, the solvent volume was reduced in vacuo, and the resulting toluene solution (3 mL) was precipitated in acetone (6 mL) twice to afford the activated polymer 7. Yield: 0.19 g, 89%. $^1$H NMR (400 MHz, CDCl$_3$, δ, ppm): 8.27 (d, $J$ = 8.5 Hz, 2H), 7.40 (d, $J$ = 8.5 Hz, 2H), 5.28 (s, 1H), 5.12 (br s, 1H), 5.02 (s, 1H), 1.42 (s, CH$_2$ PIB, 26H), 1.11 (s, CH$_3$ PIB, 78H). M$_w$ = 541 kg/mol, PDI = 2.0.

Synthesis of Copolymer 3.8

PEO-NH$_2$ with a MW of 750 g/mol (1.2 g, 1.6 mmol) was dissolved in dry toluene (30 mL). In a separate flask, the activated IIR 3.7 (1.3 g, 1.3 mmol) was dissolved in dry toluene (20 mL) and was then added to the PEO-NH$_2$ solution dropwise. Next, DMAP (0.24 g, 2.0 mmol) was dissolved in dry toluene (10 mL) and the solution was added to the reaction mixture. The resulting mixture was stirred overnight at 60 °C. The solvent was removed in vacuo and the resulting rubbery material was washed once with deionized water and then purified by precipitation from THF (10 mL) into water (100 mL). Yield: 1.4 g, 70%. $^1$H NMR (400 MHz, CDCl$_3$, δ, ppm): 5.20 (br s, 1H), 5.12 (s, 1H), 5.06 (br s, 1H), 4.87 (s, 1H), 3.65 (s, 44H), 3.39 (s, 2.4H), 1.42 (s, CH$_2$ PIB, 26H), 1.11 (s, CH$_3$ PIB, 78H). PEO content (from $^1$H NMR): 40 wt%; $T_g$ = -60 °C; $T_m$ = 20 °C; M$_w$ = 741 ± 53 kg/mol.

Synthesis of Copolymer 3.9

The procedure described above for the preparation of copolymer 3.8 was followed except that PEO-NH$_2$ with a MW of 2000 g/mol was used and the resulting copolymer was washed once with distilled water and then purified by precipitation from THF (15 mL) into diethyl ether (150 mL). Yield: 1.6 g, 69%. $^1$H NMR (400 MHz, CDCl$_3$, δ, ppm): 5.20
(br s, 1H), 5.12 (s, 1H), 5.06 (br s, 1H), 4.87 (s, 1H), 3.65 (s, 99H), 3.38 (s, 2.6H), 1.41 (s, CH₂ PIB, 26H), 1.11 (s, CH₃ PIB, 78H). PEO content (from ¹H NMR): 60 wt%; T_g = -57 °C; T_m = 44 °C, M_w = 1740 ± 20 kg/mol.

**Synthesis of Copolymer 3.10**

The procedure described above for the preparation of copolymer 3.9 was followed except that PEO-NH₂ with a MW of 5000 g/mol was used and the water washing was performed twice. Yield: 1.9 g, 78%. ¹H NMR (600 MHz, CDCl₃, δ, ppm): 5.20 (br s, 1H), 5.12 (s, 1H), 5.06 (br s, 1H), 4.87 (s, 1H), 3.65 (s, 319H), 3.38 (s, 3H), 1.41 (s, CH₂ PIB, 26H), 1.11 (s, CH₃ PIB, 78H). PEO content (from ¹H NMR): 83 wt%; T_m = 60 °C, M_w = 5040 ± 670 kg/mol.

**Evaluation of Film Stability by Mass Loss Measurement**

Melt pressed films were prepared using a hydraulic heated press (Hydraulic Unit Model #3912, Carver, Inc., Wabash, IN). All copolymers were pressed at 120 °C within a pressure of 300 kPa for 15 seconds to a thickness of approximately 0.2 mm. The melt pressed films were punched into circles of 5 mm diameter and weighed. They were then immersed in a vial containing 1 mL of deionized water. The films were agitated at a rate of 5 rpm using a GyroTwister (Labnet International Inc.) over a period of four weeks. Each week, the films were rinsed well with deionized water, dried under vacuum at 40 °C overnight, and weighed. This experiment was carried out in triplicate for each time point.

**Release of an Encapsulated Dye from Films**

First 35 mg/mL solution of IIR in toluene was prepared and drop cast on glass cover slips (circular, 25 mm diameter). Next, 1 mL of a 35 mg/mL solution of each copolymer in toluene was prepared. Added to each of these solutions was 39 µL of a 1.0 mM Rhodamine B solution in THF. Then the copolymer solution was drop cast onto the IIR coated cover slips (3 coats of 100 µL each). The films were dried under vacuum. The film thickness as measured by profilometry was found to be approximately 20 µm (KLA Tencor P-10 Surface Profiler). The films were then immersed in a 100 mM, pH 7.4 phosphate buffer containing 0.1 wt% NaN₃. They were agitated at a rate of 5 rpm using a
GyroTwister (Labnet International Inc.). The quantity of released dye was determined by UV-visible measurements at 550 nm using a Cary Bio 300 UV spectrophotometer (Varian). The concentration of rhodamine in the solution was calculated using an extinction coefficient $\varepsilon$ of 98000 M$^{-1}$cm$^{-1}$ determined from a calibration curve prepared from the dye in solution. This experiment was carried out in triplicate for each copolymer.

**Evaluation of Cell Growth on Films**

C2C12 cells were maintained at 37 °C and 5% CO$_2$ in Dulbecco’s Modified Eagle Medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and supplemented with 1% Glutamax (100×) solution and 1% Penstrep (100×). First microscope glass cover slips (circular, 25 mm diameter) were coated using a 35 mg/mL solution of IIR in toluene. Next, a 35 mg/mL solution of the copolymers containing 18 wt% and 34 wt% PEO in toluene was drop cast onto the IIR coated cover slips (3 coats of 100 µL each time). Each polymer was studied in triplicate. The surfaces were sterilized by submersion in 70% ethanol, and were then left to dry. The sterilized samples were placed in the wells of a 6-well plate and 5 x $10^5$ cells in 2 mL of cell culture medium were seeded onto each surface. The samples were incubated for 48 hours, and then fixed with 4% paraformaldehyde solution for 10 min. The samples were washed twice with PBS (Invitrogen) at pH 7.2, and then treated with 2 mL of acetone at -20 °C for 5 minutes to permeabilize the membrane. After that, they were washed again with PBS, stained with Alexa Fluor 568 phalloidin (Invitrogen) and DAPI (Invitrogen) following the manufacturer’s directions. The samples were washed again with PBS and placed face down onto glass microscope slides with ProLong® Gold Antifade Reagent (Invitrogen) and sealed. Confocal images were obtained using a confocal laser scanning microscope (LSM 510 Duo Vario, Carl Zeiss) using a 20x objective and excitation wavelengths of 405 (DAPI) and 578 nm (phalloidin). Cell were counted using Image Pro Plus software (3 images on each of 3 surfaces per polymer). Statistical analyses (one-way ANOVA test followed by Tukey’s test) were performed using the software Prism.

**Preparation of Aqueous Assemblies of Copolymers 3.1-3.3 and 3.8-3.10**
1 mL of a 10 mg/mL solution of the copolymer was prepared in THF that had been passed through a 0.2 µm pore size polytetrafluoroethylene syringe filter (Dikma, ProMax™). To this solution, 0 mL, 0.4 mL, 1 mL, or 2 mL of filtered (0.2 µm pore size Tuffryn® syringe filter, PALL) deionized water was added rapidly via syringe to provide solutions containing either 0%, ~30%, ~50%, or ~70% water in THF. The resulting solutions were then dialyzed overnight against water using a regenerated cellulose membrane (Spectra/Por) with a molecular weight cut-off of 12000 – 14000 g/mol. Dynamic light scattering was then performed on ZetaSizer Nano instrument from Malvern Instruments. For the experiment in which the concentration was investigated the same procedure described above was followed except that the initial THF solution was prepared at a concentration of 2 mg/mL. For long-term stability experiments, the samples were stored in vials at room temperature and the light scattering measurements were repeated after 6 months.

**TEM**

A drop of the nanoparticle suspension (~3 mg/mL) was transferred via pipette to a carbon formvar grid and was left for 1 min. The excess solution was removed. The grid was stained with the vapor of OsO₄ in a sealed container, for 2 hours. Imaging was performed using a Phillips CM10 microscope operating at 80 kV with a 40 µm aperture. The assembly diameters were determined by measuring the diameters of the assemblies in each image (magnified) relative to the scale bar and then calculating the average and standard deviation.

**Nile Red Encapsulation**

10 mg/mL solution of copolymer 3.1 in THF was prepared. To 0.3 mL of this solution was added 0.5 mg of solid nile red followed by a rapid addition of 0.7 mL of deionized water. The resulting solution was mixed thoroughly and then dialyzed against water overnight. A control sample of nile red in water was prepared by sonicating 0.5 mg of nile red in 1 mL deionized water. The fluorescence of each sample was obtained on a QM-4 SE spectrofluorometer equipped with double excitation and emission monochromators from Photon Technologies International. An excitation wavelength of
485 nm was used for nile red and the emission spectra were recorded from 520 and 700 nm.

**In Vitro Cytotoxicity Assay**

C2C12 mouse myoblast cells were cultured in growth medium composed of Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and supplemented with 1% Glutamax (100×) solution and antibiotics (Penicillin and Streptomycin, 100 units/mL each). Cells were seeded onto a 96-well plate (Nunclon TC treated) at a density of \(2 \times 10^3\) cells per well with a final volume of 100 µL of culture medium. Cells were allowed to adhere for 24 hours at 37 °C in a humidified incubator with 5% CO₂. After 24 hours the growth media was aspirated. Control cells were grown in growth media alone, nanoparticle samples were incubated at two-fold decreasing concentrations for 10 different concentrations from 2 mg/mL to 0.0039 mg/mL in growth media with 8 replicates at each concentration for 48 hours. All media was aspirated, and then 100 µL of fresh media and 10 µL of MTT solution (5 mg/mL) were added to each well. After incubation for 4 hours, the media was aspirated and the formazan product was solubilized by addition of 50 µL of DMSO to each well. The absorbance of each well was measured at 540 nm using a plate reader (Tecan Safire) and after subtraction of the blank, the result was compared to that of the control cells that were not exposed to micelles in order to calculate the relative cell viability.
3.5 References


Chapter 4

4 Synthesis and Properties of \textit{arb}-PIB-g-PEO Graft Copolymers: A Comparison of Linear and Arborescent Graft Copolymer Architectures\textsuperscript{*}

4.1 Introduction

The development of polymeric materials with controlled architectures has been a highly active area of polymer science and engineering over the past few decades. This interest is motivated by the significant effect that polymer architecture can impart on the properties of materials. For example, the degree of branching in PE, the most widely used commodity plastic, dictates the strength of the intermolecular interactions, affecting its crystallinity, density, tensile strength, resilience, and consequently its applications. A wide array of branched molecular architectures including hyperbranched,\textsuperscript{1,2} star,\textsuperscript{3,4} comb-like,\textsuperscript{5} arborescent,\textsuperscript{6} dendrimer-like polymers,\textsuperscript{3,7} and dendrimers\textsuperscript{8,9} are currently available through a variety of synthetic approaches. Pioneered by Gauthier and Möller in the 1990s,\textsuperscript{10} arborescent polymers combine the characteristics of both hyperbranched polymers and dendrimers but comprise linear polymeric chains between branching sites, allowing the molecular weights and dimensions to be orders of magnitude larger than dendrimers at similar grafting generations.\textsuperscript{6} They can be prepared by the successive grafting of polymeric building blocks in a step-wise manner,\textsuperscript{6,11,12} or alternatively in a “one-pot” living polymerization using an “\textit{inimer}”,\textsuperscript{13} a molecule that has both initiating and polymerizable moieties.\textsuperscript{14,15}

PIB-based elastomers possess many attractive properties including high elasticity, impermeability to gas and water, and good thermal and chemical stability. These properties have enabled its use in many diverse commercial applications including automobile tires, the bladders of sporting equipment, lubricating oils, sealants, and chewing gum. PIB has also been used in block copolymers where its properties can be

\textsuperscript{*}This section contains work that has been submitted to LANXESS for approval prior to publication.
tuned to incorporate those of the other block. A noteworthy example is the development of polystyrene (PS)-PIB-PS linear triblock copolymers, commonly referred to as SIBS.\textsuperscript{16} Incorporation of the glassy PS blocks imparts thermoplastic properties, making SIBS a TPE.\textsuperscript{17-19} This material has been commercialized and is used clinically as a drug-eluting coating on the TAXUS\textsuperscript{®} vascular stent.\textsuperscript{20,21} More recently, arborescent versions of PIB-PS have been synthesized and their properties have been compared to the linear SIBS.\textsuperscript{15,22-26} The arborescent analogues have been demonstrated to retain the desirable biocompatibility of SIBS in certain applications, while at the same time exhibiting different rheological and mechanical properties, such as lower creep and improved fatigue life.\textsuperscript{23,26,27}

We have recently explored PIB-PEO graft copolymers prepared by the derivatization and subsequent grafting of PEO onto the IP units in PIB-co-PIP containing $2 - 7$ mol\% IP, a polymer commonly referred to as IIR.\textsuperscript{28-30} The synthesis was well controlled and highly reproducible, allowing a wide range of PEO contents to be explored. At lower PEO content, these copolymers exhibited unusual micrometer-scale patterning when spin coated on surfaces.\textsuperscript{28,29} At higher PEO content, films of the material resisted the adsorption of proteins and the growth of cells.\textsuperscript{29,30} In addition, it was possible to prepare stable nanosized assemblies in aqueous solution.\textsuperscript{30} With the aim of understanding how changes in molecular architecture affect the synthesis and properties of PIB-PEO copolymers, we describe here the synthesis of arborescent PIB-PEO graft copolymers (arb-PIB-g-PEO). Several arb-PIB-g-PEO copolymers were prepared and their properties were studied and compared to the analogous linear PIB-PEO graft copolymers (lin-PIB-g-PEO).

4.2 Results and Discussion

4.2.1 Synthesis and Chemical Characterization of arb-PIB-g-PEO

The synthesis of the arb-PIB-g-PEO began with arb-PIB-PIPs \textbf{4.1a – 4.1c} (Scheme 4.1). These copolymers were prepared as previously reported,\textsuperscript{31} by first the synthesis of arb-PIB from IB and 4-(2-methoxyisopropyl)styrene inimer, followed by the sequential addition of IP, leading to short end blocks of PIB-co-PIP (Figure 4.1). Three
different \textit{arb}-PIB-PIP copolymers containing varying IP content of 1.5 mol\%, 2.9 mol\% and 5.5 mol\% were used (Table 4.1).

**Table 4.1. Characteristics of \textit{arb}-PIB-PIP starting materials.**

<table>
<thead>
<tr>
<th>Polymer (\textit{arb}-PIB-PIP)</th>
<th>% 1,4 IP content ± 0.04</th>
<th>% Branching ± 0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1a</td>
<td>1.53</td>
<td>Not available</td>
</tr>
<tr>
<td>4.1b</td>
<td>2.94</td>
<td>0.26</td>
</tr>
<tr>
<td>4.1c</td>
<td>5.48</td>
<td>0.37</td>
</tr>
</tbody>
</table>

As shown in Scheme 4.1, the same route previously reported by our group for the synthesis of PIB-PEO graft copolymers from linear IIR was used, but with some key modifications. The sequence of synthetic steps involved epoxidation of the double bond moieties of the IP groups using \textit{m}-CPBA, epoxide opening using aqueous HCl in toluene, and finally activation of the resulting allylic alcohols using 4-NPC to provide the activated polymers 4.2a-4.2c. We have recently reported that the extension of our initial synthetic approach from IIR containing 2 mol\% IP to IIR containing 7 mol\% IP required some modifications due to the insolubility of an intermediate hydroxyl-functionalized rubber upon drying. This was hypothesized to result from hydrogen bond-mediated cross-linking of the material within the hydrophobic environment of the IIR. Despite their relatively low IP content, this issue was also prevalent in the derivatization of the \textit{arb}-PIB-PIP and even extended to challenges in isolating the epoxide-functionalized materials in good yields. This can likely be attributed to the functionalized IP units being concentrated on the periphery of the arborescent polymer where they can play a larger role in the solubility and other properties, rather than being randomly distributed throughout the backbone as in linear random PIB-PIP. Indeed, Puskas et al. have recently shown that \textit{arb}-PIB-PIP has very different properties than the linear material.
To address the solubility challenges described above in the functionalization of 
*arb*-PIB-PIP, a one-pot sequence was used for the conversion of 4.1a-c to 4.2a-c, in 
comparison the 2 – 3 pot sequences previously reported.\textsuperscript{28-30} The entire sequence can be performed in less than 5 h, with purification of 4.2a-c by precipitation in acetone. Compounds 4.2a-c were characterized by NMR spectroscopy, infrared (IR) spectroscopy, and SEC. Interestingly, while the aromatic peaks corresponding to the 4-nitrophenyl moiety were clearly visible in the \textsuperscript{1}H NMR spectra at 8.3 and 7.4 ppm, the peaks corresponding to the alkenes in the region of 4.5-5.5 ppm were very broadened (see appendix). IR spectra had characteristic absorption bands for the carbonyl stretching mode (at 1766 cm\textsuperscript{-1}), the \textit{exo} double bond stretching mode (at 1600-1622 cm\textsuperscript{-1}) and the nitro group asymmetric stretching mode (at 1535 cm\textsuperscript{-1}). \textsuperscript{1}H NMR and IR spectra of the intermediate epoxide and allylic alcohol were also obtained by withdrawing aliquots of the reaction mixture and precipitating the polymer into acetone. While the same issue regarding the broadness of the alkene peaks was observed for these intermediates, the spectra showed the expected characteristic functional group peaks, high conversions, and the absence of side reactions (see appendix). SEC results showed a modest increase in $M_\text{w}$ for 4.2a-4.2c relative to 4.1a-4.2c, and no significant changes in the PDIs.
The last synthetic step involved the reaction of the activated rubbers 4.2a-4.2c with amine-terminated PEO (PEO-NH₂) of varying MWs including 750, 2000, and 5000 g/mol. The resulting copolymers were purified by precipitation into acetone or diethyl ether. As shown in Table 4.2, a small library of arb-PIB-PEO with varying PEO content ranging from 8 to 54 wt% was prepared. The theoretical PEO content was calculated based on the IP content of the starting arb-PIB-PIP, while the actual PEO content was determined by 1H NMR spectroscopy based on the relative intensities of the PIB and PEO peaks at 1.42 ppm and 3.65 ppm respectively (see appendix). In general, the actual PEO content was somewhat lower than the theoretically possible content. On one hand, the presence of the functionalized IP units at the terminus of the arb-PIB-PIP copolymer may result in greater accessibility. However, the close proximity of the IP units in these terminal blocks, in comparison to those in IIR, may also introduce steric hindrance as nearby PEO chains may hinder the grafting of subsequent chains. The latter factor seemed to dominate as the difference between the theoretical PEO content and the actual PEO content became greater for the 5000 g/mol PEO-NH₂ in comparison with the 2000 g/mol PEO-NH₂.
Table 4.2. Chemical properties of *arb*-PIB-g-PEO copolymers.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>IP content in starting <em>arb</em>-PIB-PIP (mol%)</th>
<th>Grafted PEO-NH$_2$ MW (g/mol)</th>
<th>Theoretical PEO content (wt%)</th>
<th>Obtained PEO content$^a$ (wt%)</th>
<th>$T_g^b$ (°C)</th>
<th>$T_m^b$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>1.5</td>
<td>750</td>
<td>17</td>
<td>8</td>
<td>-65</td>
<td>18</td>
</tr>
<tr>
<td>4.4</td>
<td>1.5</td>
<td>2000</td>
<td>35</td>
<td>23</td>
<td>-65</td>
<td>46</td>
</tr>
<tr>
<td>4.5</td>
<td>1.5</td>
<td>5000</td>
<td>58</td>
<td>24</td>
<td>-65</td>
<td>47</td>
</tr>
<tr>
<td>4.6</td>
<td>2.9</td>
<td>2000</td>
<td>52</td>
<td>48</td>
<td>-64</td>
<td>44</td>
</tr>
<tr>
<td>4.7</td>
<td>5.5</td>
<td>2000</td>
<td>67</td>
<td>54</td>
<td>-64</td>
<td>52</td>
</tr>
<tr>
<td>4.8</td>
<td>5.5</td>
<td>5000</td>
<td>84</td>
<td>31</td>
<td>-66</td>
<td>50</td>
</tr>
</tbody>
</table>

$^a$ Calculated from $^1$H NMR spectroscopy; $^b$ Obtained from DSC.

Thermal properties of the *arb*-PIB-g-PEO copolymers were investigated using DSC. Each copolymer exhibited a sharp melting transition ($T_m$) due to crystalline PEO blocks in addition to a glass transition temperature ($T_g$) corresponding to the amorphous PIB (see appendix). As previously reported,$^{29,30}$ it was found that while the $T_g$ remained relatively constant ($\sim$ -65 °C), the $T_m$ of the PEO decreased upon incorporation into the copolymer in comparison to that of the PEO homopolymer. For example, copolymer 4.3 containing 8 wt% PEO with a MW of 750 g/mol had a $T_m$ of 18 °C, in comparison to PEO homopolymer of 750 g/mol which has a $T_m$ of 32 °C. Similarly, copolymers 4.4, 4.5, 4.6 and 4.8 exhibited lower $T_m$s compared to their corresponding PEO homopolymers of 2000 g/mol and 5000 g/mol which have $T_m$s of 52 and 59 °C respectively. Similar to the *lin*-PIB-g-PEO,$^{29}$ the analysis of *arb*-PIB-g-PEO by SEC was problematic. However, DSC confirmed the covalent grafting of the PEO to the *arb*-PIB-PIP as well as the absence of ungrafted PEO in the purified copolymers. The presence of ungrafted PEO leads to an extra melting transition at the same $T_m$ as the homopolymer, whereas only a single melting transition was observed for each *arb*-PIB-g-PEO (see appendix).
4.2.2 AFM Studies

The microphase separation of SIBS and its arborescent analogues have been extensively studied using techniques such as AFM and TEM as the presence of plastic phases within a continuous elastomeric phase can provide physical cross-links, making the materials TPEs. TPEs behave like cured rubbers at room temperature, while at higher temperatures they can be processed like plastics. In addition, we have previously found that the topographies and morphologies of *lin*-PIB-*g*-PEO copolymers have implications in terms of protein adsorption to their surfaces. For these reasons, it was of interest to study the microphase separation of the *arb*-PIB-*g*-PEO.

From the library of new copolymers, 4.3, 4.4, and 4.6 having 8, 24, and 48 wt% PEO respectively were selected to study as they cover a range of PEO content. Solutions of these polymers, as well as *arb*-PIB-PIP 1b in toluene were spin coated onto silicon wafers and imaged by AFM before and after annealing. Figure 4.2 shows representative AFM phase images for the different copolymers. Similar to the observations of Puskas and coworkers for *arb*-PIB-poly(*p*-methylstyrene)(PpMS) with short terminal blocks of PpMS, irregularly distributed spherical domains were observed for *arb*-PIB-PIP (Figure 4.2a). This observation of phase separation is consistent with the reinforcing properties of the terminal PIB-PIP blocks in these materials. In contrast, films of copolymers 4.3 and 4.4 were very smooth (see appendix) and showed no signs of phase separation in the phase images (Figure 4.2b and 4.2c). It is possible that the surfaces of these materials were covered with a thin layer of PIB that migrated to the surface due to its lower surface energy relative to that of PEO. In general, PIB chains, being above their T_g can relax more readily than the crystalline PEO chains. However, films of copolymer 4.6 exhibited phase separation with a cylindrical/lamellar morphology similar to that observed for SIBS with about 30 wt% PS (Figure 4.2d). Upon annealing at 110 °C for 25 hours, this morphology completely disappeared. This was unexpected as annealing has often been shown to increase phase separation and ordering in copolymer films, even in our previous work. A more detailed investigation will be required to explore this phenomenon.
In previous work, we have observed microphase separation of *lin*-PIB-g-PEO, particularly with higher PEO content (e.g. 65 wt%), when spin coated from CH$_2$Cl$_2$ onto silicon wafers. In the current work, we spin coated toluene solutions of *lin*-PIB-g-PEO having 18 wt%, 34 wt%, and 65 wt% onto silicon wafers for comparison with the arborescent materials described above. These polymers were prepared from linear PIB- *co* -PIP having 2 mol% IP by the grafting of PEO of molecular weight 750, 2000, and 5000 g/mol respectively. Topographical images (see appendix) showed highly smooth surfaces, and no signs of phase separation were observed in the phase images (Figure 4.3), except for the 65 wt% PEO copolymer which showed a small degree of nanoscale texture (Figure 4.3d). Thus, it appears that phase separation is less apparent for these polymers when cast from toluene versus CH$_2$Cl$_2$ solutions. This may result from the solubility differences between these solvents or from their different rates of evaporation. In addition, from this comparison using toluene as the solvent, phase separation was less apparent for the linear polymers in comparison with the arborescent polymers.
Figure 4.3. AFM phase images of: a) IIR (2 mol% IP), b) lin-PIB-g-PEO with 18 wt% PEO, c) lin-PIB-g-PEO with 34 wt% PEO, d) lin-PIB-g-PEO with 65 wt% PEO.

4.2.3 Tensile Properties of \textit{arb}-PIB-g-PEO Copolymers

As described above, microphase separation in copolymers composed of soft and hard blocks can have a significant impact on their mechanical properties. Therefore, it was of interest to evaluate and compare the tensile properties of the \textit{arb}-PIB-g-PEO with those of the PIB starting polymers (\textit{arb}-PIB-PIP and IIR containing 2 mol% IP) and the \textit{lin}-PIB-g-PEO. Figures 4.4a and 4.4b compare representative stress-strain curves for \textit{arb}-PIB-g-PEO and \textit{lin}-PIB-g-PEO respectively and Table 4.3 compares the tensile strength at break, percent elongation at break, Young’s modulus, and toughness for these materials. In both cases, the elongation at the break values for unfunctionalized \textit{arb}-PIB-PIP and butyl rubber were much higher than those of the \textit{lin}-PIB-g-PEO and \textit{arb}-PIB-g-PEO copolymers. At the same time, their mechanical strengths were very low and they mainly exhibited elastomeric properties.
Figure 4.4. Representative stress-strain plots of: a) *arb*-PIB-PIP and *arb*-PIB-g-PEO copolymers, b) IIR and *lin*-PIB-g-PEO copolymers.

From Table 4.3, it is apparent that increasing the PEO content increases the modulus and the tensile stress at break while reducing the elongation at the break. For example, copolymer 4.6 containing 48 wt% PEO exhibits a tensile strength of 5.6 MPa and 141% elongation at break in comparison with a tensile strength of 1.1 MPa and 264% elongation at break for copolymer 4.3 having only 8 wt% PEO. These results indicate the reinforcing effect of the PEO domains in the *arb*-PIB-g-PEO copolymers and suggest that these materials can behave as TPEs. This could be beneficial in terms of different applications where depending on the required mechanical properties; different graft copolymers could be selected. In comparing the *arb*-PIB-g-PEO to *lin*-PIB-g-PEO, it was
noted that similar trends were observed in the mechanical properties, with the tensile strength at break, Young's modulus, and toughness also increasing with increasing PEO content and the percent elongation at break decreasing. The percent elongation at break as well as the toughness were generally higher for lin-PIB-g-PEO in comparison with arb-PIB-g-PEO for similar PEO content. However, from Figure 4.4 it is clear that they exhibit increased yielding and plastic behavior. These differences can likely be attributed to the differences in microphase separation for these polymers having different architectures.

Table 4.3. Comparison of the tensile properties of arb-PIB-P, arb-PIB-g-PEO, IIR, and lin-PIB-g-PEO.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tensile strength at break (MPa ± std dev)</th>
<th>Elongation at break (% ± std dev)</th>
<th>Young’s modulus (MPa ± std dev)</th>
<th>Toughness (MPa ± std dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copolymer 4.1a</td>
<td>0.1 ± 0.1</td>
<td>1001 ± 201</td>
<td>0.1 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Copolymer 4.1b</td>
<td>0.1 ± 0.2</td>
<td>680 ± 175</td>
<td>0.1 ± 0.3</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>Copolymer 4.3</td>
<td>1.1 ± 0.3</td>
<td>246 ± 61</td>
<td>0.5 ± 0.1</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Copolymer 4.4</td>
<td>2.0 ± 0.6</td>
<td>138 ± 24</td>
<td>0.9 ± 0.7</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Copolymer 4.6</td>
<td>5.6 ± 1.1</td>
<td>141 ± 33</td>
<td>5.3 ± 1.4</td>
<td>5.8 ± 1.3</td>
</tr>
<tr>
<td>IIR (2 mol% IP)</td>
<td>0.2 ± 0.1</td>
<td>800 ± 187</td>
<td>0.5 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>lin-PIB-g-PEO (18 wt% PEO)</td>
<td>3.3 ± 0.3</td>
<td>1042 ± 83</td>
<td>0.3 ± 0.1</td>
<td>27 ± 0.4</td>
</tr>
<tr>
<td>lin-PIB-g-PEO (34 wt% PEO)</td>
<td>4.1 ± 1.2</td>
<td>609 ± 150</td>
<td>9 ± 1.9</td>
<td>29 ± 0.6</td>
</tr>
</tbody>
</table>

^ Standard deviations on tensile measurements correspond to 4 measurements on different melt pressed samples.
4.2.4 Adsorption of Proteins to \textit{arb}-PIB-g-PEO Copolymer Surfaces

We have previously demonstrated that surfaces coated with \textit{lin}-PIB-g-PEO having low PEO content exhibited unusual micrometer-scale patterns upon the adsorption of fluorescently labeled proteins, while those with PEO content $\geq 18 \text{ wt\%}$ resisted the adsorption of these proteins.\textsuperscript{29} It was of interest to determine whether \textit{arb}-PIB-g-PEO would also exhibit these properties. To evaluate this, solutions of \textit{arb}-PIB-PIPs \textsuperscript{4.1a} and \textsuperscript{4.1b}, as well as copolymers \textsuperscript{4.3}, \textsuperscript{4.4}, and \textsuperscript{4.6} were coated on silicon wafers, then immersed in a solution of rhodamine-labeled fibrinogen\textsuperscript{28} for 35 minutes. As in previous work,\textsuperscript{28,29} fibrinogen was selected because it is a prevalent protein from plasma, involved in the clotting of blood. It has previously received considerable interest in the area of biomaterials research because it plays a pivotal role in the process of surface-induced thrombosis.\textsuperscript{34} Following immersion in the protein solution, the surfaces were washed and imaged by confocal fluorescence microscopy. As shown in Figures \textsuperscript{4.5a} and \textsuperscript{4.5b}, \textit{arb}-PIB-PIP containing 1.5 and 2.9 mol\% IP exhibited significant adsorption of fibrinogen. Unlike the homogeneous protein adsorption that was previously observed for IIR, the adsorption of protein on \textit{arb}-PIB-PIP exhibited micrometer-scale patterns, as we have previously observed for \textit{lin}-PIB-g-PEO with low PEO content. This can perhaps be attributed to the phase separation that is possible for these polymers, whereas phase separation is not possible in IIR, where the IP units are dispersed randomly throughout the backbone.
Figure 4.5. Fluorescence confocal microscopy images (543 nm) of thin films following adsorption of a rhodamine-fibrinogen conjugate: a) copolymer 4.1a, b) copolymer 4.1b, c) copolymer 4.3, d) copolymer 4.4 and, e) copolymer 4.6 (Each image represents an area of 0.45 mm × 0.45 mm). f) Relative fluorescence values obtained by confocal microscopy, corresponding to the adsorption of rhodamine-fibrinogen conjugate (*P<0.001 by one-way ANOVA test followed by Tukey’s test), error bars represent the standard deviation of 8 measurements on 3 different samples of each surface).

By the incorporation of PEO, even at the low content of 8 wt% in copolymer 4.3, the level of protein adsorption was reduced, as shown qualitatively in Figures 4.5c-e. In addition, the fluorescence was quantified by fluorescence microscopy for at least 8 random regions on each surface and at least 3 surfaces of each type were measured for statistical reasons. As shown in Figure 4.5f, all of the copolymers exhibited significantly (p<0.001) reduced levels of protein adsorption relative to the arb-PIB-PIP, with copolymer 3 having an ~3-fold lower average fluorescence, and copolymers 4.4 and 4.6 having >10-fold lower fluorescence. Therefore, like the lin-PIB-g-PEO, a critical value of about 20 wt% PEO seems to be required to achieve a significant reduction in protein adsorption. This reduction in protein adsorption can be attributed to the well-known
ability of PEO to confer resistance to protein adsorption\textsuperscript{35,36} and implies that under the conditions of the experiment, it was possible for the PEO blocks to be presented on the surfaces of these materials.

4.2.5 Assembly of \textit{arb}-PIB-g-PEO in Aqueous Solution

Despite the high hydrophobicity of PIB, we were able to demonstrate in recent work that upon the incorporation of sufficient PEO content, it was possible to disperse the \textit{lin}-PIB-g-PEO in water through the preparation of nanometer-sized spherical micelles.\textsuperscript{30} Micelles with different diameters in the range of \textasciitilde30 – 500 nm were obtained by dissolving the copolymers in THF, rapidly adding water, and then the removing the THF by dialysis against water. Depending on the THF/water ratio prior to dialysis, it was possible to control the sizes of assemblies, with the rapid addition of a large percentage of water to the THF prior to dialysis generally resulting in assemblies with smaller diameters through a kinetic trapping process.

To compare the aqueous assembly properties of the \textit{arb}-PIB-g-PEO with those of the linear analogues, they were studied under the same conditions. Copolymers 4.3, 4.4, and 4.6 were first dissolved in THF, and then varying quantities of water were added rapidly via syringe, resulting in suspensions containing 0, 30, 50 or 70\% water prior to dialysis. After dialysis, DLS was performed to assess the diameters of the assemblies. As shown in Figure 4.6a, in contrast to the results for the \textit{lin}-PIB-g-PEO, the diameters of the assemblies did not generally depend on the percentage of water added prior to dialysis. Copolymer 4.3 formed large assemblies of \textasciitilde170 – 180 nm in diameter, likely a result of this copolymer's low PEO content, which would not be sufficient to stabilize the larger interfacial surface area associated with smaller assemblies. Copolymer 4.4 exhibited a modest dependence of the micelle diameter on the preparation method, with the diameter decreasing from \textasciitilde130 to \textasciitilde80 nm upon the addition of 30\% water prior to dialysis, but then the diameter remained relatively constant, independent of an increase in the percentage of water. Copolymer 4.6 did not exhibit any dependence on the method of preparation and formed micelles with diameters of 70 – 80 nm regardless of the method. This suggests that 70 – 80 nm may represent the lower size limit for the assemblies and may correspond to unimolecular micelles formed from these very high MW polymers. In
comparison with the *lin*-PIB-\(g\)-PEO, the architecture of the branched PIB core with grafted PEO chains on the periphery may make *arb*-PIB-\(g\)-PEO ideal for the formation of unimolecular micelles.

**Figure 4.6.** a) Z-average diameter for aqueous assemblies as a function of the amount of water added rapidly to a THF solution of different copolymers prior to dialysis against water: Error bars represent the standard deviation on measurements from 3 separately prepared samples of assemblies; b) and c) TEM image of assemblies formed from copolymer 4.6 by dialysis from 100% THF (scale bar = 100 nm in b and 20 nm in c). (The THF/water ratio prior to dialysis was 100/0 (v:v)).

The assemblies were also imaged by TEM, with staining by OsO\(_4\). These images confirmed that the assemblies were indeed spherical in nature for each copolymer (see appendix). In addition, due to the selectivity of the OsO\(_4\) for double bonds of the
functionalized IP units, it was possible visualize that the PEO functionalized IP units were on the peripheries of the assemblies with unstained PIB at the core (Figure 4.6b and 4.6c).

In order to further probe the formation of micelles from copolymers 4.3, 4.4, and 4.6, and explore the possibility that copolymer 3 formed multimolecular micelles while 4.4 and 4.6 formed unimolecular micelles, the critical micelle concentrations (CMCs) for copolymers 4.3, 4.4, and 4.6 were probed using pyrene. Pyrene experiences a polar environment in aqueous media in the absence of micelles (i.e., below the CMC). As micelles are formed, pyrene preferentially partitions into the hydrophobic cores of the micelles, a nonpolar environment. While a number of changes in the fluorescence emission spectra of pyrene are associated with the partitioning of pyrene between the two phases,\(^{37}\) in this study we evaluated the change \(I_1/I_3\) as a function of copolymer concentration, where \(I_1\) is the intensity of the vibronic emission band at 372 nm and \(I_3\) is the intensity of the vibronic emission band at 382 nm.

The micelle samples were prepared as described above, with no addition of water to the THF solution prior to dialysis. As shown in Figure 4.7a, for copolymer 4.3, a plot of \(I_1/I_3\) versus the log of the copolymer concentration has two distinctive slopes, with a corresponding CMC of 20 mg/L. In contrast, as shown in Figures 4.7b and 4.7c, no CMC was detectable for copolymers 4.4 or 4.6, suggesting that these polymers exhibit a tendency to form unimolecular micelles as a result of their arborescent architectures.
Figure 4.7. $I_1/I_3$ from emission spectra of pyrene in aqueous solution of a) copolymer 4.3, b) copolymer 4.4, and c) copolymer 4.6 ($T = 25 \, ^\circ C$).
4.3 Conclusions

We have successfully prepared a small library of arb-PIB-g-PEO graft copolymers and compared their synthesis and properties to those of lin-PIB-g-PEO. In the synthesis, it was found that although the presence of IP units at the peripheries of the arborescent molecules may make them more available for the grafting of PEO-NH$_2$, the close proximities of the IP units due to their concentration in the peripheral blocks made complete functionalization challenging due to steric hindrance from nearby PEO chains. This was particularly evident in the grafting of PEO-NH$_2$ with a MW of 5000 g/mol, but high grafting yields were still obtained using 2000 g/mol PEO-NH$_2$. When microphase separation was probed by AFM, it was found that it was most evident for the higher PEO content copolymer with 48 wt% PEO, and was more pronounced than for lin-PIB-g-PEO of comparable PEO content under the conditions investigated. While the microphase separation was not evident from AFM for all copolymers, the tensile properties including tensile strength at break, Young's modulus, elongation at break, and toughness did change in a systematic manner for both the arborescent and linear graft copolymers, with the linear materials exhibiting higher elongation at break and higher toughness, but increased yielding and plastic behavior. As previously observed for the lin-PIB-g-PEO, arb-PIB-g-PEO resisted the adsorption of fluorescently-labeled fibrinogen. Lastly, the assembly of arb-PIB-g-PEO in water was investigated and it was found that unlike lin-PIB-g-PEO, the size was not generally dependent of the method of preparation and there was a tendency for these polymers to form unimolecular micelles. Thus, while the inherent properties of the arb-PIB-g-PEO are similar to those of lin-PIB-g-PEO, there are key differences in their abilities to self-assemble on surfaces and in solution that result from their differences in architecture. This emphasizes the importance of controlling the molecular architecture in order to fine-tune the properties of materials for specific applications.

4.4 Experimental Section

General Procedures and Materials:
arb-PIB-PIP containing 1.5 mol% IP (M_w = 375 kg/mol, PDI = 2.1), 2.9 mol% IP (M_w = 312 kg/mol, PDI = 1.9) and 5.5 mol% IP (M_w = 275 kg/mol, PDI = 1.8) were prepared as previously reported. Solvents were purchased from Caledon (Georgetown, Canada) and all other chemicals were purchased from Sigma Aldrich and were used without further purification unless otherwise noted. DMAP was purified by recrystallization in toluene before use. m-CPBA was dissolved in toluene and dried with MgSO_4 before use. Pyridine was distilled over CaH_2 before use. Dry toluene was obtained from a solvent purification system based on aluminium oxide columns. ^1H NMR spectra were obtained in CDCl_3 at 600 MHz on a Varian INOVA 600 spectrometer. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of CDCl_3 (δ 7.26). IR spectra were obtained using a Bruker Tensor 27 instrument as films from toluene on KBr plates. SEC was performed in THF with a flow rate of 1 mL/min at 25 °C using an SEC instrument equipped with a Viscotek Max VE2001 solvent module and a Viscotek VE3580 RI detector operating at 30 °C. The separation technique employed two Agilent Polypore (300 mm × 7.5 mm) columns connected in series to a Polypore guard column (50 mm × 7.5 mm). The calibration was performed using polystyrene standards. DSC measurements were acquired under nitrogen using a DSC Q20 calorimeter from TA instruments at heating rate of 10 °C/min from -100 °C to 125 °C. The reported transitions were obtained from the second of two heating cycles.

Synthesis of Polymer 4.2a

arb-PIB-PIP 4.1a (1.0 g, 0.26 mmol of IP units) was dissolved in toluene (35 mL). To this solution, a solution of m-CPBA (0.10 g, 0.58 mmol) in toluene (3 mL) was added and the reaction mixture was stirred for 1 hr at room temperature. Then, HCl (37% in water, 22 µL, 0.26 mmol) was added and the reaction mixture was stirred for 1.5 hours at room temperature to form hydroxyl-functionalized arb-PIB-PIP. In order to remove water, 0.3 g of anhydrous MgSO_4 anhydrous was added and the solution was stirred for 30 minutes. After filtration, to the filtrate, 4-NPC (0.22 g, 1.1 mmol) was added followed by pyridine (0.13 mL, 1.6 mmol). The reaction mixture was stirred for 2 hours at room temperature, and the pyridine salts were removed by filtration. The filtrate was concentrated under reduced pressure to a volume of 10 mL and then the resulting activated arb-PIB-PIP
copolymer 4.2a was purified by precipitation into acetone (50 mL). The precipitation from toluene into acetone was repeated three times and then the product was dried under vacuum. Yield: 90%. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm): 8.28 (br s, 2H), 7.38 (br s, 2H), 1.42 (s, CH$_2$ PIB, 131H), 1.11 (s, CH$_3$ PIB, 394H); $M_w$ = 498 kg/mol, PDI = 2.3.

Synthesis of polymer 4.2b

The procedure described above for the preparation of polymer 4.2a was followed except that $arb$-PIB-PIP 4.1b (1.0 g, 0.51 mmol of IP units) was used. Yield: 88%. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm): 8.28 (br s, 2H), 7.38 (br s, 2H), 1.42 (s, CH$_2$ PIB, 67H), 1.11 (s, CH$_3$ PIB, 201H); $M_w$ = 339 kg/mol, PDI = 2.1.

Synthesis of polymer 4.2c

The procedure described above for the preparation of polymer 4.2a was followed except that $arb$-PIB-PIP 4.1c (1.0 g, 0.97 mmol of IP units) was used. Yield: 87%. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm): 8.27 (br s, 2H), 7.40 (br s, 2H), 1.42 (s, CH$_2$ PIB, 34H), 1.12 (s, CH$_3$ PIB, 103H); $M_w$ = 356 kg/mol, PDI = 2.2.

Synthesis of Copolymer 4.3

Activated copolymer 4.2a (1.0 g, 0.25 mmoL) was dissolved in toluene (40 mL). In a separate flask PEO-NH$_2$ with a MW of 750 g/mol (0.26 g, 0.35 mmoL) was dissolved in toluene (4 mL). To this solution, the activated copolymer solution was added dropwise, followed by a solution of DMAP (46 mg, 0.38 mmol) in toluene (1 mL). The resulting mixture was stirred at 60 °C for 12 hours. Next, the solvent was removed in vacuo and the unreacted PEO-NH$_2$ was removed by washing the rubbery material once with deionized water. The resulting material was then dissolved in a minimum volume of dichloromethane and the product was purified by precipitation into a 4-fold excess volume of acetone. Yield = 86%. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm): 3.65 (s, 29H), 3.38 (s, 3H), 1.42 (s, CH$_2$ PIB, 131H), 1.12 (s, CH$_3$ PIB, 403H). PEO content (from $^1$H NMR): 8 wt%; $T_g$ = -65 °C; $T_m$ = 18 °C.

Synthesis of Copolymer 4.4
The procedure described above for the preparation of copolymer 4.3 was followed except that PEO-NH2 with a MW of 2000 g/mol was used and the resulting copolymer 4.4 was purified by precipitation from THF into cold diethyl ether. Yield = 84%. $^1$H NMR (600 MHz, CDCl3, δ, ppm): 3.65 (s, 105H), 3.38 (s, 3H), 1.42 (s, CH2 PIB, 131H), 1.12 (s, CH3 PIB, 394H). PEO content (from $^1$H NMR): 24 wt%; Tg = -65 °C; Tm = 46 °C.

**Synthesis of Copolymer 4.5**

The procedure described above for the preparation of copolymer 4.3 was followed except that PEO-NH2 with a MW of 5000 g/mol was used and the resulting copolymer was purified by precipitation from THF into cold diethyl ether. Yield = 81%. $^1$H NMR (600 MHz, CDCl3, δ, ppm): 3.65 (s, 98H), 3.38 (s, 3H), 1.42 (s, CH2 PIB, 131H), 1.12 (s, CH3 PIB, 387H). PEO content (from $^1$H NMR): 23 wt%; Tg = -65 °C; Tm = 47 °C.

**Synthesis of Copolymer 4.6**

Starting from activated copolymer 4.2b, the procedure described above for the preparation of copolymer 4.3 was followed except that PEO-NH2 with a MW of 2000 g/mol was used and the resulting copolymer was purified by precipitation from THF into cold diethyl ether. Yield = 80%. $^1$H NMR (600 MHz, CDCl3, δ, ppm): 3.65 (s, 154H), 3.38 (s, 3H), 1.41 (s, CH2 PIB, 67H), 1.11 (s, CH3 PIB, 200H). PEO content (from $^1$H NMR): 48 wt%; Tg = -64 °C; Tm = 44 °C.

**Synthesis of Copolymer 4.7**

Starting from activated copolymer 4.2c (0.50 g), the procedure described above for the preparation of copolymer 4.3 was followed except that PEO-NH2 with a MW of 2000 g/mol was used and the resulting copolymer was purified by precipitation from THF into cold diethyl ether. Yield = 78%. $^1$H NMR (600 MHz, CDCl3, δ, ppm): 3.65 (s, 101H), 3.38 (s, 3H), 1.42 (s, CH2 PIB, 34H), 1.12 (s, CH3 PIB, 102H). PEO content (from $^1$H NMR): 54 wt%; Tg = -64 °C; Tm = 53 °C.

**Synthesis of Copolymer 4.8**
Starting from activated copolymer 4.2c (0.92 g), the procedure described above for the preparation of copolymer 4.3 was followed except that PEO-NH$_2$ with a MW of 2000 g/mol was used and the resulting copolymer was purified by precipitation from THF into cold diethyl ether. Yield = 79%. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm): 3.65 (s, 38H), 3.39 (s, 3H), 1.42 (s, CH$_2$ PIB, 34H), 1.12 (s, CH$_3$ PIB, 102H). PEO content (from $^1$H NMR): 31 wt%; $T_g$ = -66 °C; $T_m$ = 50 °C.

**AFM**

Silicon wafers were cut into small pieces (1.5 cm × 1.5 cm), then immersed in a 1:2 Piranha solution to clean organic residue off the surface. This was followed by a thorough rinse in water and then ethanol. The wafers were then dried under a stream of air. Thin films of copolymers were prepared by spin casting 3 wt% solutions of the samples in toluene onto silicon wafers. Spin casting conditions were 150 $\mu$L for 1.5 cm$^2$ of silicon wafer, 6000 rpm, 30 s. The spin coated surfaces had a final thickness of ~70 nm as measured by AFM using a scratch test. The surfaces were imaged using an atomic force microscope (XE-100, Park Systems) in the dynamic force mode with rectangular-shaped silicon cantilevers having a spring constant of 40 N/m. Data were then refined using the software Nanoscope.

**Mechanical Properties**

Melt pressed copolymer films were obtained using a hydraulic heated press (Hydraulic Unit Model #3912, Carver, Inc., Wabash, IN). ~1 g of polymer was placed on a folded Teflon sheet. The temperature in the press was raised to 190 °C. The Teflon sheet containing the polymer was then placed between the heated platens and preheated for 2 min at 190 °C and then pressed at 2 MPa for 3 min to form a compression molded sheet. The sample was then allowed to cool to room temperature and then removed from the press. After that, the sheets were cut into 25 mm × 5 mm × 0.3 mm (length × width × thickness) strips. The stress-strain properties of the samples were measured according to ASTM D 882-09 on an INSTRON universal testing machine 3300 series, with a crosshead speed of 250 mm/min at 25 °C. For each copolymer, at least 4 samples were tested in separate analyses, and the data reported is the calculated mean.
Protein Adsorption and Confocal Laser Scanning Microscopy

A 20 mg/mL solution of copolymer in toluene was prepared and was drop cast on a circular glass cover slip (25 mm diameter). 3 coats of 100 µL each were applied to the surface. The films were then dried under vacuum. A 1 mg/mL solution of the rhodamine-fibrinogen conjugate\textsuperscript{38} in 5 mM phosphate buffer, pH 7.2 was prepared. The polymer coated glass cover slips were then immersed in this protein solution. After 35 minutes, nonadsorbed proteins were removed by washing the surface with buffer and then water. The fluorescence was then evaluated by using an LSM 510 multichannel point scanning confocal microscope (Laser 543 nm and band-pass filter of 560 – 615 nm, magnification 20×). The settings on the instrument were kept constant for the comparison of all surfaces. The fluorescence was evaluated by averaging 8 randomly selected regions of the surface for each sample. Linear operation of the camera was ensured, and the constant exposure time used during the image collection permitted quantitative analysis of the observed fluorescent signals. The fluorescence microscopy images were analyzed using Image-Pro Plus software version 7.0 which yielded the mean and standard deviation of the fluorescence intensity within a given image. For all the samples, three surfaces were prepared and measured.

Preparation of Aqueous Assemblies of \textit{arb-PIB-g-PEO}

1 mL of a 10 mg/mL solution of the copolymer was prepared in filtered THF (THF was passed through a 0.2 µm pore size polytetrafluoroethylene syringe filter (Dikma, ProMax\textsuperscript{TM}) prior to use). To this solution, 0 mL, 0.4 mL, 1 mL, or 2 mL of filtered deionized water was added rapidly via syringe to provide solutions containing either 0%, ~30%, ~50%, or ~70% (v:v) water in THF. The resulting solutions were then dialyzed overnight against water using a regenerated cellulose membrane (Spectra/Por) with a molecular weight cut-off of 12000 – 14000 g/mol. Dynamic light scattering was then performed on ZetaSizer Nano instrument from Malvern Instruments.

TEM Imaging
A drop of the nanoparticle suspension (~10 mg/mL) was transferred via pipette to a carbon formvar grid and was left for 2 min. The excess solution was removed. The grid was stained with the vapor of OsO₄ in a sealed container, for 1 hr. Imaging was performed using a Phillips CM10 microscope operating at 80 kV with a 40 µm aperture. The assembly diameters were determined by measuring the diameters of the assemblies in each image (magnified) relative to the scale bar and then calculating the average and standard deviation.

**Determination of the CMC**

The CMC of the arb-PIB-g-PEO copolymers was evaluated by means of a fluorescence spectroscopy method with pyrene as a probe. 1 mg Pyrene was dissolved in 10 mL of dichloromethane, and 0.1 mL of the solution was transferred to each vial and dried under a stream of air. Then, a 10 mg/mL solution of a selected copolymer in THF was prepared and dialyzed against water overnight using a regenerated cellulose membrane (Spectra/Por) with a molecular weight cut-off of 12000 – 14000 g/mol. From this aqueous solution, a series of concentrations from 0.01 mg/mL to 1.11 mg/mL in 0.1 M phosphate buffer solution was made and transferred to the vials containing solid pyrene. The vials were covered by foil. Then, the fluorescence spectrum of each sample was obtained on a QM-4 SE spectrofluorometer equipped with double excitation and emission monochromators from Photon Technologies International. An excitation wavelength of 334 nm was used for pyrene and the emission spectra were recorded from 365 nm to 450 nm with the \( I_1 \) and \( I_3 \) values obtained at 372 nm and 384 nm, respectively.
4.5 References

Chapter 5

5 Synthesis and Application of Cinnamate-Functionalized Rubber for the Preparation of UV-Curable Films∗

5.1 Introduction

IIR, a copolymer of IB and small percentages of IP, is a high performance synthetic elastomer with many attractive properties including high elasticity, impermeability to gas and water, high damping, and good thermal and chemical stability. Because of these properties it is used in a diverse array of commercial applications ranging from automobile tires to sporting equipment. In addition, due to its low toxicity, food grade IIR has been commercialized and is used in a variety of chewing gums.

In the past couple of decades there has also been increasing interest in the development of PIB-based materials for biomedical applications.1-5 The most noteworthy example has been the clinical use of a PIB-polystyrene (SIBS) triblock copolymer as a drug eluting coating on the TAXUS® vascular stent.3,6 Similar polymers have also been investigated in corneal shunts for the treatment of glaucoma,7,8, synthetic aortic valves9, and hydrophobic electrospun fiber mats.10 PIB-poly(methyl methacrylate) (PMMA) composites have been shown to have enhanced properties relative to commercial bone cements due to the incorporation of the elastomeric PIB into the glassy PMMA material.11,12 Multiarm PIB-cyanoacrylate (CA) copolymers have been reported as promising materials for intervertebral disk replacement13,14 and tissue adhesives.15 PIB-based polyurethanes have been demonstrated to exhibit unprecedented combinations of mechanical properties and high oxidative, hydrolytic and biological stability.4 Furthermore, copolymers of PIB with hydrophilic polymers such as poly(N,N-dimethylacrylamide) or PEO have been used to form membranes that can encapsulate cells while allowing the exchange of oxygen, nutrients, and secreted proteins such as

∗This chapter contains work that has been submitted: Wu, W.; Karamdoust, S.; Turowec, B. A.; Gillies, E. R. EUR. POLYM. J. 2013, pending. See Co-Authorship Statement for specific contributions from each author.
insulin across the membrane.\textsuperscript{16} Our group has shown that IIR-PEO films resist the adsorption of proteins and the growth of cells, providing non-fouling properties.\textsuperscript{17,18} IIR-PEO graft copolymers can also assemble into micellar structures in aqueous solution.\textsuperscript{19}

In traditional, non-biomedical applications, IIR is chemically cross-linked using additives such as zinc oxide or elemental sulfur with additives such as thiuram or thio-carbamates, in order to provide properties such as improved creep resistance, resilience and modulus in comparison to the non-cross-linked rubber. The inclusion of photoinitiators in various formulations of IIR and PIB has provided UV-curable rubbers for applications such as coatings, sealants and adhesives.\textsuperscript{20-24} IIRs that cure with peroxide initiators in the presence of co-agents have also been developed in recent years.\textsuperscript{25,26} The above cross-linking processes are not ideal for biomedical applications due to the possibility of leaching toxic additives from the resulting materials. In the case of the SIBS triblock copolymers used in the stent coating applications, the incorporation of the styrene blocks imparts thermoplastic properties to the rubber, allowing the material to behave as a cross-linked rubber at room temperature and melt like plastic at temperatures above the \(T_g\) of polystyrene segments.\textsuperscript{3} However, to the best of our knowledge, a IIR derivative that is capable of undergoing chemical cross-linking without the use of chemical additives has not yet been reported.\textsuperscript{27}

Cinnamate modified polymers have been demonstrated to undergo cross-linking upon UV irradiation via a \([2+2]\) cycloaddition mechanism in the absence of chemical additives such as photoinitiators.\textsuperscript{28} For example, Visconte and coworkers prepared cross-linked films of natural rubber by maleation, reaction with various cinnamate derivatives, and then UV treatment.\textsuperscript{29-31} The cinnamate-based UV curing approach has also been used for a variety of applications including photopatternable surfaces\textsuperscript{32} and also to covalently fix noncovalent polymer assemblies in solution.\textsuperscript{33} The low toxicity of cinnamic acid makes this a particularly attractive approach for the development of curable IIR films for biomedical application.\textsuperscript{34} In addition, the simple preparation of hydroxyl functionalized butyl rubber derivatives recently reported by our group makes the preparation of IIR-cinnamate derivatives a synthetically facile process.\textsuperscript{17}
We report here the synthesis and chemical characterization of cinnamate functionalized IIR derivatives starting from both low (2 mol%) and high (7 mol%) IP content IIRs in order to provide different loadings of cinnamate. The preparation of polymer films is described, followed by studies of their UV curing kinetics, gel content, and swelling ratios. Mammalian cell culture studies demonstrate that toxic molecules do not leach from the cross-linked materials. In addition, it is shown that the approach can be extended to the preparation of cross-linked films of other functional IIR derivatives including IIR-PEO graft copolymers.

5.2 Results and Discussion

5.2.1 Polymer Synthesis and Characterization

The synthesis of cinnamate functionalized IIR began from the previously reported epoxidized IIR derivatives 1.27\(^\text{17}\) and 3.5\(^\text{19}\), which were prepared in a single step from IIR containing 2 mol% and 7 mol% IP respectively. As shown in Scheme 5.1, the epoxide moiety of 1.27 was opened to provide the allylic alcohol 1.28 as previously reported\(^\text{17}\). This alcohol was then reacted with cinnamoyl chloride in the presence of pyridine at room temperature, to provide the target cinnamate derivative 5.1 and 5.2.

\[
\begin{align*}
1.27 & \quad (2.2 \text{ mol\% IP}) \\
3.5 & \quad (7.0 \text{ mol\% IP}) \\
\text{Toluene, Pyridine} & \quad \overset{\text{HCl, RT, Toluene}}{\longrightarrow} \\
1.28 & \quad (2.2 \text{ mol\% IP}) \\
3.6 & \quad (7.0 \text{ mol\% IP, not isolated}) \\
\end{align*}
\]

In the case of the higher IP content, the allylic alcohol 3.6 was not isolated as the workup and drying of this polymer resulted in an insoluble material, likely a result of
hydrogen bond-mediated cross-linking due to the high concentration of hydroxyls within the hydrophobic matrix of the polymer. Instead, following HCl neutralization and drying of the reaction mixture, pyridine and cinnamoyl chloride were added and 3.6 was converted to the cinnamate functionalized polymer 5.2 in a pseudo-one-pot sequence from 3.5.

The polymers were characterized by a number of techniques including NMR and IR, SEC, and DSC. NMR spectroscopy showed clean conversion to the cinnamate functionalized polymers as shown in Figure 5.1 for the conversion of 3.5 to 5.2. This is unlike much of the previously reported chemistry on IIR and its brominated derivatives where the requirement for high reaction temperatures often leads to multiple products resulting from the desired reaction as well as side reactions such as eliminations. Infrared spectroscopy also confirmed the presence of the cinnamate moieties, as indicated by distinctive C=O and C=C stretches at 1720 and 1640 cm\(^{-1}\) respectively. SEC showed that the polymer molecular weight did not significantly change throughout the process. Polymer 5.1 exhibited a \(T_g\) of -63 °C, very similar to that of IIR itself, while 5.2 exhibited a modestly elevated \(T_g\) of -52 °C likely resulting from the aromatic cinnamate moieties.

### 5.2.2 Preparation of Polymer Films

Several methods were explored for the preparation of polymer films. It was found that films with thicknesses ranging from approximately 10 to 250 nm could be prepared by spin coating solutions of the polymers in hexanes at different concentrations onto quartz plates. Alternatively, Meyer rods were used to prepare films with micrometer scale thicknesses. The film thicknesses were measured by profilometry and UV-visible absorbance measurements were performed on the same surfaces. The optical density increased linearly with film thickness allowing a calibration curve to be generated for optical density versus film thickness (see appendix). Based on these measurements, the optical densities of the films are 0.6 a.u./µm of thickness for 5.1 and 2.7 a.u./µm for 5.2 at 274 nm, the absorption maximum (\(\lambda_{\text{max}}\)) for the cinnamate moiety.
Figure 5.1. $^1$H NMR spectra of a) polymer 3.5 and b) polymer 5.2 in the downfield region from 2.5-8 ppm, showing clean conversion from the epoxide to cinnamate functionalized rubber (CDCl$_3$, 600 MHz).

5.2.3 Kinetics of UV Curing

The irradiation of films was performed using a mercury lamp, and quartz glassware was used to permit the penetration of UV light. In general, the optical densities of the films at 274 nm decreased with increased irradiation as shown in Figure 5.2. Examination of the infrared spectra of films before and after irradiation also showed a reduction in the peaks corresponding to the cinnamate moiety and a new C=O peak emerging (see appendix). This is consistent with the occurrence of the photoinduced cross-linking mechanism.$^{28,30}$ As the optical density is directly proportional to the concentration of cinnamate moieties in the films, these values can be used to quantitatively study the kinetics of the cross-linking reaction. $A_0/A$ where $A_0$ is the initial absorbance and $A$ is the absorbance at time $t$, can be used in place of $C_0/C$, where $C_0$ is the initial concentration of cinnamate moieties and $C$ is the concentration at time $t$.\textsuperscript{30} Thus, consumption of cinnamate moieties can be calculated as $[(A_0-A)/A_0] \times 100\%$. The kinetic analysis was performed for films from 5.1 and 5.2 with varying thicknesses (Table 5.1) and the % consumption of cinnamate versus time is shown in Figure 5.3.
Figure 5.2. A decrease in optical density is observed for a film (thickness = 670 nm) of 5.1 upon UV irradiation. Irradiation time increases from 0 min (top) to 40 min (bottom).

Table 5.1. Thicknesses and optical densities of the rubber films studied.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Designation</th>
<th>Thickness</th>
<th>Optical Density (274 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>5.1-1</td>
<td>670 nm</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>5.1-2</td>
<td>2.3 µm</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>5.1-3</td>
<td>9.5 µm</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>5.2</td>
<td>5.2-1</td>
<td>220 nm</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>5.2-2</td>
<td>850 nm</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Figure 5.3. Consumption of cinnamate groups as a function of UV irradiation time for films of 5.1 and 5.2 with varying film thickness.
The UV curing process for cinnamate functionalized polymers has been previously summarized by the steps shown in Scheme 5.2,\textsuperscript{39} where C and C* denote the ground state and excited cinnamate moieties, respectively. A quasi-steady-state assumption is made for C*, that is, [C*] remains constant during the cross-linking process, and the rate of cross-linking is limited by the dimerization step (k_2[C*][C] \ll k_4[C*]).

Scheme 5.2. Proposed mechanism for the photoinduced cross-linking of cinnamate functionalized polymers.

The reaction kinetics is proposed to differ, depending on the film thickness.\textsuperscript{40} For films with low optical density (generally lower than 0.5), where the intensity of incident light is virtually unchanged after penetrating the film, equation 1 is expected to apply, where \( h \) is Plank’s constant, \( \nu \) is the wave number, \( \varepsilon \) is the extinction coefficient, \( I_0 \) is the intensity of the incident light, \( k_{obs} \) is the observed rate constant.\textsuperscript{40} Equation 1 can be rearranged to equation 2 and thus a linear relationship between \( [C_0]/[C] \) versus time is expected. This was indeed the case for films 5.1-1 and 5.2-1 as shown in Figure 5.4a.

\[
\begin{align*}
\frac{1}{[C]} - \frac{1}{[C_0]} &= k_{obs}t \\
\text{where } k_{obs} &= 4.60 \times \frac{1}{6.023 \times 10^{23}h\nu} \times I_0\varepsilon \frac{k_2}{k_3 + k_4} \\
\frac{[C_0]}{[C]} &= k_{obs} [C_0] t + 1
\end{align*}
\]
Figure 5.4. Time dependence of a) \([C_0]/[C]\) for films 5.1 – 1 and 5.2 – 1, b) \(\ln([C_0]/[C])\) for 5.1 – 2, 5.1 – 3, and 5.2– 2.

For films with high optical density (significantly larger than 0.5), where the absorbed light intensity is nearly equal to that of incident light, equation 3 is expected to apply, where \(l\) denotes the thickness of the film. As shown in Figure 5.3b, \(\ln([C_0]/[C])\) versus time was indeed linear over the entire time measured. This result differed from that of Azuma et al. on cinnamate modified polydienes, where \(\ln([C_0]/[C_1])\) versus time was linear in the early reaction stages, but plateaued in the later stages. This was attributed to a retardation of the cross-linking reaction upon the formation of a rigid cross-linked network. However, these modified PIPs had much higher cinnamate content than 5.1 and 5.2. In the current work, kinetic data indicate that the UV curing doesn’t
have a significant effect on the mobility of the polymer chains. This may be explained by the relatively low content of cinnamate functionalities (2 or 7 mol%).

\[
\ln \frac{[c_0]}{[c]} = k_{obs} t
\]  
(3)

where \(k_{obs} = 4.60 \times \frac{1}{6.023 \times 10^{23} h v} \times I_0 e^{-\frac{k_2}{k_3 + k_4}} \frac{1}{l}

5.2.4 Properties of Cross-linked Films

While relatively thin films were required for the above studies, it was also of interest to investigate whether the UV curing could be applied to thicker films and also to use these materials to investigate properties such as the gel content, \(T_g\) and volume swelling ratio. Thus, films with a thickness of \(~0.1\) mm were prepared by melt pressing. As described above, these films were irradiated for different time periods. A portion of each resulting film was subjected to thermal analysis and the remainder was extracted using toluene to remove soluble polymer. The weight of material was measured before extraction and after extraction both in the wet and dry state. The gel content was calculated as the weight percentage of insoluble polymer relative to total polymer. The volume swelling ratio \(^{41}\) of the insoluble polymer was calculated according to equation 4, where \(q_w\) is the ratio of the wet weight to the dry weight of the insoluble material from each sample, \(\rho_{\text{polymer}}\) is the density of IIR (0.92 g/cm\(^3\)) and \(\rho_{\text{solute}}\) is 0.87 g/cm\(^3\) for toluene in this study.

\[
q_v = 1 + \frac{(q_w - 1)\rho_{\text{polymer}}}{\rho_{\text{solute}}}
\]  
(4)

As shown in Figure 5.5a, the evolution of the gel content versus irradiation time was markedly different for 5.1 versus 5.2. The gel content of 5.1 increased gradually with irradiation time while that of 5.2 rapidly increased and plateaued. This can likely be attributed to the differences in cinnamate content, as to achieve the critical degree of cross-linking in the case of 5.2 would require a lower percentage of the cinnamate groups to react. As shown in Figure 5.5b, the volume swelling ratios followed the trends expected based on the gel content study. The volume swelling ratio for 5.1 was initially
much higher than that of 5.2, likely due to the very loose network of cross-links, and then decreased in the later stages of the cross-linking, reaching a value approximately two-fold higher than that of 5.2. Similar to the gel content, the volume swelling ratio of 5.2 plateaued almost immediately, indicating that a critical number of cross-links were formed immediately, restricting the swelling of the material. It is also noteworthy that in studies of various control polymers such as 5.1 or acetylated 5.1 or 5.2, no evidence of cross-linking was detected as the materials remained fully soluble following photoirradiation.

Figure 5.5. a) Gel content versus irradiation time for melt pressed films of polymer 5.1 (♦) and 5.2 (□), b) Volume swelling ratio versus time for melt pressed films of polymer 5.1 (♦) and 5.2 (□).
Interestingly, although the gel content is relatively high, the $T_g$ of 5.1 and 5.2 remained almost constant over different irradiation times (Figure 5.5). According to Ueberreiter and Kanig, $\Delta T_{g,c} = Z\chi'$, where $\Delta T_{g,c}$ is the change of $T_g$ with increasing cross-linking, $Z$ is a constant (for example $3.2 \times 10^4$ for NR), and $\chi'$ is moles of cross-link per gram of polymer.\textsuperscript{42} Considering 5.1 and 5.2 have relatively low cinnamate content (maximum of 2 mol% and 7 mol%, respectively), the resulting $\Delta T_{g,c}$ is expected to be small, provided the cinnamate dimerization is the only reaction that leads to cross-linking.

![Graph showing $T_g$ versus cure time for polymers 5.1 and 5.2](image)

**Figure 5.6.** $T_g$ versus irradiation time for melt pressed films of polymers 5.1 (●) and 5.2 (□) showing no significant change in $T_g$.

### 5.2.5 Toxicity of Cross-linked Polymer Films

As described above, one of the key advantages to the cinnamate-based curing approach is that no chemical additives are required and cinnamic acid is considered to be non-toxic, even if ester hydrolysis were to result in its release from the films. To investigate this, melt pressed films of 5.1 and 5.2 were incubated in cell culture media for a period of 24 hours. This media, containing possible leachates was then added at varying concentrations to C2C12 mouse myoblast cells and they were incubated for 24 hr. An MTT assay was then performed to assess cell viability in comparison with control cells that were grown in cell culture media that was not exposed to polymer films.\textsuperscript{43,44} As shown in Figure 5.7, no toxicity was observed at any leachate concentrations, suggesting
that this cross-linking approach does not result in the leaching of toxic chemical additives from the polymer films.

Figure 5.7. Viabilities of cells incubated in serial two-fold dilutions of culture medium that was incubated with cross-linked films of polymers 5.1 and 5.2, showing that toxic chemicals did not leach from the films into the culture medium.

5.2.6 Application of Cinnamate-based Cross-linking to IIR-PEO Graft Copolymers

Having developed a method for curing films of cinnamate functionalized IIR, it was also of interest to apply this to other functional butyl rubber derivatives. In particular, our group has recently shown that IIR-PEO graft copolymers exhibit interesting properties when cast into films.\textsuperscript{17-19} For example, copolymers with low PEO content form micrometer-scale patterns,\textsuperscript{17,18} while those with higher (i.e. > 24\%) PEO content tend to resist the adsorption of proteins and the growth of cells.\textsuperscript{18,19} However, the curing of these films was not performed, and they exhibited gradual mass loss into aqueous solution as a result of the high aqueous solubility of PEO.\textsuperscript{19} To demonstrate the applicability of our new cross-linking approach to IIR graft copolymers, a IIR derivative having both cinnamate moieties and grafted PEO chains was prepared. This was accomplished as shown in Scheme 5.3 by first ring opening polymer 3.5, and then functionalizing approximately 40\% of the resulting hydroxyls with cinnamoyl chloride. The remaining hydroxyls were then functionalized with 4-NPC and then this activated polymer was
reacted with amine terminated PEO (PEO-NH$_2$) of MW 5000 g/mol. The product was purified by precipitation from dichloromethane into methanol to remove any free PEO (see appendix).

**Scheme 5.3.** Synthesis of cinnamate functionalized IIR-PEO graft copolymer.

Using the methods described above for **5.1** and **5.2**, films of copolymer **5.4** were prepared. Similar to polymers **5.1** and **5.2**, copolymer **5.4** had an absorption maximum at 274 nm due to the cinnamate moieties, and the absorbance decreased with photoirradiation, consistent with the occurrence of a cross-linking reaction (see appendix). Using a film with a thickness of ~ 0.1 mm, the gel content was found to be 70% and the volume swelling ratio was 17 after a 60 min irradiation. These results are similar to those obtained in the absence of the grafted PEO chains, suggesting that despite the possible phase separation induced by the PEO, the UV curing is still effective.

It was also of interest to determine whether the cross-linked IIR-PEO films still exhibited the properties of the uncross-linked films. To evaluate this, the growth of cells on films of IIR, and UV cured **5.1**, **5.2**, and **5.4** were evaluated. C2C12 mouse myoblast cells were seeded onto the surfaces, incubated for 2 days, fixed, washed, and their nuclei were stained with DAPI, while their cytoskeletons were stained with Alexa Fluor 568 phalloidin. As shown in Figure 5.8, both the number of adhered cells and their appearance varied across the different polymer surfaces. The appearance of cells on the films of IIR (Figure 5.8a) and UV cured polymer **5.1** (Figure 5.8b) were quite similar to one another, though there was a statistically significant reduction in the number of cells on **5.1** (Figure 5.8e). Based on the toxicity assays described above, this reduction in the cell count is not likely a result of the leaching of toxic molecules from the films, but may
be due to the modest changes in the polymer structure that affect the adhesion of the cells to the surface. That the introduction of cinnamate moieties and subsequent curing does lead to a change in the number of adhered cells was further suggested by the results for 5.2 (Figure 5.8c), though the differences with 5.1 were not statistically significant. Very few cells adhered to films of UV cured polymer 5.4 (Figure 5.8d). In addition, these cells exhibited significant changes in their cytoskeletons and tended to grow in clusters and on top of one another suggesting that they preferred to adhere to one another than to the surface. This was expected based on our previous results for uncured surfaces of IIR-PEO graft copolymers,19 as well as the known ability of PEO to resist the adsorption of proteins, and consequently the adhesion of cells.45 The number of adhered cells was significantly less than on IIR (Figure 5.8e). There were also fewer cells than on 5.1 or 5.2 though these differences were not statistically significant due to the high standard deviations associated with these measurements. Nevertheless, these results suggest that by using the cinnamate cross-linking approach it is possible to maintain the properties of the IIR-PEO graft copolymers after UV curing.

**Figure 5.8.** a-d) Confocal microscopy images of C2C12 cells adhered to a) IIR (control); b) UV cured polymer 5.1, c) UV cured polymer 5.2, d) UV cured polymer 5.4. The cell nuclei are stained blue with DAPI and cytoskeletons are stained in green with Alexa Fluor 568 phalloidin (note – nonhomogeneity in the background of d) results from some three-dimensional topography to the surface and some adsorption of the dyes). Each image represents an area of 0.45 x 0.45 mm. e) number of adhered cells per mm² for control surface of IIR and UV cured films of 5.1, 5.2, and 5.4 (*P <0.05).
5.3 Conclusions

In conclusion, a UV curable cinnamate-functionalized IIR was developed. This allows the curing of IIR to be performed for the first time without chemical additives, preventing the possible leaching of additives from the resulting material, making this method particularly attractive for potential biomedical applications. These rubbers were useful for the preparation of films by various methods including spin coating, Meyer rods, and melt pressing. The kinetics of cross-linking in the films was also studied. In studies of thicker films prepared by melt pressing, it was found that the derivative 5.1 exhibited a steady increase in gel content with increased photoirradiation time over a period of 40 minutes, while 5.2 having a higher cinnamate content reached a gel content greater than 80% in less than 10 minutes. Toxicity assays suggested that neither 5.1 or 5.2 leached any toxic compounds following UV curing. The method was also extended to allow for the UV curing of IIR-PEO graft copolymers and it was demonstrated that this process allowed the properties of these graft copolymers to be maintained, while obtaining a cross-linked film. Thus, this approach appears to be a highly versatile and non-toxic approach for generating cross-linked films of IIR.

5.4 Experimental Section

Materials and General Procedures:

IIR containing 2 mol% IP (M_w = 388 kg/mol, PDI = 3.4) and 7 mol% IP (M_w = 423 kg/mol, PDI = 2.9) were provided by LANXESS Inc. Polymers 1.27, 3.5, and 1.28 were prepared as previously reported.\(^ {17,19}\) Cinnamoyl chloride (Sigma-Aldrich, 96%), reagent grade K_2CO_3, MgSO_4 anhydrous were purchased from Caledon and used as received. Reagent grade pyridine (99%, Caledon) was refluxed over CaH_2 for 2 hours and distilled prior using. \(^ 1\)H NMR spectra were obtained at 600 MHz on a Varian INOVA 600 Spectrometer. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of CDCl_3 (δ 7.26) and coupling constants (J) are reported in ppm. Infrared (IR) spectra were obtained using a Bruker Tensor 27 instrument as films from toluene on
KBr plates. The UV-visible absorption measurements were performed on a Varian Cary 300 Bio UV-visible spectrophotometer. SEC was performed in THF with a flow rate of 1 mL/min at 30 °C using a Viscotek GPCmax VE2001 GPC solvent/sample module equipped with a Viscotek VE 3580 differential refractive index detector and two PolyPore (300 mm × 7.5 mm) columns from Agilent. MW data is given relative to polystyrene standards. DSC measurements were acquired under a nitrogen atmosphere with a DSC Q20 calorimeter from TA instruments at heating rate of 10 °C/min from -100 °C to 125 °C, and the reported T_g were generated from the second of two heating cycles.

**Synthesis of Polymer 5.1**

The hydroxyl functionalized polymer 1.28 (1.0 g, 0.39 mmol of hydroxyl) was dissolved in 30 mL of dry toluene and 2 mL pyridine was added, followed by cinnamoyl chloride (0.33 g, 2.0 mmol, 5.0 equiv. per hydroxyl). The reaction was shielded from light with aluminum foil and stirred overnight at room temperature. The resulted polymer was precipitated three times in acetone, then dried under vacuum. Yield: 92%. ^1^H NMR (600 MHz, CDCl₃, δ, ppm): 7.71 (d, J = 15.8, 1H), 7.53-7.38 (m, 5H), 6.48 (d, J = 15.8, 1H), 5.32 (br s, 1H), 5.20 (s, 1H), 4.93 (s, 1H), 1.42 (s, CH₂ PIB, 26H), 1.11 (s, CH₃ PIB, 78H). IR (thin film, cm⁻¹): 2951 (-CH₃ stretch), 2918 (-CH₂- stretch), 1718 (-C=O stretch), 1639 (-C=C-C=O stretch), SEC: M_w = 355 kg/mol, PDI = 2.5; T_g = -63 °C.

**Synthesis of Polymer 5.2**

The epoxidized polymer 3.5 (1.0 g, 1.2 mmol of epoxide) was dissolved in 30 mL of toluene and 1-2 drops of 10 M HCl was added into the solution. After 3 hours, 0.25 g of K₂CO₃ was ground into a fine powder and added to the solution to neutralize excess HCl. The solution turned clear, then 0.25 g of anhydrous MgSO₄ anhydrous was added to further remove water. The mixture was stirred for 1 hr and then centrifuged to remove all insoluble solids. The supernatant was transferred into a dry flask and 2 mL of dry pyridine was added to the solution followed by cinnamoyl chloride (1.0 g, 6.25 mmol, 5.2 equiv.). The reaction was shielded from light with aluminum foil and stirred overnight at room temperature. The resulting polymer was precipitated three times in acetone and then
dried under vacuum. Yield: 90%. $^1$H NMR (600 MHz, CDCl$_3$, δ, ppm): 7.71 (d, J = 15.8, 1H), 7.53-7.38 (m, 5H), 6.48 (d, J = 15.8, 1H), 5.32 (br s, 1H), 5.20 (s, 1H), 4.93 (s, 1H), 1.42 (s, CH$_2$ PIB, 26H), 1.11 (s, CH$_3$ PIB, 78H). IR (thin film, cm$^{-1}$): 2951 (-CH$_3$ stretch), 2918 (-CH$_2$- stretch), 1718 (-C=O stretch), 1639 (-C=C-C=O stretch), SEC: $M_w$ = 428 kg/mol, PDI = 3.4; $T_g$ = -52 ºC.

**Synthesis of Polymer 5.3**

Starting from polymer 3.5 (0.92 g, 1.1 mmol of epoxide), the procedure described above for the preparation of polymer 5.2 was followed except that cinnamoyl chloride was added in increments of 0.1 g and the reaction conversion was monitored by $^1$H NMR (2 additions required). Upon reaching 40% conversion, 4-NPC (0.48 g, 2.4 mmol, ~ 2 equiv. per hydroxyl group) was added and the reaction mixture was stirred for 2 hours at room temperature. Pyridine salts were then removed by filtration and the resulting solution was precipitated three times in acetone to afford the polymer 5.3. Yield: 87%. $^1$H NMR (600 MHz, CDCl$_3$, δ, ppm): 8.28 (d, J = 8.8, 1.5H), 7.70 (d, J = 15.8, 0.4H), 7.53-7.38 (m, 0.9H), 7.40 (d, J = 8.2, 3H), 6.48 (d, J = 15.8, 0.4H), 5.32 (br s, 0.4H), 5.28 (s, 0.6H), 5.21 (s, 0.4H), 5.13 (br s), 5.03 (s, 0.6H), 4.94 (s, 0.4H), 1.42 (s, CH$_2$ PIB, 26H), 1.11 (s, CH$_3$ PIB, 78H). IR (thin film, cm$^{-1}$): 2951 (-CH$_3$ stretch), 2918 (-CH$_2$- stretch), 1770 (-C=O stretch), 1639 (-C=C-C=O stretch), 1531 (-NO$_2$ asymmetric stretch), 1391(-NO$_2$ symmetric stretch), SEC: $M_w$ = 474 kg/mol, PDI = 3.2; $T_g$ = -54 ºC.

**Synthesis of Polymer 5.4**

PEO-NH$_2$ with a MW of 5000 g/mol (0.40 g, 80 µmol, 0.29 equiv. relative to activated carbonates) was dissolved in 10 mL of dry toluene. Polymer 5.3 (0.40 g, 0.28 mmol of activated carbonates) was dissolved in 30 mL dry toluene and was then added to the solution of PEO-NH$_2$. The resulting mixture was stirred overnight. The solvent was removed in vacuo and the resulting rubbery material was washed with methanol couple of times until the polymer film turned white. The resulting product was then purified by precipitation from dichloromethane into methanol 3 times. Yield: 75%. PEO content (from $^1$H NMR): 40 wt%; $^1$H NMR (600 MHz, CDCl$_3$, δ, ppm): 8.28 (d, J = 8.8, 0.8H),
7.71 (d, $J = 15.8, 0.4H$), 7.53-7.38 (m, 1H), 7.40 (br s, 2.4H), 6.48 (d, $J = 15.8, 0.4H$), 5.35-4.80 (m, 3H), 3.65 (s, 44H), 3.39 (s, 3H), 1.42 (s, CH$_2$ PIB, 26H), 1.11 (s, CH$_3$ PIB, 78H). IR (thin film, cm$^{-1}$): 3360 (-NH stretch), 2951 (-CH$_3$ stretch), 2918 (-CH$_2$- stretch), 1770 (-C=O stretch), 1718 (-C=O stretch), 1639 (-C=C=C=O stretch), 1531 (-NO$_2$ asymmetric stretch), 1391 (-NO$_2$ symmetric stretch), 1234 (-C-O stretch). As in previous reports, it was not possible to perform SEC on this IIR-PEO graft copolymer.$^{18}$ $T_g = -53 ^\circ C$, $T_m = 39 ^\circ C$.

**Preparation of Polymer Films**

Polymer films were prepared by three different methods. Films with thicknesses in the nanometer range were prepared by spin coating onto quartz plates ($3 \times 1 \times 1/16$ inch) at a spin rate of 5000 rpm for 20 s. The film thickness was controlled by changing concentration of the polymer solution (5 mg/mL, 10 mg/mL, 25 mg/L and 50 mg/mL; for polymer 5.2, 50 mg/mL was not used due to high viscosity). Films with thicknesses in the micrometer range were cast onto quartz plates using Meyer rods. The film thicknesses were varied by changing the polymer concentration (25 mg/mL or 50 mg/mL) and the wire size (#20, #30 and #90). The films were dried under vacuum before subjecting them to UV-visible absorption measurements of the films directly on the quartz plate. To generate the calibration curve (see appendix), the thicknesses of selected films were measured using a KLA Tensor P-10 profiler equipped with a tungsten stylus with a diamond on the tip. A scratch was made in the film. The applied force was 3 mg and scan speed was 200 µm/s. Thick films (0.1 mm, measured using a caliper) were prepared by melt pressing 0.1 g of polymer between two Teflon sheets at 95 °C and a pressure of ~ 35 MPa.

**UV Curing**

The UV irradiation was carried out at 25 °C at a distance of 20 cm from a high-pressure mercury lamp (450W, ACE Glass incorporated 7830-60) kept in a water-cooled quartz trap. The films were kept in a quartz beaker that was purged with nitrogen prior to sealing with parafilm. For films on quartz plates, a black tape was attached to the back of each
quartz plates to prevent diffracted UV-light from initializing cross-linking from behind the film.

**Gel Content and Swelling Tests**

The melt pressed films (~ 0.1 mm) were punched into circles of 5 mm diameter, having weights of approximately 20 mg. They were then irradiated with UV light as described above, for different time periods. A portion of each film was subjected to thermal analysis. The remainder was weighed, then immersed in a vial containing 15 mL of toluene and the toluene was replaced with fresh toluene every 8-10 h. After 48 h, the films were removed from toluene and weighed. They were then dried *in vacuo* to constant weight. The gel content was calculated as the (extracted dry weight/initial dry weight) × 100%. The volume swelling ratio was calculated according to equation 4.

**Toxicity Assay**

Preparation of leachate: Test samples were melt-pressed to a thickness of 0.4 mm as described above. The melt pressed film was then cut into squares of 1 cm × 1 cm. Samples were sterilized by washing with 70% ethanol and subsequently dried for 2 h under UV light. They were placed in Petri dishes and incubated in 2 mL of Dulbecco’s Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 1% Glutamax (100×) solution and 1% Penstrep (100×) in an incubator at 37 °C for 24 h. The leachate was then removed and passed through a 0.2 µm filter.

MTT Assay: C2C12 mouse myoblast cells were seeded in a Nunclon® 96-well U bottom transparent polystrol plate to obtain 10,000 cells/well in 100 µL of DMEM containing serum, glutamx and antibiotics as described above. The cells were allowed to adhere in a 5% CO₂ incubator at 37 °C for 24 hours. Next, the growth medium was aspirated and was replaced with either the positive control – sodium lauryl sulfate (SDS) in the cell culture medium at concentrations of 0.2, 0.15, 0.10, or 0.05 mg/mL, serial two-fold dilutions of the leachate, or just the medium as a negative control. The cells were then incubated at 37 °C (5% CO₂) for 24 h. The medium was then aspirated and replaced with 110 µL of
fresh medium containing 0.5 mg/mL MTT reagent. After 4 h of incubation (37 °C, 5% CO₂), the MTT solution was carefully aspirated and the purple crystals were dissolved by addition of 50 µL of spectroscopic grade dimethylsulfoxide (DMSO). After shaking (1 s, 2 mm amp, 654 rpm), the absorbance of the wells at 540 nm was read using an M1000-Pro plate reader (Tecan). The absorbance of wells not containing cells but treated by all of the above steps was subtracted as a background and the cell viability was calculated relative to wells containing cells that were exposed to just culture medium. No (0%) cell viability was detected for the cells exposed to the highest concentrations of the positive control sodium lauryl sulfate, confirming the sensitivity of the assay.

**Evaluation of Cell Growth on Films**

C2C12 cells were maintained at 37 °C and 5% CO₂ in Dulbecco’s Modified Eagle Medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and supplemented with 1% Glutamax (100×) solution and 1% Penstrep (100×). Microscope glass cover slips (circular, 25 mm diameter) were coated by drop casting a 35 mg/mL solution of polymer in toluene (3 coats of 100 µL). For polymer 5.4, an additional coating of IIR was used below 5.4 as a primer layer on the glass coverslip (35 mg/ml, 100 µL drop cast, one coat) to avoid delamination of the rubber from the glass surface during the incubation period. The films were cross-linked by 60 min of UV irradiation (see above). The experiments were done in triplicate for each polymer. The surfaces were sterilized by submersion in 70% ethanol, and were then left to dry. The sterilized samples were placed in the wells of a 6-well plate and 5 × 10⁵ cells in 2 mL of cell culture medium were seeded onto each surface. The samples were incubated for 48 h, and then fixed with 4% paraformaldehyde solution for 10 min. The samples were washed three times with PBS (Invitrogen) at pH 7.2, and then treated with 2 mL of acetone at -20 °C for 5 minutes to permeabilize the membrane. After that, they were washed again with PBS, stained with Alexa Fluor 568 phalloidin (Invitrogen) and DAPI (Invitrogen) following the manufacturer’s directions. The samples were washed again with PBS and placed face down onto glass microscope slides with ProLong® Gold Antifade Reagent (Invitrogen) and sealed. Confocal images were obtained using a confocal laser scanning microscope (LSM 510 Duo Vario, Carl Zeiss) using a 20× objective and excitation wavelengths of
405 (DAPI) and 578 nm (phalloidin) for randomly selected regions of the surfaces. Cells were counted on 3 different images from each of the 3 different surfaces for each polymer (9 images per polymer). Statistical analyses (ANOVA followed by Tukey’s test) were performed using the software Prism.
5.5 References


5. Puskas, J. E.; Dos Santos, L. M.; Orlowski, E. *Rubber Chemistry and Technology* 2010.


Chapter 6
Conclusions and Future Directions

This thesis described the development of different approaches for the modification of IIR in order to provide new and enhanced properties. For example, properties such as high antibacterial activity, resistance to protein adsorption, enhanced mechanical properties and dispersion in aqueous solution were achieved through this work. This has significance for the expansion of IIR into new application areas. In particular, it was demonstrated that IIR can likely be more broadly applied in the expanding field of biomaterials.

In chapter 2, I demonstrated that antibacterial IIR surfaces could be developed without any modification of the IIR backbone. In this study, PDMAEMA was spin coated onto alkane-modified silicon wafers and IIR surfaces, then covalently cross-linked to the surfaces by HHIC. The PDMAEMA was then quarternized to provide antimicrobial activity. The surfaces were extensively characterized at each step in order to monitor changes in surface chemistry. All of the results indicated the effectiveness of using HHIC to cross-link the polymer to the surface without modifying the functionalities on the polymer as well as confirming the successful quarternization of the surfaces. The resulting IIR surfaces exhibited high antibacterial activity against both Gram-positive and Gram-negative bacteria.

The development of lin-PIB-g-PEO graft copolymers with high IP content, through a clean and mild modification of IIR was the main focus of Chapter 3. Graft copolymers containing up to 83 wt% PEO content were synthesized. At higher PEO content, the surfaces resisted the adsorption of proteins and the growth of cells, a property that is desirable for numerous applications. While the surfaces resisted dissolution into water even at the highest PEO content, it was possible to prepare stable aqueous assemblies of the materials using a solvent exchange method. The particle size was controlled by the kinetics of the solvent exchange process. The assemblies were found to be nontoxic and were capable of encapsulating hydrophobic probes. All of these properties make these graft copolymers promising candidates for variety of applications.
As the synthetic approach overcame the issues reported previously in terms of preparing \textit{lin}-PIB-g-PEO graft copolymers, it was also of interest to apply the methodology for the modification of \textit{arb}-PIB-PIP. The preparation of a small library of \textit{arb}-PIB-g-PEO copolymers was reported in Chapter 4 along with their chemical, physical and mechanical properties. Based on these results, it was apparent that differences in molecular architecture can lead to different properties. For example, both arborescent and linear graft copolymers revealed similar trend in properties such as resistance to protein adsorption and enhanced mechanical properties, however the self-assembly behavior for the \textit{arb}-PIB-g-PEO copolymers on surfaces and in solution was different.

In Chapter 5, a UV-curable IIR derivative was successfully synthesized and cross-linked by controlled exposure to UV light, in the absence of additional chemical additives. The synthetic approach was based on the reaction of hydroxyl-functionalized IIR with cinnamoyl chloride to generate a cinnamate functionalized IIR. Depending on the IP content of the starting IIR the cinnamate content was varied. The kinetics of the cross-linking was studied by UV-visible spectroscopy and it was found to vary according to the film thickness. The changes in gel content and volume swelling ratio with irradiation time were dependent on the cinnamate content. Toxicity studies suggested that the cross-linked materials do not leach toxic molecules. The approach was also applied to obtain cross-linked films of IIR-PEO graft copolymers, leading to surfaces that resisted the adhesion and growth of cells. Thus, the approach is versatile and is of particular interest when non-leaching coatings of cross-linked IIR are desired for biomedical or other applications.

Future investigations in this area of study can involve the continued development of PIB-based graft copolymers taking advantage of the clean and mild conditions employed herein. For example, other hydrophilic polymers can be used. By tuning the hydrophobic and hydrophilic character of the resulting graft copolymers, different properties can be obtained. So far, our studies were extended to high IP IIR and an \textit{arb}-PIB-PIP copolymer. However, other PIB architectures can be employed and compared
with the ones reported in this thesis. It would be desirable to synthesize alternative UV-curable PIB derivatives using other UV active moieties.
Appendices

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Appendix 2:

- $^1$H NMR spectra for PDMAEMA.
- Size exclusion chromatogram of PDMAEMA.
- AFM image of ODTS/silicon wafer.
- AFM image of PDMAEMA/ODTS/silicon wafer.
- AFM image of PDMAEMA/ODTS/silicon wafer cross-linked for 30 s.
- AFM image of PDMAEMA/ODTS/silicon wafer cross-linked for 180 s.
- AFM image of uncross-linked PDMAEMA/ODTS/silicon wafer following immersion and sonication in CH$_2$Cl$_2$/NEt$_3$.
- High resolution C 1s XPS spectrum of ODTS/silicon wafer.
- High resolution C 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)-washed.
- High resolution C 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)-washed.
- High resolution C 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)-washed.
- High resolution C 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (30 s).
- High resolution C 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (180 s).
- High resolution C 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (180 s).
- High resolution N 1s XPS spectrum of PDMAEMA/ODTS/silicon wafer-washed.
- High resolution N 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (30 s).
- High resolution N 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (30 s).
- Representative high resolution Br 3d XPS spectrum of an unquaternized surface.
- High resolution Br 3d XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer.
- Infrared spectrum of PDMAEMA.
- Infrared spectrum of quaternized PDMAEMA.
Figure A2.1. $^1$H NMR spectrum of PDMAEMA (CDCl$_3$, 400 MHz).

Figure A2.2. Size exclusion chromatogram of PDMAEMA (differential refractive index detection).
Figure A2.3. AFM image of ODT/silicon wafer.

Figure A2.4. AFM image of PDMAEMA/ODT/silicon wafer. A film thickness of ~20 nm was measured as the average height difference between the film (top right) and the scratch (lower left).
Figure A2.5. AFM image of PDMAEMA/ODTS/silicon wafer cross-linked for 30 s. A film thickness of ~ 20 nm was measured as the average height difference between the film (left) and the scratch (right).

Figure A2.6. AFM image of PDMAEMA/ODTS/silicon wafer cross-linked for 180 s. A film thickness of ~ 20 nm was measured as the average height difference between the film (top left) and the scratch (bottom right).
Figure A2.7. AFM image of un-cross-linked PDMAEMA/ODTS/silicon wafer following immersion and sonication in CH$_2$Cl$_2$/NEt$_3$, showing that the film was removed.

Figure A2.8. High resolution C 1s XPS spectrum of ODTS/silicon wafer.
**Figure A2.9.** High resolution C 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (30 s)-washed.

**Figure A2.10.** High resolution C 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (180 s)-washed.
Figure A2.11. High resolution C 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (30 s).

Figure A2.12. High resolution C 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (180 s).
Figure A2.13. High resolution C 1s XPS spectrum of PDMAEMA/ODTS/silicon wafer - washed.

Figure A2.14. High resolution N 1s XPS spectrum of HHIC treated PDMAEMA/ODTS/silicon wafer (30 s).
Figure A2.15. High resolution N 1s XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer (30 s).

Figure A2.16. Representative high resolution Br 3d XPS spectrum of an unquaternized surface.
Figure A2.17. High resolution Br 3d XPS spectrum of quaternized PDMAEMA/ODTS/silicon wafer.

Figure A2.18. Infrared spectrum of PDMAEMA.
Figure A2.19. Infrared spectrum of quaternized PDMAEMA.
Appendix 3:

- $^1$H NMR spectra of copolymers 3.8, 3.9, and 3.10.
- DSC traces of copolymers 3.8, 3.9, and 3.10.
- Debye plots for copolymers 3.8, 3.9, and 3.10.
- Release of Rhodamine B from a film prepared from copolymer 3.2 under sink conditions.
- Representative DLS traces for aqueous assemblies.
- Additional DLS data for concentration dependence and stability of assemblies.
Figure A3.1. $^1$H NMR spectrum of copolymer 3.8 (CDCl$_3$, 400 MHz).

Figure A3.2. $^1$H NMR spectrum of copolymer 3.9 (CDCl$_3$, 400 MHz).
Figure A3.3. $^1$H NMR spectrum of copolymer 3.10 (CDCl$_3$, 600 MHz).

Figure A3.4. DSC trace for copolymer 3.8.
Figure A3.5. DSC trace for copolymer 3.9.

Figure A3.6. DSC trace for copolymer 3.10.
Figure A3.7. Debye plot for copolymer 3.8.

Figure A3.8. Debye plot for copolymer 3.9.
**Figure A3.9.** Debye plot for copolymer 3.10.

**Figure A3.10.** Release of rhodamine B from films prepared from copolymers 3.2 as a function of time upon incubation of films in pH 7.4, 100 mM phosphate buffer at 25 °C. Error bars represent the standard deviations on measurements from 3 different surfaces. The experiment was performed using the same procedure as for Figure 3.3, except that the incubation buffer was replaced with fresh buffer at each time point and the cumulative release was measured.
Figure A3.11. Representative size distributions obtained by dynamic light scattering for copolymers a) 3.1, b) 3.2, c) 3.3, d) 3.8, e) 3.9, f) 3.10 after dialysis (against water) of the assemblies from THF/water 30/70.
Figure A3.12. Representative size distributions obtained by dynamic light scattering for copolymers a) 3.1, b) 3.2, c) 3.3, d) 3.8, e) 3.9, f) 3.10 after dialysis (against water) of the assemblies from 100% THF.

Figure A3.13. Comparison of the Z-average diameters obtained from copolymer solutions of different concentrations. The concentration corresponds to that of the initial THF solution of the copolymer 3.1. Error bars represent the standard deviations on measurements from 3 separately prepared samples of the assemblies.
Figure A3.14. Z-average diameters for aqueous assemblies at t = 0 and t = 6 months storage at room temperature, showing the stabilities of the assemblies over time: a) copolymer 3.1 and b) copolymer 3.2.
Appendix 4:

- $^1$H NMR spectra of intermediate functionalized derivatives of 4.1c.
- IR spectra of intermediate functionalized derivatives of 4.1c.
- $^1$H NMR spectra of copolymers 4.3-4.8.
- DSC traces of copolymers 4.3-4.8.
- AFM topography images of copolymers 4.1b, 4.3, 4.4 and 4.6.
- AFM topography images of IIR and lin-PIB-g-PEO copolymers.
- Representative size distributions obtained by dynamic light scattering.
Figure A4.1. $^1$H NMR spectra (CDCl$_3$, 600 MHz) of a) copolymer 4.1c, b) epoxidized 4.1c, c) hydroxyl functionalized 4.1c, and d) 4-NPC 4.1c.
Figure A4.2. IR spectra of: a) copolymer 4.1c, b) epoxidized 4.1c, c) hydroxyl functionalized 4.1c, d) 4-NPC 4.1c.
Figure A4.3. $^1$H NMR spectrum of copolymer 4.3 (CDCl$_3$, 600 MHz).

Figure A4.4. $^1$H NMR spectrum of copolymer 4.4 (CDCl$_3$, 600 MHz).
Figure A4.5. $^1$H NMR spectrum of copolymer 4.5 (CDCl$_3$, 600 MHz).

Figure A4.6. $^1$H NMR spectrum of copolymer 4.6 (CDCl$_3$, 600 MHz).
Figure A4.7. $^1$H NMR spectrum of copolymer 4.7 (CDCl$_3$, 600 MHz).

Figure A4.8. $^1$H NMR spectrum of copolymer 4.8 (CDCl$_3$, 600 MHz).
Figure A4.9. DSC trace for impure copolymer 4.6 showing the presence of a second $T_m$ corresponding to excess ungrafted PEO.

Figure A4.10. DSC trace for copolymer 4.3.
Figure A4.11. DSC trace for copolymer 4.4.

Figure A4.12. DSC trace for copolymer 4.5.
Figure A4.13. DSC trace for copolymer 4.6.

Figure A4.14. DSC trace for copolymer 4.7.
Figure A4.15. DSC trace for copolymer 4.8.

Figure A4.16. AFM topography images of: a) copolymer 4.1b, b) copolymer 4.3, c) copolymer 4.4, and d) copolymer 4.6.
Figure A4.17. AFM topography images of: a) IIR (2 mol% IP), b) lin-PIB-g-PEO with 18 wt% PEO, c) lin-PIB-g-PEO with 34 wt% PEO, d) lin-PIB-g-PEO with 65 wt% PEO.

Figure A4.18. Representative size distributions obtained by dynamic light scattering for copolymers a) 4.3, b) 4.4, c) 4.6, and d) 4.8 after dialysis (against water) of the assemblies from 100% THF.
Figure A4.19. Representative size distributions obtained by dynamic light scattering for copolymers a) 4.3, b) 4.4, c) 4.6, and d) 4.8 after dialysis (against water) of the assemblies from 70% THF.

Figure A4.20. Representative size distributions obtained by dynamic light scattering for copolymers a) 4.3, b) 4.4, c) 4.6, and d) 4.8 after dialysis (against water) of the assemblies from 50% THF.
Figure A4.21. Representative size distributions obtained by dynamic light scattering for copolymers a) 4.3; b) 4.4; c) 4.6; d) 4.8; after dialysis (against water) of the assemblies from 30% THF.

Figure A4.22. TEM images of assemblies formed from a) copolymer 4.3, b) copolymer 4.4, c) copolymer 4.6. (Solvent was 100% THF prior to dialysis in all cases; scale bars correspond to 500 nm)
Appendix 5:

- $^1$H NMR spectra of polymers 5.1, 5.2, and 5.3.
- Calibration curves for optical density vs film thickness.
- IR spectra of films before and after UV curing.
- DSC traces for copolymer 5.3.
- UV-visible spectra of copolymer 5.3 at different irradiation times.
**Figure A5.1.** $^1$H NMR spectrum of polymer 5.1 (CDCl$_3$, 600 MHz) in the downfield region from 2.5-8.5 ppm showing clean conversion to the cinnamate functionalized polymer.

**Figure A5.2.** $^1$H NMR spectrum of polymer 5.3 (CDCl$_3$, 600 MHz) in the downfield region from 4.5-8.5 ppm showing partial conversion of the hydroxyl functionalized polymer to about 40:60 cinnamate:4-nitrophenyl carbonate.
Figure A5.3. $^1$H NMR spectrum of polymer 5.4 (CDCl$_3$, 600 MHz) showing grafting of PEO.

Figure A5.4. Optical density (274 nm) versus film thickness (as measured by profilometry) for thin films of 5.1 (●) and 5.2 (□). Films were prepared by spin coating.
Figure A5.5. Optical density versus film thickness (as measured by profilometry) for films of 5.1 (●) and 5.2 (□). Note that the O.D was measured at 305 nm instead of 274 nm in order to stay within the detector range for the thickest films. Films were prepared using Meyer rods.

Figure A5.6. Infrared spectrum of polymer 5.1 before (black) and after (red) curing, showing a reduction in the peak at 1640 cm\(^{-1}\) corresponding to the C=C of the cinnamate moieties and a reduction and broadening of the peak at 1720 cm\(^{-1}\) (suggestive of a new peak at 1730 cm\(^{-1}\)) corresponding to the C=O of the cinnamate moieties.
Figure A5.7. Infrared spectrum of polymer 5.2 before (black) and after (red) curing, showing a reduction in the peak at 1640 cm$^{-1}$ corresponding to the C=C of the cinnamate moieties and a reduction and broadening of the peak at 1720 cm$^{-1}$ (suggestive of a new peak at 1730 cm$^{-1}$) corresponding to the C=O of the cinnamate moieties.

Figure A5.8. Differential scanning calorimetry trace for polymer 5.4 (second heating cycle). The presence of a single melting transition at a temperature different than that of pure PEO with a molecular weight of 5000 g/mol (59 °C) confirms the absence of free PEO in the product.
Figure A5.9. UV spectra of copolymer 5.4 at different irradiation times. The film was prepared by using a Meyer rod #20 and a polymer concentration of 50 mg/mL. Using profilometry the film thickness was measured to be 1 µm.
Curriculum Vitae

EDUCATION

• 09/2008 - 09/2013 The University of Western Ontario, Canada, Ph.D. in Organic Chemistry
  Research Supervisor: Dr. E. R. Gillies

• 09/2003- 09/2006, University of Tehran, Iran, M.Sc. in Organic Chemistry
  Thesis title: Polymer Nanocomposite Synthesis via Sol-Gel Chemistry
  Research Supervisor: Dr. S. Khoee

• 09/1998-09/2002 University of Tehran, Iran, B. Sc. in Applied Chemistry

Scholarships and ACADEMIC HONOURS

• Western Research Graduate Scholarship valued $38,311, 2008-present
• CSC 2004 Travel award valued $360, 2012
• M.Sc. thesis award, Iranian Nanotechnology Initiative valued $1,200, 2006

PUBLICATIONS

Patent Application


Book Chapter


Referral Journal Publications


**CONFERENCE PRESENTATIONS**


TEACHING EXPERIENCE

• Teaching Assistant, The University of Western Ontario
  09/2008 – present
  Laboratory instructor and help room assistant for Chemistry 2213a and Chemistry 2223b

• Undergraduate Student Mentor, The University of Western Ontario
  09/2010 - 04/2011
  CBE 4415 student Jesse Lam

COMMUNITY/OUTREACH ACTIVITIES

• Served as a safety committee member of the department of chemistry, Western University, 2013-present.
• Volunteer member of “Let’s talk science” Since 2012-present.
• Participated as a judge in the SCI-TECH ENCOUNTERS, Regional Science and Inventor’s Fair, 2011-2013.
• Participated as a volunteer member in coordinating “on-site” registration, providing technical support to presenters at the “Western Conference on Science Education” (WCSE), Western University, 2011.
• Served as a member of the scholarships committee of the “Graduate Teaching Assistant Union”, April-May 2011 and April-May 2012.