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SURFACE AND BULK MODIFICATION OF POLY(LACTIC ACID)

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SURFACE AND BULK MODIFICATION OF POLY(LACTIC ACID)

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Chemical Engineering

by
Rahul M. Rasal
May 2009

Accepted by:
Dr. Douglas Hirt, Committee Chair
Dr. Anthony Guiseppi-Elie
Dr. David Bruce
Dr. Ken Webb
The major drawbacks of PLA are its poor toughness and lack of readily reactable groups. Unfortunately, typical methods of PLA toughening are associated with significant modulus and/or ultimate tensile strength (UTS) loss. The main objective of this research was to toughen PLA, with minimal modulus and/or UTS loss, and introduce reactive groups into the PLA matrix in one step. Initially, this objective was divided into two separate parts: PLA surface modification followed by toughening.

PLA film was solvent cast from chloroform solution and was surface modified using a sequential two-step photografting approach. Benzophenone was photografted onto the film surface in Step 1 followed by photopolymerization of hydrophilic monomers, acrylic acid and acrylamide, from the film surface. The resultant films were characterized using ATR-FTIR spectroscopy, water contact angle goniometry, transmission FTIR microspectroscopy, and tensile testing. The effect of the reaction solvent (ethanol and water) in Step 2 on PLA film surface and bulk properties was also studied. There was significant penetration of monomers into the films when ethanol was used as the reaction solvent, resulting in significant toughness loss. This monomer penetration into the films was successfully reduced by using water instead of ethanol as the reaction solvent in Step 2 and resultant films showed higher toughness than films surface-modified using ethanol as the reaction solvent in Step 2. It was also observed that solvent cast PLA film retained approximately 13 wt% chloroform, as characterized using thermogravimetric analyses (TGA). The presence of residual chloroform in the film
specimens is undesirable from a biocompatibility standpoint. Therefore, further work was conducted on melt-processed films where residual solvent from the film-formation method would not be an issue.

Addition of a small amount of poly[(3-hydroxybutyrate)-co-(3-hydroxyhexanoate)] (PHBHHx) to PLA improved the toughness of the resultant melt-processed blend from 4 ± 2 MPa for neat PLA to 175 ± 35 MPa for PLA-PHBHHx blend (90 wt% PLA). PLA-PHBHHx blend films were melt-processed using a single screw extruder. These polyblend films appeared to be non-compatible as characterized using dynamic mechanical analyses (DMA). PLA-PHBHHx blend films underwent rapid physical aging losing their toughness from 175 ± 35 MPa (right after extrusion) to 68 ± 34 MPa (day 3). The blend films were surface modified using the sequential two-step photografting protocol using water as the reaction solvent in Step 2. PLA-PHBHHx blend films lost approximately 95% of their toughness on surface modification due to UV-assisted solvent induced crystallization as characterized using wide angle X-ray diffraction (WAXD) analyses.

A novel reactive blending approach was developed to toughen PLA with minimal modulus and UTS loss and introduce reactive groups into the PLA matrix. PLA was reactive blended with a stiffening polymer, poly(acrylic acid) (PAA), followed by physical blending with a toughening polymer, poly(ethylene glycol) (PEG), in solution. The modified PLA was extruded into films using a co-rotating twin-screw extruder and characterized using tensile testing, differential scanning calorimetry (DSC), DMA, and toluidine-blue-surface-staining. This material exhibited, for the first time, approximately
10 fold increase in PLA’s toughness without significant modulus and/or UTS loss and also introduced a controlled concentration of surface modifiable reactive acid groups into the PLA matrix in one step.
DEDICATION

I dedicate this work to my beloved Guru, H H Swami Shivkrupanandji, parents, Mrs. and Mr. Maruti Rasal, and sister, Mrs. Shambhavi Kadam. Without their unconditional support, love, and motivation, it would not have been possible.
ACKNOWLEDGMENTS

I am grateful to my parents and sister for their motivation and encouragement. Thank you very much for being there whenever I needed you.

I am very thankful to Dr. Douglas Hirt for his precious guidance, flexibility, and support. A fantastic friend who has not only helped me through my Ph.D. dissertation research but also played a vital role in shaping-up my personality as a researcher. I am also thankful to my dissertation committee members (Dr. Guiseppi-Elie, Dr. Bruce, and Dr. Webb) for their time and energy. I would like to sincerely acknowledge Dr. Drews and Ms. Kim Ivey for their expert opinions on several analytical techniques.

I am thankful to the Center for Advanced Engineering Fibers and Films (CAEFF) for the financial support. This work was supported by the Engineering Research Centers Program of the National Science Foundation under NSF Award Number EEC-9731680.

Special thanks to my close friends at Clemson (Amol, Shamik, Sourabh, Carrie, Halil, and Ward) for making Clemson my second home. I express my sincere thanks to Nripen for being an excellent friend and making Clemson-life a fun experience. Past and present Hirt group graduate and undergraduate students: Amol, Keisha, Jeffery, Richard, Mary, Irl, and Courtney, thank you very much for your time on this research. All past and present office-mates: Amol, Keisha, Jeffery, Richard, Greg, Esteban, and Fiaz, thank you for making our office a fun-place to work. I must thank my friends in the Surabhi group, Dr. Ogale and Mrs. Ogale for their enthusiasm, support, and guidance.
Finally I would like to acknowledge all my friends who have not been mentioned here. Thank you very much!!!!
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CHAPTER ONE

INTRODUCTION

Environmental concerns and sustainability issues associated with petrochemical-based polymers have driven considerable engineering and scientific efforts devoted to the discovery, development, and modifications of biodegradable and renewably-derived polymers over the past several decades [1-2]. One such polymer is poly(lactic acid) or poly(lactide) (PLA), a thermoplastic polyester that is renewably-derived (from corn, starch, sugar, etc.), biodegradable, recyclable, and compostable [3]. PLA is biocompatible with non-toxic degradation products (at low concentrations), making it a natural choice for many biomedical applications [4]. Table 1.1 provides a chronological list of PLA in-vivo studies conducted over last four decades, demonstrating its satisfactory biocompatibility. The Food and Drug Administration (FDA) has also approved PLA for direct contacting with biological fluids [5]. In addition to this, PLA has excellent stiffness, comparable to that of poly(ethylene terephthalate) (PET) [6]. These attractive properties serve to make PLA a suitable substitute for many petrochemical-based polymers.
Table 1.1 PLA *in-vivo* biocompatibility testing (adapted, in part, from ref [17])

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<td>[15]</td>
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<tr>
<td>Fracture fixation of rabbit femur</td>
<td>Insignificant inflammatory response</td>
<td>[16]</td>
</tr>
</tbody>
</table>
Ankle fracture fixation in human: Found safe and effective, no complications [17]

Implants in the repair of goat osteochondral defects: No obvious histological abnormalities [18]

Fracture fixation of dog femur: No inflammatory reaction [19]
Fixation of osteochondral fractures of the femoral condyle: Complete bony healing without clinically relevant complications [20]
Bone defect coverage in sheep: Good biocompatibility [21]

However, the main drawback of PLA is its brittleness (poor toughness), which limits its use in many applications. Another drawback of PLA is lack of readily reactable side-chain groups. This makes PLA’s surface modification a challenging task. PLA needs to be surface modified in many applications such as friction modification, anti-fogging, adhesion, implantable biomaterials, and biopolymer-based drug delivery.

**PLA PRODUCTION AND APPLICATIONS**

Figure 1.1 illustrates the various reactions involved in PLA production. Carbohydrates (primarily sucrose and starch) derived from renewable resources are bacterially fermented to produce lactic acid. All the carbon, hydrogen, and oxygen atoms in carbohydrates and final PLA product have their origin in carbon dioxide and water, photosynthesis reactants.
Figure 1.1 Reaction schemes to produce PLA (reproduced with permission from ref. [22]).
There are two primary routes to produce PLA from lactic acid: direct condensation polymerization of lactic acid and ring opening polymerization through a lactide intermediate. The first approach involves the removal of water, the use of solvent under high vacuum and temperature, and can produce only low to intermediate molecular weight polymers. In addition to this, it requires relatively large reactors and leads to increased color and racemization. Because of these disadvantages, the ring opening polymerization has been more favored. In this approach, a low molecular weight prepolymer is first produced by removing water under mild conditions and without the use of a solvent. A cyclic intermediate dimer, lactide, is then produced by catalytically depolymerizing this prepolymer. The lactide monomer is further subjected to a solvent free ring opening polymerization to produce PLA [22].

Due to PLA’s bioresorbability and biocompatibility in the human body, it has been used for resorbable sutures and prosthetic devices [20]. PLA has been finding increasing consumer applications mainly due to its renewability, biodegradability, transparency, processibility, and mechanical properties. Although PLA has been shown to be a practically feasible packaging material, its higher cost has confined its use to limited packaging application only [6]. Dannon and McDonald’s (Germany) pioneered the use of PLA as a packaging material in yogurt cups and cutlery [6]. NatureWorks LLC polymers have been used for a range of packaging applications such as high-value films, rigid thermoformed containers, and coated papers [23]. Ingeo™, a PLA-based fiber, has been designed for apparel, furnishings, and nonwovens applications [24]. BASF’s Ecovio®, which is a derivative of petrochemical-based biodegradable Ecoflex® and
contains 45 wt% PLA, has been used to make carrier bags, compostable can liners, mulch film, and food wrapping. Commercially available PLA films and packages have been found to provide mechanical properties better than polystyrene (PS) and comparable to PET [6]. The extensive utilization of PLA in consumer and biomedical applications will be dictated mainly by cost reductions as well as fine control over PLA bulk and surface properties.

***PLA MATERIALS SCIENCE***

Lactide has three stereoisomers: L-lactide, D-lactide, and meso-lactide. The stereochemical composition of the PLA has a significant effect upon its melting point, crystallization rate, extent of crystallization, and mechanical properties [25]. Pure poly(D-lactide) or poly(L-lactide) have an equilibrium crystalline melting point of 207 °C [26, 27]. However, due to small and imperfect crystallites, slight racemization, and impurities, typical PLA melting points are 170-180 °C [28]. A 1:1 mixture of pure poly(L-lactide) and poly(D-lactide) exhibited a higher melting temperature (230 °C) and better mechanical properties than either pure polymer (the UTS for the 1:1 stereocomplex was 50 MPa while that for pure poly(L-lactide) was 31 MPa [28-30]). Although stereochemical composition had a significant effect on melting point, glass transition temperature was not as significantly affected (e.g., glass transition temperature of pure poly(L-lactide) was found to be 55-60 °C for M_v ~ 23-66 kDa and that of poly(D,L-lactide) was found to be 49-52 °C for M_v ~ 47.5-114 kDa) [31].
Rheological characteristics of PLA make it suitable for cast and blown film extrusion and fiber spinning. PLA has relatively poor melt strength and its melt viscosity is not very shear-sensitive. This has been overcome by employing branching by treatment of PLA with peroxide or by introduction of multifunctional initiators or monomers. Branched PLA displays high viscosity (melt-strength) at low shear rates, making it more suitable for applications such as extrusion coating, extrusion blow-molding, and foaming (Figure 1.2) [25]. Typical properties of NatureWorks PLA 2002D resin (designed for extrusion/thermoforming applications) are shown in Table 1.2. NatureWorks PLA grades differ in sterochemical composition, molecular weight, and additive packages [25].

![Complex Viscosity vs Frequency (rad/s)](image_url)

**Figure 1.2** Rheology of linear and branched NatureWorks PLA (reproduced with permission from ref. [25]).
PLA optical properties, more specifically transmission of UV and visible wavelengths, are very important in designing the right packaging material to protect and preserve products. Figure 1.3 shows the optical properties of PLA compared to standard packaging materials. PLA shows significant UV light transmission at 225 nm. At 250 nm, 85% of the UV light is transmitted, while at 300 nm, 95% of UV light is transmitted. Effective UV stabilizers are able to absorb UV and thus prevent damage to the UV sensitive packaged products [6].

Table 1.2 PLA 2002D properties [32]

<table>
<thead>
<tr>
<th>Physical/Mechanical Property</th>
<th>PLA 2002D</th>
<th>ASTM Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>1.24</td>
<td>D792</td>
</tr>
<tr>
<td>Melt Index (210 °C/2.16 kg)</td>
<td>5-7</td>
<td>D1238</td>
</tr>
<tr>
<td>Tensile Strength @ Break, psi (MPa)</td>
<td>7700 (53)</td>
<td>D882</td>
</tr>
<tr>
<td>Tensile Yield Strength, psi (MPa)</td>
<td>8700 (60)</td>
<td>D882</td>
</tr>
<tr>
<td>Tensile Modulus, kpsi (GPa)</td>
<td>500 (3.5)</td>
<td>D882</td>
</tr>
<tr>
<td>Tensile Elongation, %</td>
<td>6.0</td>
<td>D882</td>
</tr>
</tbody>
</table>
PLA dissolves in many common organic solvents such as acetone, benzene, chloroform, dichloromethane, dioxane, dimethylformamide, ethyl acetate, tetrahydrofuran, toluene, trichloromethane, and p-xylene. PLA does not dissolve in water, alcohols, and unsubstituted hydrocarbons [33]. Solubility parameters for polylactides from the literature are reported in Table 1.3.

**Figure 1.3** Percent transmission versus wavelength for PLA (98% L-lactide), PS, LDPE, PET and cellophane films. (PLA samples were obtained from Cargill Dow LLC) (reproduced with permission from ref. [6]).
Table 1.3 Solubility parameters for PLA [6]

<table>
<thead>
<tr>
<th>Determination Method</th>
<th>Solubility Parameter (cal⁰.⁵cm⁻¹.⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density in Solution</td>
<td>10.25 ± 0.16</td>
</tr>
<tr>
<td>Limiting Viscosity Number</td>
<td>10.00 ± 0.20</td>
</tr>
</tbody>
</table>

**Group Contribution Methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Solubility Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>9.7</td>
</tr>
<tr>
<td>Hoy</td>
<td>9.9</td>
</tr>
<tr>
<td>Van Krevelen</td>
<td>9.4</td>
</tr>
</tbody>
</table>

**PLA MODIFICATIONS**

In order to overcome main drawbacks associated with PLA, mainly brittleness and lack of readily reactable groups, PLA has been bulk and surface modified. Each of these modifications has been aimed at either modifying mechanical properties or surface properties.
Several bulk modification methods have been employed to improve mechanical properties (mainly toughness), degradation behavior, processibility, and crystallinity of PLA. With respect to structure-property relationships, crystallinity is an important characteristic that affects PLA degradation rate [34] and mechanical properties [31]. Kolstad [35] observed approximately 40% increase in the crystallization half time for every 1 wt% increase in the meso-lactide content in poly(L-co-meso-lactide). He also observed that the addition of 15 wt% or more meso-lactide rendered the resulting polymer significantly non-crystallizable. Perego et al. [31] studied the effect of molecular weight and crystallinity on the mechanical properties of PLA. Poly(L-lactide) (M_v ~ 23-66 kDa) and poly(D,L-lactide) (M_v ~ 47.5-114 kDa) exhibited small changes in the tensile strength at break, which varied from 55 to 59 MPa for poly(L-lactide) and from 40 to 44 MPa for poly(D,L-lactide) in the given molecular weight range. It was also observed that the tensional and flexural modulii of elasticity, Izod impact strength, and heat resistance (the measure of polymer’s resistance to distortion under a given load at elevated temperature) increased with crystallinity. Crystallinity not only affects the bulk properties but also the surface roughness. Washburn et al. [36] applied a linear temperature gradient to produce a crystallinity gradient across a PLA film and observed that MC3T3-E1 osteoblasts proliferated faster on the smoother regions than on the rougher regions. The critical rms roughness, above which a statistically significant reduction in proliferation rate occurred, was found to be approximately 1.1 nm.
Different processing methodologies have been applied to control orientation and, hence, bulk properties of polymers. These approaches influence the bulk properties without altering the PLA chemistry or introducing additives. Injection molded samples of amorphous PLA showed higher tensile strength at break and notched Izod impact strength upon drawing [37]. An injection molding process that applied an oscillating shear flow to orient the semi-solid melt improved the Charpy impact strength [37]. Bigg [38] observed a substantial increase in % elongation and tensile strength at break of PLA with different ratios of L-lactide to D,L-lactide upon biaxial orientation. For L-lactide to D,L-lactide copolymer ratio of 80/20, % elongation at break increased from 5.7 to 18.2% and tensile strength at break increased from 51.7 to 84.1 MPa upon biaxial orientation at 85 °C. The literature on stereochemical and processing manipulations of PLA indicates that these bulk modifications have not been very effective in toughening PLA.

PLA has been copolymerized with a range of polyesters and other monomers either through polycondensation of lactic acid with other monomers, producing low molecular weight copolymers, or ring opening copolymerization of lactide with cyclic monomers like glycolide, ε-caprolactone, δ-valerolactone, trimethylene carbonate, etc. as well as linear monomers like ethylene glycol [33] producing high molecular weight copolymers. Fukuzaki et al. [39] copolymerized L-lactic acid and ε-caprolactone without any catalyst to produce low molecular weight (M_w ~ 6.8-8.8 kDa) copolymers for biomedical applications. These copolymers showed excellent in-vitro (enzymatic) and in-vivo degradation properties. A key advantage that condensation copolymerization offers is control over polymer end groups. Lactic acid has been condensation copolymerized
with diols or diacids in such a way that the resulting copolymer has either hydroxyl or acid end groups and a particular molecular weight. Although polycondensation produces low molecular weight polymers ($M_w < 10$ kDa), this control over the end groups is a valuable tool in addition-type chemistry [40]. Ring opening copolymerization (ROC) of L-lactide is a common approach for PLA copolymer synthesis, initiated with hydroxyl groups, such as alcohol or polyol [41]. The ring opening lactide copolymerization route has been used extensively due to its precise chemistry control and resulting favorable copolymer properties [33]. The polymerization mechanism can be ionic, co-ordination, or free radical depending on the type of catalyst system involved [33, 42]. The transition metal compounds of tin [43, 44], aluminum [45], lead [46], zinc [47], bismuth [46], iron [48], and yttrium [49] have been reported to catalyze lactide ROC. Haynes et al. [50] copolymerized lactide with another commercially available biodegradable and renewably derived thermoplastic polyester, poly(hydroxyalkanoate) (PHA). The resulting copolymer was found to have a lower complex viscosity compared to neat PLA. Also, the storage and loss modulii of this copolymer underwent less change with frequency (0.1-100 radians/sec) compared to neat PLA. PLA has been copolymerized extensively with PEG due to PEG’s biocompatibility and hydrophilicity. An alternating copolymer of lactic acid and ethylene oxide produced from the ring opening of the cyclic ester monomer 3-methyl-1,4-dioxan-2-one has been used to plasticize PLA [51]. Diblock and triblock PLA-PEG copolymers were also synthesized to improve hydrophilicity and drug-delivery properties of PLA. However, PLA and PEG underwent phase separation leading to poor mechanical properties of the copolymers [52]. To improve the compatibility between
PLA and PEG components, PLA-PEG copolymers were produced by copolycondensation of PLA-diols and PEG-diacids using carbodiimide-based wet chemistry. The resultant copolymer did not phase separate and exhibited improved mechanical properties [53].

In addition to copolymerization, PLA has been extensively bulk modified using blending. Blending is probably the most widely used methodology to improve PLA mechanical properties. PLA has been blended with different plasticizers and polymers (biodegradable and non-biodegradable) to achieve desired mechanical properties. PLA is a glassy polymer that has poor elongation at break (< 10%) [54]. Different biodegradable as well as non-biodegradable plasticizers have been used to lower the glass transition temperature, increase ductility, and improve processibility [55]. Martin and Avérous [56] used glycerol, citrate ester, PEG, PEG monolaurate, and oligomeric lactic acid to plasticize PLA and found that oligomeric lactic acid and low molecular weight PEG (M_w ~ 400 Da) gave the best results while glycerol was found to be the least efficient plasticizer. Citrate esters (molecular weight 276-402 Da) derived from naturally occurring citric acid were found to be miscible with PLA at all compositions. For these blends with citrate esters, elongation at break was significantly improved accompanied with considerable loss of tensile yield strength [57]. Baiardo et al. [58] used acetyl tri-n-butyl citrate and PEGs with different molecular weights (M_w ~ 0.4-10 kDa) to plasticize PLA. Acetyl tri-n-butyl citrate miscibility limit was found to be 50 wt% while PEG miscibility decreased with increasing molecular weight. These researchers also observed a significant increase in elongation at break at the expense of strength and tensile modulus. Hillmyer et al. [59, 60] blended PLA with low density poly(ethylene) (LDPE)
to improve the toughness. Recently, DuPont has commercialized Biomax® Strong non
degradable PLA additives to improve toughness without significant transparency loss.
These additives are designed to have “special chemistry” for PLA, so even small amounts
(1-5 wt %) provide significant toughness benefits [61]. NatureWorks LLC studied
different commercial toughening agents for PLA [3]. In their work, Blendex™ 338, an
acrylonitrile-butadiene-styrene terpolymer containing 70% butadiene rubber, was found
to significantly improve notched Izod impact strength and elongation at break of PLA.
Another additive, Pellethane™ 2102-75A (a commercial grade polyurethane from Dow
Chemical Company), was also found to significantly improve these properties [3]. PLA
blends with biodegradable polymers have been extensively investigated because they
offer property improvements without compromising biodegradability. For example, PHA
is a bacterially produced family of biodegradable aliphatic homo or copolyesters with
more than 150 different types consisting of different monomers [62]. Addition of a small
amount (typically < 20 wt %) of Nodax copolymer to PLA remarkably improved the
toughness of the resultant blend without significantly affecting the optical clarity [63].
PLA/PCL is another extensively studied biodegradable PLA blend system. PCL is a
rubbery polymer with low T<sub>g</sub> and degrades by hydrolytic or enzymatic pathways. Broz et
al. [64] tuned modulus, strain at break, and ultimate tensile strength through the blend
composition. Jiang et al. [65] blended PLA with a biodegradable thermoplastic
poly(butylene adipate-co-terephthalate) (PBAT) to improve toughness and processibility
of PLA. PLA has also been blended with chitosan, a naturally occurring biodegradable,
biocompatible, edible, and nontoxic biopolymer, to improve wettability [66, 67].
Although PLA/collagen blends had reduced tensile and bending strengths compared to neat PLA, they underwent faster degradation under enzymatic conditions. The weight decreased to half the original weight of a PLA/collagen blend (30 wt% collagen) after five weeks, but neat PLA and PLA/collagen blends (10 wt% collagen) did so after eight and six weeks, respectively [68]. PLA has been toughened by physically blending with a variety of rubbery polymers, which was associated with significant modulus and/or UTS loss [69-71].

In summary, efforts to improve PLA’s toughness have resulted in a decrease of other important mechanical properties. The work presented in Chapter 5 addresses this issue.

**Surface modifications**

PLA surface interactions with other materials play an important role in numerous consumer and biomedical applications. Special surface chemical functionalities, hydrophilicity, roughness, and topography are often required and need to be controlled. While, undoubtedly, there has been work done to surface modify PLA for commodity applications (e.g., packaging films), there is a scarcity of data in the literature related to such things as friction modification, adhesion, and anti-fogging. However, there is abundant research reported in the literature on PLA surface modification for biomedical applications, so this portion of the introduction will focus on those investigations with the notion that some of the approaches could also be suitable for other commodity
applications. PLA has been surface modified using coating, entrapment, migratory additives, plasma treatment, chemical conjugation using wet chemistry, and photografting. The first four methods are non-permanent (non-covalent attachment of functional groups) while the later two are permanent (covalent attachment) surface modification methods.

Surface coating involves the deposition/adsorption of the modifying species onto the polymer surface. Typically, PLA has been coated with biomimetic apatite [72]; extra cellular matrix (ECM) proteins like fibronectin, collagen, vitronectin, thrombospondin, tenascin, laminin, and entactin [52, 73]; and RGD peptides [74] to control PLA-cell interactions. Although coating is a simple and convenient surface modification protocol, passive adsorption could induce competitive adsorption of other materials in the system and change the configuration of adsorbed species [52].

Entrapment is another surface modification methodology that can be used to incorporate molecules that do not adsorb onto PLA and does not require readily reactable side chain groups. Biomacromolecules such as alginate [75], chitosan [75], gelatin [75], poly(L-lysine) (PLL) [76], PEG [76-78], and poly(aspartic acid) [79] have been entrapped during the reversible swelling of the PLA surface region upon exposure to a solvent/nonsolvent mixture. This method typically requires a miscible mixture of a solvent and nonsolvent for PLA, with the surface-modifying molecules being soluble in the mixture and the nonsolvent [76]. Cai et al. [79] modified PLA surfaces by entrapping poly(aspartic acid) (PASP) in order to enhance their cell affinity. Rat osteoblasts were seeded onto the modified surfaces to examine their effects on cell adhesion and
proliferation. The findings showed that PASP-modified PLA surfaces may enhance the cell-surface interactions. The solvent-nonsolvent mixtures used in these entrapment protocols consisted of acetone or 2,2,2-trifluoroethanol as a solvent for PLA. Typically, most of the PLA solvents are not biocompatible. These studies have not reported on the amounts of the residual solvent left behind in surface-modified films. From a biocompatibility standpoint, surface-modification protocols should involve more benign solvents or removal of non-biocompatible solvents from the film bulk without affecting surface properties.

Migratory additives, carrying specific functional groups, are blended with PLA as a way to tailor PLA surface properties. Yu et al. [80] blended poly(D,L-lactic acid)-block-poly(ethylene glycol) (PLE) copolymer and RGD derivatives with PLA to engineer the surface properties of the resultant blend to promote chondrocyte attachment and growth. The blends prepared by this methodology showed enhanced hydrophilicity compared to neat PLA. The water contact angle decreased from 76° for neat PLA to 50° for PLA/PLE blends (75 wt % PLA). The chondrocyte cultures showed significant improvement of chondrocyte attachment and viability on the PLA films modified with PLE and RGD derivatives.

Plasma surface treatment of polymers began in the 1960s [81] and, within the last decade, has been applied to improve PLA surface hydrophilicity and cell affinity. The term “plasma” refers to a mixture of positive ions and electrons produced by ionization [82]. Yang et al. [83] used anhydrous ammonia (NH₃) plasma treatment to improve hydrophilicity and cell (human skin fibroblast) affinity of complex shapes like porous
PLA scaffolds prepared using a particulate leaching technique. The NH₃ plasma generated reactive amine groups on PLA scaffolds that anchored collagen through polar and hydrogen bonding interactions. These surface-modified scaffolds showed enhanced cell adhesion [84]. The main disadvantage of this technique is that the effectiveness of the surface modification is partially lost due to surface rearrangement [85]. The surface-modifying species rearrange by thermally activated macromolecular motions to minimize the interfacial energy, making the effect of plasma treatment non-permanent [83, 85-87]. Yang et al. [83] found that the modifying effects could be maintained by preserving samples at a low temperature (0-4 °C). The mobility of surface molecular chains was significantly decreased at temperatures much less than the Tg of PLA (55 °C). Since this temperature range (0-4 °C) is much lower than physiological as well as room temperature, this stabilization approach might not be practical. Apart from the rearrangement tendency of the modifying species introduced using plasma treatment, the treatment can also affect degradation of PLA. The NH₃ plasma-modification depth increased with treatment time, while the plasma power (20 to 80 W) influenced the depth only slightly. It was observed that the PLA degradation increased with an increase of plasma power and treatment time [88]. Although plasma treatment has been used to improve wettability and cell affinity of PLA, the issues related to non-permanent surface modification potentially make it unsuitable for certain biomedical and consumer applications.

Chemical conjugation using wet chemistry has been used to surface modify PLA extensively. Alkaline surface hydrolysis is a simple way to create reactive functional
groups, e.g., carboxylic acids (-COOH) and hydroxyls (-OH), on PLA [52]. The resulting carboxylic acid groups on PLA can readily be conjugated with surface-modifying species containing amine (-NH$_2$) or hydroxyl (-OH) groups. Typically acid groups are first activated with phosphorous pentachloride (PCl$_5$) [89], thionyl chloride (SOCl$_2$) [90], or water soluble carbodiimides [91] and subsequently conjugated with amines or hydroxyls (see Figure 1.4). Aminolysis is another way to introduce reactive amine groups onto PLA surfaces. 1,6-hexanediame has been used for aminolysis followed by conjugation with biocompatible macromolecules like gelatin, chitosan, or collagen [92]. The aminolysis reaction was performed by immersing PLA in hexanidine-propanol solution (0.06 g/mL) at 50 °C (below PLA’s Tg) for 8 min. PLA surface hydrophilicity (as measured using a sessile drop method) decreased slightly after aminolysis and further after biomacromolecule immobilization.

Janorkar et al. [93] introduced amine groups on the PLA film surface by photoinduced grafting of 4,4’-diaminobenzophenone followed by wet chemistry to create branched architectures containing amine functionalities on the periphery of the grafted layers. The grafted branched architectures were created by subsequent carbodiimide mediated reactions with succinic acid and tris(2-aminoethyl) amine. MC3T3 fibroblast attachment and viability improved with the grafting of amine terminated branched architectures.
Figure 1.4 Generalized reaction scheme for carboxylic acid activation using PCl₅, SOCl₂, or water soluble carbodiimides followed by chemical conjugation with amine (-NH₂) or hydroxyl (-OH) functionalities.

Photografting has been used extensively to tailor PLA surface properties primarily due to the advantages it offers: low cost of operation, mild reaction conditions, selectivity of UV light, and permanent alteration of surface chemistry [94]. This approach relies on
PLA photoactivation to create reactive groups associated with or followed by grafting of selected functionalities. Since PLA does not have any readily reactable side chain groups, this approach is useful for PLA surface modification. Typically, these methods are classified as “grafting to” or “grafting from” approaches. Polymer chains of known molecular weight, composition, and architecture are covalently attached to the surface in a “grafting to” approach, which is convenient for preliminary studies [95]. However, it is difficult to achieve high grafting densities with a “grafting to” approach because of steric hindrance and diffusion limitations [96]. The “grafting from” approach, which involves growing polymer chains from the surface, overcomes the limitations of the “grafting to” approach. In “grafting from”, photoinitiators are immobilized onto the substrate to initiate subsequent polymerization of vinyl or acrylic monomers from the surface. Photografting reactions have been carried out either in liquid or vapor phases. Zhu et al. [97] used a “grafting to” approach to immobilize chitosan chains onto PLA film surfaces using a hetero-bifunctional crosslinking reagent, 4-azidobenzoic acid. The “grafting from” approach has been used more extensively than the “grafting to” approach for PLA surface modification. Typically, either plasma treatment or photoinitiator is used to activate the PLA surface followed by photopolymerization of vinyl or acrylic monomers from the surface. Janorkar et al. [34] successfully used a “grafting from” approach to create bioactive PLA surfaces. The PLA film grafted with poly(acrylic acid) (PAA) and poly(acrylamide) (PAAm) exhibited improved wettability. Another positive outcome of this research was that PLA films grafted with PAA underwent faster \textit{in-vitro} degradation, which was attributed to acrylic acid monomer migrating into the film bulk and
polymerizing. Janorkar et al. [98] have also used single-monomer and mixed-monomer systems of AA, AAm, and vinyl acetate (VAc) to produce surface-confined homopolymers and copolymers to yield a spectrum of hydrophilicities, ranging from 82° for unmodified PLA to 12° for PLA grafted with PAAm. In order to avoid detrimental solvent effects on PLA, Edlund et al. [99] used a single-step vapor phase photografting route to covalently attach poly(acrylamide), poly(maleic anhydride), and poly(N-vinylpyrrolidone) to PLA-film surfaces. PLA film was exposed to the vapor phase mixture of monomer and benzophenone (photoinitiator) under UV irradiation at 50 °C. These reactions were carried out below PLA’s glass transition temperature to avoid any significant bulk changes. The extent of grafting and wettability increased with UV irradiation time. The static water contact angle values of PLA changed from 80° to 50° for poly(maleic anhydride) grafting, to 35° for poly(acrylamide) grafting, and to 25° for poly(N-vinylpyrrolidone) grafting for 30 min. Källrot et al. [100] observed that PLA films grafted with poly(N-vinylpyrrolidone) using the single-step vapor phase photografting protocol provided a good substrate for normal human cells of two types, keratinocytes and skin fibroblasts, to adhere and proliferate.

**SUMMARY OF DISSERTATION RESEARCH**

In the first part of this dissertation research, PLA films were successfully surface modified using a sequential two-step photografting method (Chapter 3). PLA was then melt-blended with poly[(3-hydroxybutyrate)-co-(3-hydroxyhexanoate)] (PHBHHx) with
an ultimate aim of making it tougher. PLA-PHBHHx blend films were further surface modified using a sequential two-step photografting method. It was observed that the blend films lost their toughness on surface modification due to UV-assisted solvent induced crystallization (UVasic) during photografting reactions (Chapter 4). In the final part of this work, a novel reactive blending technology was developed to toughen PLA with minimal modulus and ultimate tensile strength loss associated with introduction of a controlled concentration of reactive acid groups into the PLA matrix (Chapter 5).
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CHAPTER TWO

ANALYTICAL TECHNIQUES

DYNAMIC MECHANICAL ANALYSES (DMA)

DMA is useful for characterizing the viscoelastic properties of polymers. DMA primarily measures the stiffness and dampening properties of a material. It is one of the most sensitive techniques to study relaxation events, such as glass transitions. DMA applies an oscillating force to the sample and analyzes the material’s response to it (Figure 2.1) [1].

Figure 2.1 The DMA supplies a sinusoidal stress to the sample, which generates a sinusoidal strain. Properties such as modulus, viscosity, and dampening can be calculated by measuring the deformation amplitude at the peak and the lag between stress and strain sine waves [1].
A SEIKO INSTRUMENTS DMS210U dynamic mechanical analyzer was used to monitor changes in the viscoelastic response of the materials as a function of temperature. A specimen (2 cm x 1 cm) was placed in mechanical oscillation at a frequency of 1 Hz and the test was conducted at a heating rate of 2 °C/min. Calibration was performed using poly(methyl methacrylate) and steel standards and polycarbonate was used to check the calibration.

**WIDE-ANGLE X-RAY DIFFRACTION (WAXD)**

WAXD patterns were obtained at room temperature in the scattering angle (2θ) range of 10 – 35° by using an XDS 2000, SCITAG INC., USA, instrument. The X-ray generator produced Cu Kα radiation (wavelength = 1.5418 nm), which was used as an X-ray source (40 kV, 30 mA). WAXD analyses were performed to monitor the crystallization of PLA-PHBHHx blend films occurring during photografting reactions.

**THERMOGRAVIMETRIC ANALYSIS (TGA)**

Thermogravimetric analyses were performed using a TA Instruments TGA 2950 operated with Thermal Advantage software version 1.1. TGA enabled us to characterize the amount of residual solvent in various samples. A film sample was loaded into a platinum pan and scanned under a nitrogen purge at a flow rate 40 ml/min. The samples
were heated from room temperature to 200 °C at a heating rate 10 °C/min to monitor weight loss.

**DIFFERENTIAL SCANNING CALORIMETRY (DSC)**

DSC was used to monitor crystallization and physical aging of PLA-based films. A Pyris 1 PerkinElmer Instrument was used to obtain DSC data from 30 to 190 °C at a heating rate 10 °C/min. The degree of crystallinity was calculated from the measured heat of fusion relative to an estimated 93 J/g [3] for a 100% crystalline PLA and 164 J/g [4] for a 100% crystalline PHB.

**ATTENUATED TOTAL REFLECTANCE (ATR) FTIR SPECTROSCOPY**

Attenuated total reflectance (ATR) FTIR spectroscopy was used to characterize the modified film surfaces. The characterization was performed using a Thermo Nicolet Magna 550 single bounce spectrometer equipped with a Thermo-Spectra-Tech Foundation Series Diamond ATR with DTGS detector. 32 scans at a resolution of 4 cm⁻¹ were collected and averaged. OMNIC software version 6.2 was used to process spectra for ATR and baseline corrections.
CONTACT ANGLE GONIOMETRY

A Krüss G10 static contact angle apparatus was used to perform static water contact angle measurements using a sessile drop method. Using a syringe, an approximately 1 μL water drop volume was placed on the film surface and allowed to stabilize for 2 min. Water contact angle values were measured using Drop Shape Analysis software. The reported water contact angles are an average of 10 readings with ±95% confidence intervals.

X-RAY PHOTON ELECTRON SPECTROSCOPY (XPS)

XPS spectra (appendix A) were obtained using a Kratos Axis Ultra Photoelectron Spectrometer with Al Kα radiation (15 kV, 225 W) and an overall instrument resolution of 1.1 eV. All spectra were collected at an electron take-off angle of 90° to the sample surface. Survey spectra were collected over the 0-1200 eV range, using a pass energy of 40 eV. High resolution spectra of the C 1s core levels were also collected using a pass energy of 40 eV. CasaXPS software was used for spectral analysis. The binding energies were corrected by referencing the C 1s binding energy to 285 eV.
OPTICAL AND FLUORESCENCE MICROSCOPY

A PAA micropatterned PLA specimen was immersed in toluidine blue (0.1 mg/ml) solution in water and observed with a Zeiss Axiovert 135 optical microscope (Appendix A). An Insight color digital camera with SPOT image acquisition software (Diagnostics Instruments, Sterling Heights, MI) was used to capture images. The optical micrographs were analyzed using Image-Pro 4.1 software. Images of streptavidin adsorbed on biotin modified PAA patterns on PLA were recorded with the aid of a fluorescence microscopy filter at 515 nm.

MICROWAVE ASSISTED EXTRACTION (MAE)

A select few surface-modified films were subjected to microwave assisted extraction in a MARS 5 microwave accelerated reaction system from CEM Corporation. These surface-modified films were subjected to the extraction in water at 95 °C for 1 h.

TRANSMISSION-FTIR MICROSCOPIC ANALYSIS WITH DIAMOND COMPRESSION CELL

Transmission-FTIR microspectroscopic analyses were conducted using a Thermo Nicolet Magna 550 FTIR spectrometer equipped with a Thermo Nicolet Ni-Plan FTIR microscope. The microtomed sections (typically 50 μm thick) were compressed using a
Thermo-Spectra-Tech micro sample plan with diamond windows. 32 scans at a resolution of 4 cm\(^{-1}\) with a KBr background were collected for each sample. An adjustable aperture was used to form an analysis area approximately 25 microns wide and 100 microns long with the long dimension parallel to the long axis of the microtomed section (i.e., the 25-micron-wide beam was used for analysis at five discrete locations across the 125 microns thickness of the film) [5].

**STATISTICAL ANALYSIS**

Statistical evaluation of the toughness data was performed using t-test. All results are reported as mean ±95% confidence intervals (level of significance = 0.025 and n=5).
REFERENCES


CHAPTER THREE

EFFECT OF THE PHOTOREACTION SOLVENT ON SURFACE AND BULK PROPERTIES OF PLA AND PHBHHx FILMS


INTRODUCTION

Poly(lactic acid) (PLA) and poly(hydroxyalkanoate) (PHA) are biodegradable thermoplastics that show great potential in consumer and biomedical applications. PLA is a well known biomaterial [1-11]. PHA is a bacterially produced homopolymer or copolymer family with more than 150 different types consisting of different monomers [12]. Poly[(3-hydroxybutyrate)-co-(3-hydroxyhexanoate)] (PHBHHx) is a member of the PHA family and has been studied extensively for biomedical applications [13-21]. PLA is relatively brittle but shows improved toughness when blended with a small amount of PHBHHx [22].

Even though PLA-PHBHHx blends exhibit enhanced toughness over neat PLA, the two polymers are hydrophobic and do not contain readily reactable groups, which limit their use in many consumer and biomedical applications. These polymers need to be surface modified to introduce readily reactable functional groups and to control their hydrophilicity. Solvent cast PLA and PHBHHx films were surface modified using photoinduced grafting because of the following advantages: low cost of operation, mild
reaction conditions, selectivity of UV light, and irreversible covalent grafting [23]. Acrylic acid and acrylamide were chosen as monomers because of their ability to make surfaces hydrophilic on photopolymerization from the film surface [24]. Moreover, acrylic acid could be subsequently conjugated to various synthetic and biomolecules. The method consisted of two steps. Step 1 involved benzophenone (photoinitiator) photografted on the film surface and Step 2 involved photopolymerization of hydrophilic monomers from the film surface. In both steps, ethanol was used as the reaction solvent and the procedure resulted in increased hydrophilicity of the films using acrylic acid and acrylamide as the monomers [24-25].

PLA and PHBHHx films used in this study had very low crystallinity initially. It was observed that the films underwent solvent-induced crystallization during the surface reactions. We have previously surmised that monomer penetrated into the film, photopolymerized, and subsequently influenced the degradation rate when ethanol was used as the reaction solvent in Step 2 [24]. In this work, the extent of monomer penetration was evaluated using FTIR microspectroscopy. We have also used water as well as ethanol to investigate the effect of reaction solvent in Step 2 on surface photografting, monomer penetration, solvent-induced crystallization, and resultant mechanical properties of solvent-cast PLA and PHBHHx films.
MATERIALS

The chemical structures of PLA and PHBHHx repeat units are shown in Figure 3.1. PLA pellets ($M_n \sim 110,000$ g/mol) were supplied by NatureWorks LLC. PHBHHx comprising 6.9 mol% 3HHx units was supplied by Procter & Gamble Company. Acrylic acid (99.5% w/w) was obtained from Acros Organics. Acrylamide (99% w/w) and chloroform ($\text{CHCl}_3$) were obtained from Aldrich Chemicals. Ethanol, HPLC water, benzophenone, and $\text{H}_2\text{O}_2$ (30% w/w) were obtained from Fisher Scientific. All chemicals were used as received.

(a) (b)

Figure 3.1 Chemical structures of (a) PLA and (b) PHBHHx repeat units.
METHODS

Film Solvent Casting: Approximately 1.1 g polymer was dissolved in 60 ml of chloroform. The films were cast in a glass petri dish, which was cleaned by exposing it to Pirahna solution (concentrated H$_2$SO$_4$ and 30% H$_2$O$_2$ 75:25 v/v) for 1 h. Extreme care must be taken when using Pirahna solution. The dish was then washed using copious amounts of distilled water and dried using nitrogen. The cleaned petri dish was aligned perfectly horizontal to ensure that the resultant film had uniform thickness. The polymer-chloroform solution was poured in the petri dish and kept in a chemical hood for 24 h to allow the chloroform to evaporate. The resultant film was removed from the petri dish using a razor blade [24].

Sequential two step photografting: Figure 3.1 shows the reaction scheme for the sequential two-step photografting reaction of acrylic acid onto a PLA film surface. A film specimen (approximately 3 cm x 1 cm x 125 μm) was dip coated in 5% w/w benzophenone solution in ethanol. The film was allowed to stand at room temperature for 30 min to ensure that ethanol was evaporated. The benzophenone dip-coated film was sealed in a quartz cuvette using parafilm in a glove box with a nitrogen atmosphere. Each side of the film was exposed to UV irradiation for 5 min in a UV processor (OAI Model No. 200; table top series, mask aligner and UV exposure system). The processor was equipped with a 350 W bulb having a wavelength range of 290-500 nm and intensity of 25 mW/cm$^2$ at 365 nm. After UV exposure, the resultant film was sonicated in ethanol for
5 min to remove unreacted benzophenone. Benzophenone-grafted film was put in a Pyrex test tube containing 10% v/v of the chosen monomer in ethanol or water. The test tube was purged with nitrogen for 30 min and exposed to UV for 3 h. The resultant film was sonicated in the solvent used for Step 2 for 5 min to remove physically adsorbed polymer from the surface [24].

**Figure 3.2** Reaction scheme for the sequential two-step photografting reaction of acrylic acid onto a PLA film surface [24].

*Mechanical Testing:* Mechanical properties were measured using an Applied Test System Inc. (ATS) mechanical tester on the film samples (3 cm x 1 cm x 125 μm) according to American Society for Testing and Materials Standard (ASTM D882) specifications. The measurement values averaged for five specimens are reported.
RESULTS AND DISCUSSION

The neat, solvent cast PLA and PHBHHx films are hydrophobic, with water contact angles $\sim 82 \pm 0.2^\circ$. Hydrophilic monomers, acrylamide and acrylic acid, were successfully polymerized from the film surface using the sequential, two-step photografting procedure discussed in detail elsewhere [24]. As mentioned above, surface-modified films were sonicated in ethanol for 5 min to remove any physically adsorbed species from the film surfaces. To ensure that sonication was sufficient, a few films were also subjected to a more aggressive microwave assisted extraction in water at 95 °C for 1 h to make sure that unreacted monomer was removed and that the grafted layers were covalently attached and not just physically adsorbed. The reason we chose water as a solvent for the microwave assisted extraction is because free poly(acrylamide) (PAAm) and poly(acrylic acid) (PAA) chains dissolve in water, while PAAm and PAA grafted to PLA or PHBHHx do not dissolve in water. So the microwave assisted extraction promoted the removal of physically adsorbed monomer, PAAm, or PAA from the surfaces. Comparing sonicated with sonicated plus microwave-extracted films, there were no significant changes in water contact angle or ATR-FTIR spectra, which confirmed the removal of unbound species as well as the covalent grafting of PAAm and PAA from the film surfaces. For all subsequent experiments, sonication was used but the use of microwave assisted extraction was discontinued.

PLA and PHBHHx films reacted with benzophenone (Step 1) did not show any significant change in the water contact angle values. A control experiment was also
performed where films were dip coated in ethanol (no benzophenone) in Step 1 and subjected to UV exposure in the chosen monomer solution in Step 2. The reacted films did not show any grafting from the film surfaces as evidenced from contact angle and ATR-FTIR measurements. Figures 3.3 and 3.4 show the effect of UV exposure time on water contact angle of the PLA and PHBHHx films grafted with benzophenone and exposed to UV irradiation in 10% v/v monomer solution in ethanol. It was observed that the water contact angle values plateaued after 3 h exposure in Step 2, so for all subsequent experiments, UV exposure time in Step 2 was maintained at 3 h. The surface-modified films are hereafter referred to as PLA-g-PAA, PLA-g-PAAm, PHBHHx-g-PAA, and PHBHHx-g-PAAm, where g = grafted. It was observed that the static water contact angle for modified PHBHHx was ultimately greater than that for modified PLA. This behavior is in agreement with the fact that the benzophenone preferentially abstracts tertiary hydrogens on the polymer [28], and the concentration of tertiary H-atoms in PLA is greater than that in PHBHHx (see Figure 3.1).
Figure 3.3 The effect of the UV exposure time on the static water contact angle of PLA (○) and PHBHHx (■) films grafted with acrylic acid with ethanol as the solvent in Step 2. The error bars represent 95% confidence intervals.
Figure 3.4 The effect of the UV exposure time on the static water contact angle of PLA (○) and PHBHHx (■) films grafted with the acrylamide with ethanol as the solvent in Step 2. The error bars represent 95% confidence intervals.
Typical ATR-FTIR spectra for neat and surface-modified films are shown in Figure 3.5. Figure 3.5a represents the neat PLA film with the –C=O peak for the PLA ester at wave number 1756 cm$^{-1}$. Figure 3.5b represents the neat PHBHHx film with the –C=O peak for the PHBHHx ester at 1721 cm$^{-1}$. Figure 3.5c represents the PLA film grafted with PAA, showing a shoulder at 1720 cm$^{-1}$, which is the –C=O acid peak. Figure 3.5d represents the PHBHHx film grafted with PAA, showing the broadening of the 1721 cm$^{-1}$ peak. The amide I –C=O peak of PAAm was observed at 1670 cm$^{-1}$ and an amide II peak was observed at 1550 cm$^{-1}$ in Figures 3.5e and 3.5f, representing PLA-g-PAAm and PHBHHx-g-PAAm, respectively.
Figure 3.5 Representative ATR-FTIR spectra of the (a) neat PLA, (b) neat PHBHHx, (c) PLA-g-PAA, (d) PHBHHx-g-PAA, (e) PLA-g-PAAm, and (f) PHBHHx-g-PAAm. Spectrum (a) shows the –C=O peak for the PLA ester at 1756 cm⁻¹ (♦). Spectrum (b) shows the –C=O peak for the PHBHHx ester at 1721 cm⁻¹ (●). Spectrum (c) shows –C=O acid peak at 1720 cm⁻¹ (★), which is a shoulder to the PLA ester peak. Spectrum (d) shows the widening of the PHBHHx ester peak at 1721 cm⁻¹ (★), due to the overlapping of the –C=O peak for the PHBHHx ester and the –C=O acid peak. Spectra (e-f) show the –C=O amide peak at 1670 cm⁻¹ (■).
Effect of reaction solvent on surface properties

Table 3.1 summarizes the static water contact angle values for surface modified PLA and PHBHHx films using water and ethanol as the reaction solvent in Step 2. With PAA, no significant difference in the water contact angle was observed when water or ethanol was used as the reaction solvent. For PAAm, surface-modified films showed a slightly lower water contact angle when ethanol was used as the reaction solvent. Figure 3.6 shows ATR-FTIR peak area ratio (PAR), viz. 1670 cm\(^{-1}\) –C=O amide peak area normalized by 1756 cm\(^{-1}\) –C=O ester peak area for PLA or 1720 cm\(^{-1}\) –C=O ester peak area for PHBHHx. Films surface-modified using ethanol as the reaction solvent in Step 2 showed slightly higher PAR values than the corresponding films surface-modified in water. But this difference was not significant (some of the error bars overlap). In short, the reaction solvent used in Step 2 had only a slight effect on the surface properties (static water contact angle and ATR-FTIR peak area ratio).

Table 3.1 Water contact angles of neat and surface modified PLA and PHBHHx after 3 h UV-exposure time and using water or ethanol as the reaction solvent in step 2

<table>
<thead>
<tr>
<th></th>
<th>PLA (Ethanol)</th>
<th>PLA (Water)</th>
<th>PHBHHx (Ethanol)</th>
<th>PHBHHx (Water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>82 ± 1</td>
<td>82 ± 1</td>
<td>76 ± 1</td>
<td>76 ± 1</td>
</tr>
<tr>
<td>Film-g-PAA</td>
<td>38 ± 2</td>
<td>41 ± 3</td>
<td>51 ± 3</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Film-g-PAAm</td>
<td>12 ± 2</td>
<td>17 ± 1</td>
<td>23 ± 2</td>
<td>28 ± 2</td>
</tr>
</tbody>
</table>
**Figure 3.6** ATR-FTIR peak area ratio, viz. 1670 cm\(^{-1}\) –C=O amide peak area normalized by 1756 cm\(^{-1}\) –C=O ester peak area for PLA or 1720 cm\(^{-1}\) –C=O ester peak area for PHBHHx. Black bars represent films grafted with acrylamide after sonication for 5 min in the respective solvents and white bars represent films grafted with acrylamide after sonication for 5 min in the respective solvents plus microwave assisted extraction in water at 95 °C for 1 h. The error bars represent 95% confidence intervals.

**Effect of reaction solvent on bulk properties**

Table 3.2 shows the various experiments designed to compare the effect of surface modification on the bulk properties. Experiment 1 is the typical sequential, two-step photografting process. Experiment 2 consisted of the same protocol as that of
experiment 1 except a film specimen was dip coated in ethanol instead of 5% w/w benzophenone solution in ethanol in Step 1. The specimens prepared by experiment 2 were designated as “PAA Control” and “PAAm Control” in the subsequent figures. Experiment 2 enabled us to study the effect of surface modification on bulk properties in the absence of surface-confined photografting, since it omitted the benzophenone photoinitiator. Experiment 3 involved the films subjected to the same UV treatment but soaked only in the chosen solvent with specimens designated as “Solvent Control” in the subsequent figures. Experiment 3 allowed us to study the effect of photografting solvent on bulk properties in the absence of benzophenone and monomer, since the films were subjected to the same UV treatment but soaked only in the chosen solvent.

**Table 3.2** Design of the photografting experiments to evaluate the effect of surface modification on mechanical properties of the PLA and PHBHHx films after 3 h UV-exposure time in step 2

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>Film-g-PAA/Film-g-PAAm</td>
</tr>
<tr>
<td>2</td>
<td>-(^\alpha)</td>
<td>+</td>
<td>PAA Control/PAAm Control</td>
</tr>
<tr>
<td>3</td>
<td>-(^\alpha)</td>
<td>-(^\gamma)</td>
<td>Solvent Control</td>
</tr>
</tbody>
</table>

\(^\alpha\) Pure ethanol was used instead of 5% benzophenone solution  
\(^\gamma\) Pure solvent was used instead of 10% monomer solution
PLA films

Figure 3.7a shows the Young’s modulii values for PLA films. The Young’s modulii for the PLA-g-PAA and PLA-g-PAAm films were greater than that for the neat film (experiment 1), particularly when ethanol was used as the solvent in Step 2 (black bars). In order to investigate this behavior further, experiments 2 and 3 were designed to study the effects of the chosen monomers and reaction solvent on the bulk properties. PLA films subjected to control experiment 2 show Young’s modulii the same as that of the corresponding PLA films subjected to experiment 1. This implied that omission of benzophenone in Step 1 did not have a significant impact on the modulii. Moreover, PLA films subjected to the Solvent Control experiment (experiment 3) showed modulii approximately the same as that of PLA films subjected to experiment 1. This result indicated that the reaction solvent in Step 2 was the main variable to affect modulus.
Figure 3.7 Effect of the photografting reaction solvent on the (a) modulus, (b) crystallinity, and (c) toughness of PLA films. Black bars indicate films prepared by carrying out the reaction in ethanol and white bars indicate films prepared by carrying out the reaction in water. The neat PLA film had very low % crystallinity such that the DSC spectrum did not show a definite melting peak. The error bars represent 95% confidence intervals. The toughness of PLA films grafted with PAA and PAAm using ethanol and water as the reaction solvents showed statistically significant difference from the toughness of neat PLA, as determined by a t-test.
Figure 3.7b shows the effect of the reaction variables on the % crystallinity of PLA films. The neat PLA film had very low % crystallinity such that the DSC spectrum did not show a definite melting peak. Compared to the neat PLA films, films prepared by experiments 1, 2, and 3 all showed an increase in crystallinity to around 20% when ethanol was used as the reaction solvent in Step 2 and 12% when water was used. These experiments indicated that solvent-induced crystallinity occurred and, for a given solvent, the presence of the monomer (acrylic acid or acrylamide) in the reaction solvent had little influence on the crystallinity. Solvent-induced crystallization of PLA films was more prevalent when ethanol was used as the reaction solvent in Step 2 than when water was used. Compared to water, ethanol’s solubility parameter is closer to that of PLA (Table 3.3) and therefore exhibits a greater extent of penetration to promote PLA crystallization.

**Table 3.3** Solubility parameters for PLA, PHBHHx, ethanol, and water. Solubility parameters for PLA and PHBHHx were calculated based on Fedors cohesive energy and molar volume values using group contribution method [30]

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Solubility parameter (MPa)^{0.5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>22.8</td>
</tr>
<tr>
<td>PHBHHx</td>
<td>21.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>26.0 [31]</td>
</tr>
<tr>
<td>Water</td>
<td>47.9 [31]</td>
</tr>
</tbody>
</table>
The modulus of neat PLA film indicated in Figure 3.7a was much lower than expected (typically reported as 1151 ± 10 MPa) [1], and we suspected that residual solvent remained in the film. TGA analysis of the PLA film solvent cast from a chloroform solution showed that the film retained around 13 weight percent of chloroform. PLA film solvent cast in chloroform and then modified by experiments 1, 2, or 3 showed an increased total solvent (chloroform or ethanol or both) content of around 3 to 6 weight percent when ethanol was used as the reaction solvent in Step 2. Complementary experiments using water as the reaction solvent in Step 2 exhibited a total weight increase of 9 to 11 weight percent (Figure 3.8a). These results indicated that modified PLA films lost residual chloroform on crystallization, particularly with ethanol that promoted a greater extent of PLA crystallization. Attempts were made to remove the residual solvent by heating specimens at various temperatures under vacuum. That methodology reduced the amount of residual solvent but significantly affected the sample’s surface roughness, even at temperatures as low as 50 °C. The rough surfaces were not suitable for surface modification and subsequent analysis. Our objective in this study was to assess the effect of photografting steps on the film’s mechanical properties. Even though the films contained residual solvent, the mechanical and surface properties of surface-modified films were compared to those of neat (unmodified) film.

Figure 3.7c shows the toughness (as reflected by area under engineering stress-strain curve) values for PLA films. The toughness shown by solvent cast neat PLA film is the toughness of PLA with 13 weight percent residual chloroform. PLA films lost their toughness significantly on surface modification when ethanol was used as the reaction
solvent in Step 2 (black bars). We attributed this toughness reduction to solvent-induced crystallization and the loss of the residual chloroform in the modified films. When water was used as the reaction solvent in Step 2, the toughness reduction was not as great. This results from the fact that solvent-induced crystallization was less prevalent in water than in ethanol and the solvent content in the modified films was higher when water was used as the reaction solvent in Step 2.

Figure 3.8 TGA curves of neat and surface modified (a) PLA and (b) PHBHHx. Note that the y-axis scales are different on the two plots.
PHBHHx films

TGA analysis of the PHBHHx film solvent cast in chloroform showed that the film retained around 1 weight percent of the chloroform. PHBHHx film (solvent cast in chloroform) prepared by experiments 1, 2, and 3 showed the solvent (chloroform or ethanol or both) content around 0.25 to 3 weight percent, when ethanol was used as the reaction solvent in step 2. Complementary experiments using water as the reaction solvent in Step 2 exhibited a weight gain of 0.25 to 2 weight percent (Figure 3.8b). In either case, the PHBHHx films retained significantly less solvent than the PLA films.

For PHBHHx-film results in Figure 3.9a using ethanol as the reaction solvent (black bars), a modulus increase was observed for PHBHHx-g-PAA compared to neat film and an even greater increase for PHBHHx-g-PAAm. Referring to the crystallinity data in Figure 3.9b, the % crystallinity for those same specimens remained the same, so an increase in modulus must be a result of something other than crystallinity. We speculated that monomer penetrated into the film and polymerized. PAA ($T_g = 126 ^\circ C$) and PAAm ($T_g = 188 ^\circ C$) have high glass transition temperatures and hence they are glassy at room temperature [29]. If there was significant penetration of these monomers and subsequent polymerization within the films, we speculate that those chains could contribute to an increase in stiffness. PHBHHx films subjected to experiments 1, 2, and 3 using water as the reaction solvent in Step 2 exhibited solvent-induced crystallization, and those specimens also exhibited an increase in modulus (Figure 3.9a, white bars). Most noteworthy, PHBHHx film immersed only in water (Solvent Control) showed
approximately same modulus as those for modified PHBHHx films, inferring that the observed modulus increases were due to solvent-induced crystallization during these experiments.

**Figure 3.9** Effect of the photografting reaction solvent on the (a) modulus, (b) crystallinity, and (c) toughness of PHBHHx films. Black bars indicate films prepared by carrying out the reaction in ethanol and white bars indicate films prepared by carrying out the reaction in water. The error bars represent 95% confidence intervals. The toughness of PHBHHx films grafted with PAA and PAAm using ethanol as the reaction solvent showed statistically significant difference from the toughness of neat PHBHHx, as determined by a t-test.
The penetration of acrylamide into the bulk of the surface-modified films was characterized by first microtoming the films to expose their cross-sections, which were then analyzed using transmission FTIR microspectroscopy. Acrylamide was used because of its distinct \(-\text{C}=\text{O}\) amide I peak, whereas acrylic acid did not show a distinct peak. Figure 3.10 represents the transmission FTIR peak area ratio, viz. \(1670 \text{ cm}^{-1} \text{ –C}=\text{O}\) amide peak area normalized by \(1452 \text{ cm}^{-1}\) asymmetric \(-\text{CH}_3\) band peak area. Based on their chemical structures, the concentration of \(\text{CH}_3\) groups (number of \(\text{CH}_3\) groups per unit volume) is greater in PLA than PHBHHx, so we multiplied the denominator of PHBHHx’s PAR by 1.36 to be able to compare results as a common basis (see Figure 3.1). In Figure 3.10, the circles represent the standard 2-step photografting process in ethanol, while the squares represent the case where the benzophenone was omitted from Step 1 (PAAm Control). A 25-micron-wide beam was used for analysis at five discrete locations across the 125 micron thickness of the film. Comparing Figures 3.10a and 3.10b, there was significantly more acrylamide penetration into PHBHHx than PLA, likely due to the lower crystallinity of the PHBHHx coupled with its low \(T_g\) (about \(-4 \degree\text{C}\) for PHBHHx vs. \(16 \degree\text{C}\) for PLA as determined by DMA for these solvent-cast films). The PAR profiles in PLA are equivalent for PLA-g-PAAm (circles) and PAAm Control (squares). The same is true for the modified PHBHHx films except at analysis positions 1 and 5, where the PARs for PHBHHx-g-PAAm are greater than that for PAAm Control in Figure 3.10b. The reason for this accentuated PAR in the near-surface regions at positions 1 and 5 is unknown at this point, but it appears to be statistically different compared to the results for PAAm Control.
Figure 3.10 Transmission FTIR peak area ratio, viz. 1670 cm$^{-1}$ –C=O amide peak area normalized by 1452 cm$^{-1}$ asymmetric –CH$_3$ band peak area, as a function of analysis position into the bulk of PLA or PHBHHx films grafted with benzophenone in step 1 and then immersed in 10% v/v acrylamide solution and exposed to UV for 3 h (○), or dip coated in ethanol and exposed each side for 5 min in step 1 then immersed in 10% v/v acrylamide solution and exposed to UV for 3 h (■). The IR beam width at each analysis position was 25 microns.
Referring back to Figure 3.9b, the crystallinities of neat PHBHHx, PHBHHx-g-PAAm, and PAAm Control were nearly identical, yet the modulus of neat PHBHHx was dramatically lower than PHBHHx-g-PAAm and PAAm Control (Figure 3.9a). This behavior is consistent with the hypothesis that acrylamide penetrated into the PHBHHx, as shown in Figure 3.10b, and polymerized to yield glassy PAAm chains that increased the stiffness of the modified PHBHHx films. In the case of PLA, there was only slight acrylamide penetration, but more substantial change in crystallinity to lead to increased stiffness for those modified PLA films. Finally, complementary experiments were conducted in water in Step 2 and very little amide was detected through the cross-section of the films.

These results are also reflected in the toughness data for PHBHHx films (Figure 3.9c). Films modified using ethanol in Step 2 (black bars) showed low toughness values coinciding with increased stiffness. PHBHHx films showed a greater toughness retention when reactions were carried out in water because there was little monomer penetration as detected by transmission FTIR microspectroscopy into the bulk of the films. There was also no significant change in the amount of residual solvent in these PHBHHx films, leading to a less significant impact on film toughness.

Finally, we did not observe significant changes in the molecular weights of PLA or PHBHHx films upon surface modification as evidenced by gel permeation chromatography (GPC).
PLA and PHBHHx were successfully surface modified using sequential, two step photografting. Although the reaction solvent used in Step 2 did not have any significant effect on surface properties, bulk properties were significantly affected. When ethanol was used as the reaction solvent in Step 2, PLA and PHBHHx films drastically lost their toughness and became stiffer. Moreover, there was significant acrylamide penetration into PLA and PHBHHx films when reactions were carried out in ethanol and the extent was greater into PHBHHx films. When water was used as the reaction solvent, transmission FTIR microspectroscopic analyses revealed very little acrylamide penetration into the bulk of these films. Solvent-induced crystallization was more prevalent when ethanol was used as the reaction solvent than when water was used as the reaction solvent for PLA films. The observed toughness loss and modulus gain of PLA films on surface modification was attributed to solvent-induced crystallization and loss of residual chloroform. The presence of residual chloroform in the film specimens is undesirable from a biocompatibility standpoint. Additionally, this work showed that the photoreaction solvent affected the bulk properties of PLA and PHBHHx films cast from a chloroform solution. Therefore, further work is being conducted on melt-processed films where residual solvent from the film-formation method will not be an issue.
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CHAPTER FOUR

TOUGHNESS DECREASE OF PLA-PHBHHX BLEND FILMS UPON SURFACE-CONFINED PHOTOPOLYMERIZATION


INTRODUCTION

PLA is an extensively studied biodegradable thermoplastic polyester derived from renewable resources, showing great potential in consumer and biomedical fields [1-9]. However, wide applicability of PLA is limited by several factors, including its brittleness and therefore poor toughness and, like many polymers, its hydrophobicity and lack of modifiable side chain groups. PLA has been blended with other polymers to improve the toughness of the resultant blend [9-17]. For example, addition of a small amount of PHBHHx to PLA has yielded a much tougher material compared with neat PLA [15]. PHBHHx belongs to a bacterially produced homopolymer or copolymer family referred to as polyhydroxyalkanoates (PHAs) [18]. PHBHHx is a biodegradable aliphatic polyester that tends not to crystallize when a small amount (typically less than 20 weight %) is blended with PLA, markedly improving the toughness of the resultant blend without losing optical clarity [15, 19]. PHAs have been investigated recently as potential biomaterials [20-24]. However, their comparatively higher production costs, thermal
degradation during melt processing, and complexities involved in film casting by extrusion limit their wide applicability [25-26].

One of the major emphases of our research is to modify the surface properties of PLA-PHBHHx films using photografting [27-29]. In the surface modification process, the films are typically immersed in various solvents. Therefore, one goal was to determine the influence of solvent immersion on film toughness. Moreover, we investigated blend films that were not subjected to the surface-modification process (i.e., no immersion in solvents) to assess the extent of physical aging on toughness reduction. Similar physical aging has been reported for PLA and its blends [30-32]. In this chapter we report, for the first time, toughness changes of PLA-PHBHHx blend films that are specifically associated with physical aging and more importantly for this work, UV-assisted solvent induced crystallization.

METHODS

Film Casting: PLA pellets were vacuum heated at 70 °C for 24 h and cooled in the vacuum oven to remove any residual moisture. PHBHHx powder was used as received. PLA, PHBHHx, and PLA-PHBHHx blend films (90 weight percent PLA) were formed using a single screw extruder. The temperature profile of the extruder for PLA and PLA-PHBHHx blend film casting was as follows: Zone 1 (located near the hopper) – 180 °C, zone 2 – 190 °C, zone 3 – 200 °C, pump – 200 °C, and die – 190 °C. The temperature profile of the extruder for PHBHHx film casting was as follows: Zone 1 – 135 °C, zone 2
– 155 °C, zone 3 – 155 °C, pump – 165 °C, and die – 155 °C. Molten polymer exiting the die was cooled on a chill roll.

*Sequential two-step photografting:* PLA-PHBHHx blend films were surface modified using a sequential two-step photografting approach discussed in detail in Chapter 3. Briefly, this method consists of photografting benzophenone which preferentially abstracts tertiary hydrogen atoms in Step 1 [33], and photopolymerizing acrylic acid or acrylamide from the film surface in Step 2. The reaction was carried out at room temperature. We chose acrylic acid and acrylamide as monomers because of their ability to improve hydrophilicity upon photopolymerization [28] and acrylic acid could be subsequently conjugated with various biomolecules depending on the intended application of the surface modified films.

*Mechanical Testing:* The film samples with a nominal thickness of 125 ± 10 μm were kept in a vacuum oven at room temperature for 24 h and subsequently annealed at 60 °C for 30 min before mechanical testing. An Applied Test System Inc. (ATS) mechanical tester was used to measure mechanical properties of the film samples (3 cm x 1 cm x 125 μm) according to American Society for Testing and Materials Standard (ASTM D882) specifications. A cross-head speed of 2.5 cm/min was used. The measurement values averaged for five specimens with ±95% confidence intervals are reported.
RESULTS AND DISCUSSION

This research focused on adding a small amount of PHBHHx (10 weight percent) to PLA with an ultimate aim of making tougher films and surface-modifying the blend films to increase their wettability. Figure 4.1 shows an engineering stress-strain curve for neat PLA and PLA-PHBHHx blend films, tested within 1 h after extrusion. The greater toughness for PLA-PHBHHx blend film, as reflected by the area under the curve, was governed by improvement in % elongation at break, from 11 ± 3 % for neat PLA to 360 ± 75 % for the blend.

Figure 4.1 Engineering stress-strain curve of extruded PLA (♦) and PLA-PHBHHx (90 weight percent PLA) blend (■) films.
Figure 4.2 (A) Tan δ as a function of temperature for extruded PLA-PHBHHx blend film (90 weight percent PLA). (B) Tan δ as function of temperature of (a) unmodified blend, (b) water control, (c) Blend-g-PAAm, and (d) Blend-g-PAA.

Figure 4.2A shows tan δ as a function of temperature of the blend film as characterized using DMA. The peak temperature of tan δ is used to denote the T_g. DMA revealed two well-defined tan δ peaks, denoting two different glass transition
temperatures at 60 and -18 °C, corresponding to the PLA and PHBHHx blend components, respectively. From this result, the PLA-PHBHHx blend appeared to be non-compatible, which is consistent with results reported previously by Furukawa and coworkers [34].

**Toughening mechanism**

Optical clarity of extruded PLA-PHBHHx blend films suggested that either PHBHHx (lower \( T_g \) component) crystallites were sufficiently small that they could not scatter light, or they were not present in the PLA matrix. This inability of PHBHHx to fully crystallize when melt blended with PLA at a low level was suggested as an explanation for the increase in toughness of the PLA-PHBHHx blend by Noda and coworkers [15]. WAXD patterns of blend films used in this study showed a reflection peak corresponding to PHBHHx at 13.4° [34], implying that PHBHHx crystallized to some extent, but not sufficient enough to scatter light (Figure 4.3).
Figure 4.3 WAXD patterns of (a) unmodified blend, (b) blend-g-PAA, (c) blend-g-PAAm, and (d) blend films prepared by water-control experiments.

However, one of the serious drawbacks of PLA-based blends is that the PLA phase tends to undergo physical aging, affecting their mechanical properties significantly [31]. Figure 4.4 shows the effect of physical aging on the toughness of our extruded blend films, where the toughness decreased as the room-temperature aging time increased. Typically, the amorphous phase in glassy or partially glassy polymers undergoes physical aging that usually occurs around its glass transition temperature [35-
Physical aging involves completely reversible spontaneous changes in the thermodynamic state of a polymer [31]. Blend films used here were cooled on a chill roll from the melt. Since the temperature was below PLA’s T<sub>g</sub> (major component), its molecular chains became frozen. The polymer was in a non-equilibrium state, having large volume, enthalpy, and entropy [31, 35]. Since room temperature was below T<sub>g</sub>, the free volume would tend to reduce spontaneously towards a thermodynamic equilibrium, resulting in an enthalpic relaxation [31, 35].

![Figure 4.4](image)

**Figure 4.4** Effect of the physical aging on toughness of extruded and annealed (at 60 °C for 30 min) blend films. The error bars represent 95% confidence intervals.

Since physical aging was an enthalpic relaxation process, occurring around T<sub>g</sub>, DSC was used to monitor physical aging in the blend films. Figure 4.5 shows the effect
of physical aging on the glass transition event in DSC scans. A peak corresponding to the excess enthalpy of relaxation of the blend can be observed at its $T_g$ (Figure 4.5b). For samples annealed at 60 °C for 30 min, this excess enthalpic relaxation peak disappeared (Figure 4.5c). Samples tested 1 day post annealing again showed the same enthalpic relaxation peak (Figure 4.5d). These results indicated that the PLA component underwent rapid physical aging after extrusion. On annealing slightly above $T_g$, there was not any significant physical aging evidence as characterized by DSC. Annealed blend samples also underwent a rapid physical aging. The endothermic enthalpic relaxation peak around $T_g$ in DSC scans for physically aged samples (Figure 4.5b and 4.5d) indicated a reduction in free volume. This reduction in free volume with physical aging would tend to reduce molecular mobility, and hence the toughness. Wang and coworkers reported a similar observation for loss of mechanical properties of PLA-starch blends due to physical aging and attributed it to the loss of interfacial interaction between two phases [31]. The loss of interfacial attraction between two phases was assigned to the shrinking of the PLA phase on physical aging [31]. For our case, the similar interfacial attraction loss might be the reason for blend toughness loss on physical aging, but that hypothesis could not be supported based on morphological studies as the PHBHHx (minor component) composition was low (10 weight percent).
**Figure 4.5** DSC thermograms of PLA-PHBHHx (90 weight percent PLA) blend films (a) right after extrusion, (b) aged for 1 day, (c) after annealing at 60 °C for 30 min, and (d) 1 day post annealing.

As shown in Figure 4.4, physically aged blend samples regained the original toughness temporarily on annealing slightly above $T_g$ (60 °C for 30 min). Physical aging being a thermodynamically reversible process, annealing at 60 °C for 30 min led the blend films to regain their original toughness. Since the main objective of this research
was to determine whether our surface modification process influenced the film’s mechanical properties, all samples were annealed at 60 °C for 30 min just prior to testing to minimize effects of physical aging. Annealing conditions were set in such a way that there was not any significant crystallization of the blend films as characterized using WAXD patterns. This enabled us to study solely the effect of photografting reactions on blend mechanical properties.

**Surface modification**

Table 4.1 shows the water contact angle values for unmodified and surface modified PLA and blend films after 3 h UV exposure in Step 2 of the surface-modification process. Unmodified PLA and blend films were hydrophobic, with water contact angle ∼ 80°. Films grafted with poly(acrylic acid) (PAA) showed water contact angle ∼ 38° and grafted with poly(acrylamide) (PAAm) showed water contact angle ∼ 23°.

**Table 4.1** Water contact angles of unmodified and surface-modified PLA and PLA-PHBHHx blend (90 weight percent PLA) films after 3 h UV-exposure time in Step 2

<table>
<thead>
<tr>
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<th>Water Contact Angle (°)</th>
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<tbody>
<tr>
<td></td>
<td>PLA</td>
</tr>
<tr>
<td>Unmodified</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Film-g-PAA</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Film-g-PAAm</td>
<td>22 ± 2</td>
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</table>
This surface chemistry was further investigated using ATR-FTIR spectroscopy. The unmodified blend film showed the –C=O peak at wave number 1747 cm\(^{-1}\) corresponding to the PLA ester. The blend film grafted with PAA showed a shoulder at wave number 1722 cm\(^{-1}\) corresponding to the –C=O acid peak. The blend film grafted with PAAm showed the –C=O peak at wave number 1670 cm\(^{-1}\) corresponding to the amide functionality of PAAm and an amide II peak at wave number 1550 cm\(^{-1}\), which is the combination of N-H bending and C-N stretching vibrational modes (spectra not shown). These peaks confirm the presence of the expected functional groups consistent with the photografting chemistry.

**Effect of the photografting reaction on bulk properties**

Figure 4.6 shows the engineering stress-strain results for unmodified and surface-modified blend films (after annealing). These films lost their toughness significantly on surface modification due to the reduction in % elongation at break upon surface modification (Figure 4.6). Figure 4.7c summarizes the toughness data for unmodified and surface modified blend films. To investigate this toughness loss further, a control experiment was performed where unmodified blend films were subjected to the same sequential two-step photografting method, except in Step 1 the films were dip coated in pure ethanol (omitting the benzophenone), and in Step 2, the films were immersed in pure water (omitting the monomer) and the samples were exposed to the same UV irradiation. These samples are referred to as “Water Control” in Figure 4.7. The water-control
specimens showed the same toughness loss as blend-g-PAA and blend-g-PAAm (where g denotes grafted). Since all films were annealed at 60 °C for 30 min before tensile testing, the observed toughness loss was attributed primarily to the surface-modification process and not due to physical aging. DMA analyses of unmodified and surface-modified films showed that that tan δ peak height decreased upon surface modification (Figure 4.2B). These DMA results suggested that the blend films might be undergoing crystallization during the photografting reaction.

![Figure 4.6](image)

**Figure 4.6** Engineering stress-strain curve of unmodified (■) and surface-modified PLA-PHBHHx (90 weight percent PLA) blend (♦) films (after annealing).
Figure 4.7 Effect of the photografting on (a) modulus, (b) ultimate tensile strength, and (c) toughness of PLA-PHBHHx (90 weight percent PLA) blend films. The error bars represent 95% confidence intervals. The toughness of blend films grafted with PAA and PAAm showed statistically significant difference from the toughness of unmodified blend, as determined by a t-test.
To analyze this behavior in detail, % crystallinity of unmodified and surface-modified blend films was measured using WAXD (Figure 4.3). Unmodified blend (Figure 4.3a) showed a reflection peak at 13.5°. Blend-g-PAA, blend-g-PAAm, and blend films prepared by water-control experiments (Figure 4.3b, 4.3c, and 4.3d) showed a new reflection peak at 16.7°, indicating crystallization during photografting reactions. Figure 4.8 summarizes the % crystallinity values for unmodified and surface-modified blend films as calculated from WAXD patterns. It was observed that % crystallinity of blend films almost doubled during photografting reactions. To investigate the cause of the observed crystallization during photografting, two other control experiments were performed. In the first, blend films were immersed in water for 3 h without UV exposure and WAXD analyses of resultant films did not show a significant crystallinity increase (labeled “Only Water” in Figure 4.8). In another control experiment, films were exposed to 3 h of UV treatment without solvent, monomer, and initiator (labeled “Only UV”) and no significant crystallinity increase was observed. These observations led us to infer that the crystallinity increase was a combined effect of reaction solvent (water) and UV irradiation, hereafter referred to as “UV-assisted solvent induced crystallization” (UVasic).
Figure 4.8 Effect of the photografting reaction on % crystallinity of PLA-PHBHHx (90 weight percent PLA) blend films. The error bars represent 95% confidence intervals.

To investigate this behavior further, the temperature of reaction solvent (water) being exposed to UV irradiation was measured with time. It was observed that the UV irradiation heated the water from room temperature (25 °C) to 35 °C (Figure 4.9). In addition to this, DMA analyses indicated that water plasticized the blend films and increased the chain mobility. The tan δ vs. temperature curve for the water control showed the onset of chain mobility around -25 °C and significant chain mobility at 35 °C.
Blend films immersed in water for 3 h without UV exposure did not undergo any significant crystallization even if there was some extent of chain mobility detected by DMA. DMA did not reveal the onset of significant chain mobility of unmodified blend films in the temperature range investigated. This may be the reason blend films exposed to UV irradiation for 3 h (no water) did not undergo any significant crystallization. The water plasticization in conjunction with the UV heating was more likely the mechanism behind UVasic. Blend films did not show any significant change in molecular weight on surface modification as characterized using gel permeation chromatography (GPC). So the observed reduction in the toughness of the blend films on surface modification was likely due to UVasic. WAXD patterns of unmodified PLA film did not show any reflection peaks, a typical characteristic of the amorphous matrix. WAXD patterns of surface-modified PLA films also did not show any reflection peaks, indicating little, if any, UVasic during photografting (spectra not shown). Since unmodified blend films underwent UVasic during photografting and PLA films did not, PHBHHx may have acted as nucleating sites for PLA-phase crystallization. It is also possible that the PHBHHx crystallizes. A WAXD spectrum for unmodified extruded PHBHHX film showed reflection peaks at 13.5° and 16.9° (spectrum not shown). A reflection peak for PLA cast from a hot chloroform solution was observed at 16.7° [34]. In short, both PLA and PHBHHx show a reflection peak at approximately 16.7°, so the new peak at 16.7° may result from PLA-phase crystallization, PHBHHx-phase crystallization, or both.
Figure 4.9 Effect of the UV irradiation on temperature of reaction solvent (water) with time. The error bars represent 95% confidence intervals.

Crystallinity developed during photografting reactions may have important ramifications with respect to biomedical applications of these films. First, the surface-reacted films lose their toughness significantly. Moreover, PLA (major phase) degrades through the hydrolysis of backbone ester groups [28], and it has been reported that the hydrolysis selectively occurs in the amorphous regions of PLA [37-38]. So the crystallinity developed may affect the PLA degradation rate.
Figure 4.10 Tan δ as function of temperature for the unmodified blend and water control.

For blend films undergoing UVasic during photografting, one would expect surface-modified films to be more brittle, reflected by an increase in modulus. Surprisingly, modulus did not change significantly upon surface modification (Figure 4.7a). A similar effect was observed for ultimate tensile strength of unmodified and surface-modified films (Figure 4.7b). To investigate this behavior further,
thermogravimetric analyses were performed. The mass retention increased from 0.21 ± 0.18 weight percent for unmodified blend films to around 1.70 ± 0.30 weight percent for surface-modified films. Blend films prepared by water control experiments showed the mass retention of 0.70 ± 0.29 weight percent. This mass retention on surface modification was more likely due to retention of the reaction solvent (water), which could act as a plasticizer. In light of these two counteracting effects, UVasic tending to increase modulus and ultimate tensile strength and “water retention” tending to reduce modulus and ultimate tensile strength, there was not any significant change in these mechanical properties on surface modification.

CONCLUSIONS

The PLA and PHBHHx components of melt processed blend films appeared to be non-compatible based on DMA analyses. Unmodified blend films were tougher (tested right after extrusion), but lost their toughness significantly due to physical aging. Physically aged films regained their toughness temporarily on annealing at 60 °C for 30 min.

The PLA-PHBHHx blend films were successfully surface modified using a sequential two-step photografting approach, and the effect of photografting on mechanical properties of blend films was studied extensively. Contribution of physical aging to the change in mechanical properties was minimized by annealing films at 60 °C for 30 min before testing. It was observed that blend films lost their toughness
significantly due to UV-assisted solvent induced crystallization. TGA data showed the presence of reaction solvent in surface-modified films. The combination of “UV-assisted solvent induced crystallization” and the plasticizing effect of residual water resulted in insignificant changes in Young’s modulus and tensile strength on surface modification. This study showed that PLA-PHBHHx blend films lost their toughness due to 1) physical aging and 2) UV-assisted solvent induced crystallization occurring during the photografting process.
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CHAPTER FIVE

POLY(LACTIC ACID) TOUGHENING WITH A BETTER BALANCE OF PROPERTIES

INTRODUCTION

The market for renewable-resource-derived, biodegradable polymers is growing due to environmental concerns and sustainability issues associated with petroleum-based polymers [1-2]. PLA is a renewably derived (from corn starch, sugar, etc.), biodegradable, and bioabsorbable thermoplastic polyester that exhibits excellent processibility and biocompatibility and requires 25-55% less energy to produce than petroleum-based polymers [3-5]. However, its use in certain applications has been limited by its poor toughness (less than 10% elongation at break) and lack of readily reactable functional groups [6].

PLA has been toughened using a variety of plasticizers, stereochemical and processing manipulations, and biodegradable as well as nonbiodegradable rubbery (low Tg) polymers [7]. These approaches often lead to significant stiffness (modulus) loss, making resultant formulations unsuitable for certain applications. Reactive groups have also been introduced onto PLA to create bioactive surfaces for biomedical applications and tailored surfaces for commodity applications (e.g., friction modification, anti-fogging, and adhesion). However, the solvents and reagents involved in these surface-modification protocols often affect PLA bulk properties, especially toughness [6, 8].
Here we report a novel reactive-blending approach that involves a combination of polymers with complementary properties, PAA and PEG, to achieve PLA toughening without significant modulus or ultimate tensile strength (UTS) losses. In addition, this technology introduces into the PLA matrix a controlled concentration of reactive acid groups that can be readily conjugated with a variety of biomolecules containing amine or alcohol groups using carbodiimide [9-10], thionyl chloride [11], or phosphorous pentachloride [12] chemistry. Moreover, PAA is known to accelerate PLA hydrolytic degradation rate [13], which may be an advantage in certain applications. PAA was chosen as a stiffening agent due to its glassy nature. PEG was chosen as a toughening agent due to its rubbery nature. The resultant reactive blends were extruded into films and analyzed using tensile testing, DSC, DMA, and toluidine-blue-dye staining.

**MATERIALS**

PLA pellets (Mn ~ 110 kDa) were supplied by NatureWorks LLC. Acrylic acid (99.5% w/w) was obtained from Acros Organics and used as received without further purification. PEG (Mn ~ 1500 Da) was obtained from Sigma. Chloroform was purchased from VWR. Benzoyl peroxide (BPO) was obtained from Fluka.
**METHODS**

*PLA reactive blending:* As shown in Scheme 5.1, a predetermined amount of PLA was dissolved in 140 mL CHCl₃ at 100 °C for 1 h followed by addition of predetermined amounts of BPO and acrylic acid. The solution was allowed to stand at 100 °C for 10 min while the acrylic acid polymerized off the PLA backbone (PLA-g-PAA). PEG was then added to the solution and kept at 100 °C for an additional hour. The solution was then cooled to room temperature and poured in a glass dish. The solution was kept at room temperature overnight and then transferred to a vacuum oven at 70 °C for 24 h and cooled in the vacuum oven to remove any residual chloroform.

![Scheme 5.1](image_url)

**Scheme 5.1** Reactive blending approach consisting of thermal polymerization of acrylic acid from PLA chains followed by PEG blending.

*Film Extrusion:* The polymer blend was immediately transferred to an extruder after drying. A twin-screw microextruder (DSM Xplore) operating in a co-rotating mode was used to cast films at 190 °C. The tapered screws were 170 mm long and the barrel volume was 15 cm³. The polymer melt exiting the die was cooled by a stream of nitrogen gas and collected on a chill roll. The resultant films had a nominal thickness 80 ± 10 μm.
Mechanical Testing: The film samples were stored at room temperature after extrusion for 24 h before mechanical testing. The mechanical properties of the film samples (7.5 cm x 1.5 cm x 80 μm) were measured using an Applied Test System Inc. (ATS) mechanical tester according to American Society for Testing and Materials Standard (ASTM D882) specifications. A cross-head speed of 1.25 cm/min was used. The measured values averaged for five specimens with ±95% confidence intervals are reported.

RESULTS AND DISCUSSION

Scheme 5.1 represents the PLA reactive blending approach consisting of thermal polymerization of acrylic acid from PLA chains followed by PEG blending. This technology offers PLA toughening with a better balance of properties associated with introduction of reactive acid groups into the PLA matrix. Briefly, PLA was thermopolymerized with acrylic acid using benzoyl peroxide (BPO) thermal initiator followed by blending with PEG in chloroform. The resultant blend was dried and extruded using a twin-screw extruder operated in a co-rotating mode. Miscibility and crystallization behavior of the films prepared using this chemistry were evaluated using DMA and DSC, respectively (Figure 5.1). We first examined the miscibility of PLA/PEG blends. Blend miscibility is governed mainly by molecular weight and composition of the constituents. Higher molecular weight and concentration of PEG showed a tendency for it to phase separate, so relatively lower molecular weight PEG (Mn ~ 1500 Da) at a
composition of 10% was used to blend with PLA, hereafter referred to as PLA/PEG(10%). PLA/PEG(10%) blends did not undergo any significant phase separation as characterized using DMA (Figure 5.1A (d)). Tan δ vs. temperature for PLA/PEG(10%) showed only one peak corresponding to PLA’s T_g. When PLA was thermopolymerized with 3 or 10 wt% acrylic acid prior to blending with PEG, hereafter referred to as PLA-g-PAA(3%)/PEG(10%) (Figure 5.1A (b)) or PLA-g-PAA(10%)/PEG(10%) (Figure 5.1A (c)), a tan δ peak corresponding to the PEG phase was observed. This observation indicated that the PEG phase showed a phase separation tendency when blended with PLA-g-PAA (‘g’ denotes grafted). When the PAA concentration was increased from 3 to 10 wt%, the tan δ peak (T_g) corresponding to the PEG phase shifted from -47 ± 2.6 °C to -32 ± 2.6 °C. Additionally, T_g corresponding to the PLA phase increased from 43 ± 2.1 °C to 48 ± 1.7 °C. These T_g shifts with composition indicated the partial miscibility of blend constituents. PLA is hydrophobic while PAA and PEG are hydrophilic. These observations also showed the possibility of favorable intermolecular interactions between PAA and PEG (as indicated by PEG’s T_g shift with PAA concentration associated with phase separation) and between PAA and PLA (as indicated by PLA’s T_g shift with PAA concentration).
Figure 5.1  (A) Tan δ as a function of temperature of (a) neat PLA, (b) PLA-g-PAA(3%)/PEG(10%), (c) PLA-g-PAA(10%)/PEG(10%), and (d) PLA/PEG(10%).

(B) DSC scans of melt quenched (a) neat PLA, (b) PLA-g-PAA(3%)/PEG(10%), (c) PLA-g-PAA(10%)/PEG(10%), and (d) PLA/PEG(10%).
The crystallization temperature ($T_c$) of PLA decreased from $129 \pm 1 \, ^\circ C$ (Figure 5.1B (a)) for neat PLA to $93 \pm 2 \, ^\circ C$ (Figure 5.1B (d)) for the PLA/PEG(10%) physical blend. The thermopolymerization of PAA with PLA, prior to blending with PEG, increased the $T_c$ to $104 \pm 3 \, ^\circ C$ (Figure 5.1B (b)) for PLA-g-PAA(3%)/PEG(10%) and to $108 \pm 1 \, ^\circ C$ (Figure 5.1B (c)) for PLA-g-PAA(10%)/PEG(10%). This increase in $T_c$ with PAA concentration supported the possibility of favorable intermolecular interactions between PAA and PLA in these blends. PLA-g-PAA(3%)/PEG(10%) and PLA-g-PAA(10%)/PEG(10%) blends dissolved in chloroform but could not be filtered through 0.2 μm teflon filter smoothly. This may have been a result of either a small extent of crosslinking during thermal polymerization or PAA homopolymerization (PAA does not dissolve in chloroform) or both. Even small extents of crosslinking could affect glass transition and crystallization events. In order to study the effect of crosslinking, if any, during PAA thermal polymerization, films were prepared using the same chemistry but excluding the PEG blending step, hereafter referred to as PLA-g-PAA(10%). It was observed that there was no significant effect of PAA thermal polymerization on PLA’s $T_g$ (as characterized using DMA). However, PLA’s $T_c$ decreased from $129 \pm 1 \, ^\circ C$ for neat PLA to $104 \pm 1 \, ^\circ C$ for PLA-g-PAA(10%). This reduction was likely a result of the nucleating effect of homopolymerized PAA domains since crosslinking and favorable intermolecular interactions would tend to increase $T_c$. These observations confirmed the possibility of favorable intermolecular interactions affecting glass transition and crystallization events in PLA-g-PAA(3%)/PEG(10%) and PLA-g-PAA(10%)/PEG(10%) blends and not the crosslinking, if any, occurring during PAA thermal polymerization.
There was only a slight increase in the toughness of the PLA/PEG(10%) physical blend over neat PLA, as represented by the area under engineering stress-strain curves (Figure 5.2). However, thermopolymerization of 3 wt% acrylic acid, prior to PEG blending, resulted in significant toughness improvement (Figure 5.2a). Figure 5.2b shows the engineering stress-strain curves of these reactive blends. It was clearly evident that the toughness improvement was due to an increase in % elongation at break from less than 10% for neat PLA to 150 ± 20% for PLA-g-PAA(3%)/PEG(10%). This could have been an outcome of favorable intermolecular interactions between PLA-g-PAA and PEG.

Figure 5.2 (a) Toughness and (b) representative stress-strain curves of neat PLA and its reactive blends. Error bars represent 95% confidence intervals.
Other methods to improve toughness result in a substantial reduction in tensile strength and/or modulus. For this reactive-blended material, as shown in Figure 5.3, Young’s modulus and ultimate tensile strength decreased slightly from $1370 \pm 130$ MPa for neat PLA to $990 \pm 100$ MPa for PLA-g-PAA(3%)/PEG(10%) and from $42 \pm 3$ MPa to $35 \pm 3$ MPa, respectively (Figure 5.3). Increase in acrylic acid content from 3 wt% to 10 wt% retained the toughness of the films with minimal Young’s modulus ($1235 \pm 70$ MPa) and ultimate tensile strength ($37 \pm 3$ MPa) loss compared to neat PLA. This modulus and ultimate tensile strength retention was attributed to glassy ($T_g \sim 125 \, ^\circ C$) PAA chains. In addition to this, increase in $T_g$ from $43 \pm 2.1 \, ^\circ C$ of PLA phase in PLA-g-PAA(3%)/PEG(10%) to $48 \pm 1.7 \, ^\circ C$ of PLA phase in PLA-g-PAA(10%)/PEG(10%), indicated the possibility of favorable intermolecular interactions between PLA and PAA.
Figure 5.3 (a) Young’s modulus and (b) ultimate tensile strength of neat PLA and its reactive blends. Error bars represent 95% confidence intervals.

Another key advantage this technology offers is the introduction of reactive acid groups into the PLA matrix for further modifications. As a proof-of-concept, these film surfaces were stained with toluidine blue dye. Toluidine blue is a cationic dye that readily binds with acid groups and not with PLA. Neat PLA did not show any significant staining (Figure 5.4a). The color intensity increased with acid concentration (Figures 5.4b and 5.4c), indicating the presence of acid groups available for subsequent binding or conjugation.
CONCLUSIONS

This simple reactive blending technology offers PLA toughening with only minimal modulus and ultimate tensile strength loss associated with the introduction of a controlled concentration of reactive acid groups into the PLA matrix. This was achieved by using a reactive-blending approach that relied on the choice of two complementary polymers, PAA (stiffening and reactive agent) and PEG (toughening agent). PLA surface and bulk properties could be controlled by varying the concentrations of PAA and PEG. PLA toughening was attributed to an increase in PLA chain mobility due to a rubbery
PEG phase and modulus and ultimate tensile strength retention was attributed to the glassy nature of PAA and favorable intermolecular interactions between PLA, PEG, and PAA phases.
REFERENCES


CHAPTER SIX

CONCLUSIONS AND FUTURE RESEARCH RECOMMENDATIONS

CONCLUSIONS

In the first part of this research, solvent cast PLA and PHBHHx films were successfully surface modified using a sequential two-step photografting method. Acrylic acid and acrylamide were photopolymerized from the film surface with ultimate aims of improving wettability and introducing readily reactable groups onto the surface. Although the reaction solvent used in Step 2, water or ethanol, had an insignificant effect on surface properties, bulk properties were significantly affected. PLA and PHBHHx films lost their toughness and became stiffer on surface modification, with the effect being more prevalent when ethanol was used as the reaction solvent. Likewise, solvent-induced crystallization was more prevalent when ethanol was used as the reaction solvent than when water was used as the reaction solvent for PLA films. The observed toughness loss and modulus gain of PLA films on surface modification was attributed to solvent-induced crystallization and loss of residual chloroform. The presence of residual chloroform in the film specimens was undesirable from a biocompatibility standpoint. Additionally, this work showed that the photoreaction solvent affected the bulk properties of PLA and PHBHHx films cast from a chloroform solution. Therefore, further work was conducted on melt-processed films where residual solvent from the film-formation method was not an issue.
Addition of a small amount of PHBHHx (10 wt %) to PLA successfully improved the toughness of the resulting melt-processed blends. PLA-PHBHHx blend films were surface modified using the same sequential two-step photografting method using water as the reaction solvent in Step 2. The PLA and PHBHHx components of melt processed blend films appeared to be non-compatible based on DMA analyses. The PLA-PHBHHx blend films lost their toughness significantly due to physical aging. It was also observed that physically aged films regained their toughness temporarily on annealing at 60 °C for 30 min. The contribution of physical aging to the change in mechanical properties was minimized by annealing films at 60 °C for 30 min before testing. This enabled us to study the effect of photografting on mechanical properties of blend films. It was observed that the blend films underwent UV-assisted solvent induced crystallization on surface modification and lost their toughness significantly. TGA data showed the presence of reaction solvent in surface-modified films. The combination of solvent heating during UV-irradiation and the plasticizing effect of residual water resulted in insignificant changes in Young’s modulus and UTS on surface modification.

Finally, a novel reactive blending approach was designed to toughen PLA with minimal modulus and/or UTS losses and to introduce a controlled concentration of reactive acid groups into the matrix in one step. This approach relied on the choice of two complementary polymers, PAA (stiffening and reactive agent) and PEG (toughening agent). PLA surface and bulk properties were controlled by varying the concentrations of PAA and PEG. PLA toughening was attributed to an increase in PLA chain mobility due
to PEG and modulus and UTS retention was attributed to the glassy nature of PAA and favorable intermolecular interactions between PLA, PEG, and PAA phases.

**RECOMMENDATIONS FOR FUTURE RESEARCH**

Physical aging is a major concern for PLA and PLA-based formulations. Results in Chapter 4 showed that melt-processed PLA-PHBHHx (90 wt % PLA) blend films lost their toughness significantly due to physical aging. The effect of physical aging can be minimized by stretching these films uniaxially and/or biaxially. These extruded films can be stretched above PLA’s glass transition temperature to induce orientation and minimize the physical aging effect. However, care should be taken to minimize PLA thermal degradation while stretching. Stretching these films slightly above PLA’s glass transition temperature may help minimize the thermal degradation. A more economical way would be to stretch these films online during extrusion.

As a proof-of-concept, PLA was successfully toughened with only minimal modulus and UTS losses and introduction of a controlled concentration of reactive acid groups into the PLA matrix. This was achieved by using a novel reactive blending approach. Following are different potential modifications that may render this technology more attractive and industrially relevant:

1. These blending reactions should be carried out in the melt-phase in an extruder.

   This could be performed in CAEFF’s microextruder (DSM Xplore) that enables
2. One of the major drawbacks of PLA is its slow degradation rate. This technology introduces controlled concentrations of hydrophilic PAA and PEG into the PLA matrix. PAA and PEG have the potential to increase the hydrolytic degradation rate of PLA. The effect of PAA and PEG concentrations on the rate of PLA’s hydrolytic degradation should be studied. This work should be performed at different pH and temperature conditions including physiological pH (7.4) and temperature (37 °C).

3. PLA-based formulations reported in Chapter 5 should be prepared using thermopolymerizable hyaluronic acid instead of acrylic acid (a more biocompatible version for biomedical applications). This may retain all the advantages of these formulations and make it more biocompatible.

4. A detailed study should be conducted to evaluate the effect of PAA, PEG, and benzoyl peroxide concentrations, reaction time, and temperature on surface and bulk properties of the resultant films. An ultimate aim of this study could be to create a library of these variables and resultant surface and bulk properties. This library may be used to produce several formulations depending on the need of the applications.

5. A detailed rheological study should be performed for these PLA-based formulations.
6. PLA-based composites have exhibited excellent shape memory properties. PLA/hydroxyapatite composite shape-memory properties have been studied above 70 °C [1]. It would be very interesting with respect to biomedical applications (such as sutures, implants, etc.) to study the dual and triple shape memory properties of these PLA-based formulations. Increasing the composition of PEG may lead to desirable shape-memory properties at physiological temperature (37 °C). Langer et al. [2-3] and Mather et al. [4] have done significant research on polymer shape-memory properties and their work should serve as a guide for further studies with our materials.
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APPENDIX A

MICROPATTERNING OF COVALENTLY ATTACHED BIOTIN ON
POLY(LACTIC ACID) FILM SURFACES

INTRODUCTION

Micropatterning biomolecules on surfaces has applications ranging from biosensors [1-2], medical implants [3], bioassays [4], and lab-on-a-chip [5]. Spatially controlled organization of biological ligands and proteins on surfaces is very important with respect to these applications. The commercial development of specific patterns relies mainly on the fabrication ease, repeatability, stability, and cost. Polymers have progressively shown the potential to be a viable alternative to the conventional microfabrication materials such as glass, silicon, or gold [4]. Environmental concerns associated with petroleum-based polymers make biodegradable polymers more attractive microfabrication candidates. Aliphatic poly(hydroxyacid) type biodegradable polymers, especially poly(lactic acid) (PLA), are of growing importance. PLA is a biodegradable and bioabsorbable thermoplastic polyester derived from renewable resources like corn, starch, or rice and exhibits excellent biocompatibility [6-12]. PLA films are commercially produced and their mechanical properties can be significantly improved by blending with other biodegradable polymers like poly(hydroxalkanoates) (PHAs) [11]. PLA production requires 25-55% less energy compared to petroleum-based polymers and estimates are that this can be further reduced to less than 10% in the future [8]. This
makes the use of PLA films in microfabrication technology potentially advantageous with respect to cost as well as degradability.

PLA has been widely researched to improve its surface [13-15] and bulk properties [16-20]. However, methods to spatially control the organization of biomolecules on PLA, which are important with respect to specific biomedical applications, are still very limited. Lin et al. [21] have applied soft lithography techniques to micropattern proteins on PLA. Briefly, poly(oligoethyleneglycol methacrylate) (poly-OEGMA) was printed on PLA to create micron-size, protein-resistant areas. Proteins adsorbed on unprinted regions leaving printed regions intact. Since the poly-OEGMA was not covalently attached to the PLA surface, these protein micropatterns might not be suitable for certain biomedical applications in which devices would require permanent surface patterns.

Micropatterning methods often consist first of a surface modification process. PLA is chemically inert with no readily reactable side chain groups making its surface modification a challenging task. PLA has been surface modified using a variety of techniques such as coating [22], migratory additives [23], plasma treatment [24-25], and entrapment [26-27]. Each has its own advantages and disadvantages, but most of these surface modifications are not permanent. In earlier reports, we have used a photoinduced grafting approach to surface modify PLA [15, 19, 20]. This approach resulted in the covalent attachment of various molecules to PLA film surfaces.

In this research, photoinduced grafting in conjunction with photolithography [4] was used to micropattern reactive poly(acrylic acid) (PAA) on PLA. These micropatterns
were confirmed by staining with toluidine blue dye, as well as characterization of surface topography using AFM. The PAA micropatterns were subsequently conjugated to amine-terminated biotin using water soluble carbodiimide chemistry. The conjugation reaction was investigated using XPS. These biotin modified PAA patterned PLA films were then subjected to fluorescent proteins to demonstrate their patterning efficiency. To our knowledge, there have not been any attempts to covalently micropattern biological ligands, such as biotin, on PLA surfaces. Therefore, the overall objective was to covalently micropattern biotin on PLA surfaces, evaluate the robustness of the micropattern, and study subsequent streptavidin adsorption.

MATERIALS

Acrylic acid (99.5% w/w) was obtained from Acros Organics and used as received without further purification. Ethanol, glass slides, HPLC water, and benzophenone were purchased from Fisher Scientific. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aldrich. (+)-Biotinyl-3,6,9-trioxaundecanediame and Alexa488 labeled streptavidin were purchased from Pierce. The Alexa488 labeled streptavidin was supplied as a yellow colored liquid at a concentration of 1 mg/ml in 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2, containing 1% BSA and 0.02% sodium azide. Dulbecco’s phosphate buffered saline (PBS) solution was purchased from Gibco.
METHODS

PLA Film Extrusion: Prior to extrusion, PLA pellets were dried in a vacuum oven at 70 °C for 24 h and cooled in the vacuum oven to remove any residual moisture. A single screw extruder (HAAKE INC.) was used to cast PLA films. The temperature profile of the extruder was as follows: Zone 1 (located near the hopper) – 180 °C, zone 2 – 190 °C, zone 3 – 200 °C, pump – 200 °C, and die – 190 °C. The polymer melt exiting the die was cooled on a chill roll. The resultant films had a nominal thickness 125 μm.

Micropatterning PAA on PLA: Scheme A.1 shows the PAA micropatterning process flow diagram. A PLA specimen was sonicated in ethanol for 5 min to clean the surface. It was subsequently dipped into benzophenone solution in ethanol (5 wt %) for 1 min and washed with copious amounts of ethanol. The benzophenone-dip-coated PLA specimen was dried using a nitrogen gas stream. This specimen was fixed on a glass slide. A couple drops of aqueous acrylic acid solution (10 wt %) were placed on the film surface. When a photomask was lowered on top of the specimen, aqueous acrylic acid solution spread uniformly. Another glass slide was placed on top to ensure that there was no air gap between photomask and aqueous acrylic acid solution. This assembly was subsequently exposed to UV irradiation for predetermined time in a UV processor (EXFO 100 W Acticure ultraviolet/visible spot-curing system). The processor had a wavelength range of 250-650 nm and intensity of 40 mW/cm² at 365 nm measured using an OAI 306 UV powermeter. The resulting PAA micropatterned PLA specimen was sonicated in water.
for 5 min and washed with copious amounts of water. The acid group surface density, $\gamma$ (acid groups/nm$^2$), was estimated using the following equation [28]

$$\gamma = \frac{\rho h_d N_A}{M} = \frac{602 \cdot 3 \rho h_d}{M}$$

(1)

where $\rho$ is density of PAA (1.22 g/cm$^3$) [29], $h_d$ is the dry layer thickness (nm) of the surface grafted PAA layer, $N_A$ is Avogadro’s number, and $M$ is the molecular weight (g/mol) of acrylic acid.

Scheme A.1 Photolithography [4] approach to micropattern PAA on PLA.
**PAA-Biotin Conjugation and Subsequent Streptavidin Adsorption:** The PAA micropatterned PLA specimen was stirred with EDC (6 wt %) and NHS (3.6 wt %) solution in PBS buffer for 3 h at room temperature. EDC and NHS concentrations were selected to give a stochiometric molar ratio of 1:1. The specimen was then washed with copious amounts of PBS buffer solution to remove any unreacted EDC or NHS. This procedure was used to activate the acid groups. The EDC/NHS activated film was then stirred in amine-terminated biotin ligand solution in ethanol (0.42 wt %) for 3 h and rinsed with ethanol and PBS buffer solution. The specimen was subsequently immersed in a solution of Alexa488 streptavidin (35 μl/ml) in PBS buffer for 3 h. It was then washed with copious amounts of PBS buffer solution and dried in a vacuum oven before examining under a fluorescence microscope. Neat PLA film was immersed in biotin solution for 3 h and then washed with copious amounts of ethanol to examine the extent of biotin adsorption on neat PLA. Neat PLA film was immersed in streptavidin solution for 3 h and then washed with copious amounts of buffer to examine the extent of streptavidin adsorption on neat PLA.
RESULTS AND DISCUSSION

Micropatterning PAA on PLA

Scheme A.1 represents the process flow diagram of PAA micropatterning on PLA using photolithography. This approach offers permanent micropatterning with a range of microfeature shapes and large patterned areas. The progression of the PAA micropatterning was characterized using ATR-FTIR spectroscopy (Figure A.1). It was observed that the –C=O acid stretch of PAA at 1720 cm$^{-1}$, which is a shoulder to the –C=O backbone PLA ester stretch at 1747 cm$^{-1}$, became stronger with UV irradiation time. This observation was supported by water contact angle goniometry data as shown in Figure A.2. Neat PLA is hydrophobic with a water contact angle $\sim 80^\circ$, and the water contact angle decreased with UV irradiation time. PAA is hydrophilic.
Figure A.1 Representative ATR-FTIR spectra revealing the progression of PAA micropatterning on PLA. The –C=O “ester stretch” of PLA is represented by a peak at 1747 cm\(^{-1}\) (♦) and the –C=O “acid stretch” of PAA is represented by a peak at 1720 cm\(^{-1}\) (●). A 2 mm wide stripe on PLA was characterized to monitor the progression of PAA micropatterning.

The decrease in water contact angle suggested that a water drop placed on a PLA-g-PAA (where g denotes grafted) region encountered a greater fraction of hydrophilic PAA than relatively hydrophobic PLA as UV irradiation time increased. For a given light intensity (40 mW/cm\(^2\) at 365 nm) and monomer concentration (10% w/w acrylic acid in
water), the surface grafted PAA molecular weight and number of PAA graft polymerization initiation sites were expected to increase with UV irradiation time. Either (or both) of those effects would cause a decrease in contact angle with increased UV irradiation time. This contact-angle decrease was the same trend observed in prior work on non-patterned PLA films (direct UV exposure without photomask) photografted with PAA [15, 19, 20].

![Figure A.2](image)

**Figure A.2** Effect of UV irradiation time on static water contact angle of 2 mm wide PAA stripe on PLA. The error bars represent 95% confidence intervals.

This behavior was further investigated by monitoring the PAA micropatterned PLA surface topography using AFM (Figure A.3). A well-defined contrast in these phase
images indicated that the AFM tip experienced distinct interactions with PLA and PAA. As shown in the upper plots in Figure A.3, the dry thickness of the PAA stripe on PLA increased with irradiation time from 60 to 170 to 360 nm, corresponding to 600 to 1700 to 3700 acid groups/nm² from equation (1). This thickness increase with UV irradiation time confirmed the increase in surface grafted PAA molecular weight, consistent with the increase in the ATR-FTIR –C=O acid stretch of PAA at 1720 cm⁻¹, which is a shoulder to the –C=O backbone PLA ester stretch at 1747 cm⁻¹ (Figure A.1), and the decrease in water contact angle (Figure A.2) with UV irradiation time. This surface-topography control could be an important consideration for biomaterial design since it is one of the cell response governing factors [30-31]. The surface topography images in Figure A.3 revealed that, for a UV irradiation time of 10 min, the PAA stripe was blunt with significant edge defects. For UV irradiation times of 15 and 20 min, the PAA stripe was sharper, with minimal edge defects. However, it was observed that for a UV irradiation time of 20 min, there was significant PAA bulk polymerization in the supernatant, which led to significant non-specific adsorption of PAA chains from the supernatant onto the PLA surface. This non-specific PAA adsorption was not acceptable for subsequent biotin conjugation. The non-specific PAA adsorption on PLA, for UV irradiation times longer than 15 min, was minimized significantly by immersing PAA micropatterned films in hot water or sonicating them in water at room temperature for 1 h. However, these methods significantly damaged the entire PAA micropatterned PLA surface topography making it unsuitable for subsequent reactions and characterization. Since a UV irradiation time of 15 min produced the best pattern quality with minimal edge defects and insignificant
non-specific PAA adsorption on PLA, the UV irradiation time was set to 15 min for subsequent experiments.

![Image of graph showing PAA microstripe height and surface topography](image)

**Figure A.3** Effect of UV irradiation time on PAA microstripe height and surface topography of PAA micropatterned PLA.

The PAA pattern quality and the extent of non-specific PAA adsorption from the supernatant onto PLA were also monitored by staining the grafted surface with toluidine blue dye and observing under an optical microscope. Toluidine blue is a cationic dye that
readily binds to the carboxylate groups of PAA, but not to inert PLA. The best pattern quality was achieved for a UV-irradiation time of 15 min (Figure A.4). For a UV-irradiation time of 10 min, the boundary between PLA-g-PAA and base PLA was not sharp, revealing some edge defects. For a UV-irradiation time of 15 min, the boundary between PLA-g-PAA and base PLA was sharper, giving the best pattern quality. These observations were consistent with the AFM results.

**Figure A.4** Optical micrographs of toluidine-blue-stained PAA micropatterned PLA (UV irradiation time 15 min) revealing micropatterns of different shapes and sizes.
PAA-Biotin conjugation

PAA has carboxylic acid (-COOH) groups that can be conjugated to -NH₂ groups of amine-terminated biotin using standard water soluble carbodiimide chemistry (Scheme A.2). EDC and NHS were used to activate the acid groups, which were subsequently reacted with amine-terminated biotin.

Scheme A.2 Scheme of the EDC/NHS mediated PAA conjugation with amine-terminated biotin.
The surface chemistry was investigated using XPS. Figures A.5a and A.5b show XPS survey scans for neat PLA and PLA-g-biotin respectively. Figure A.5a showed two peaks located at binding energies 531 and 284 eV corresponding to the O 1s and C 1s signals. Based on XPS survey scans for neat PLA, the C/O ratio was 1.49 ± 0.34, close to the theoretical value of 1.5 based on the chemical structure of PLA (see Figure A.6). XPS survey scans for PLA-g-biotin showed two additional elements: N 1s at 398 eV and S 2p at 165 eV. The presence of these two elements, especially S, confirmed successful PAA-biotin conjugation.
**Figure A.5** XPS survey scans for (a) neat PLA and (b) PLA-g-biotin.
The biotin immobilization surface chemistry was further investigated using a high resolution XPS scan of C 1s. The high resolution scan of neat PLA was deconvoluted into three C 1s component peaks (283.8, 285.7, and 288.0 eV) of approximately equal composition corresponding to the three types of carbon atoms present in PLA [32] (spectrum not shown). These results were expected based on the PLA chemical structure and agree well with previous reports [32-33]. The high resolution C 1s scan of PLA-g-biotin showed two additional peaks (Figure A.6). Peak 4 at 284.6 eV was assigned to C atoms bonded to secondary N atoms (C-NH-CO) and peak 5 at 288.5 eV was assigned to the carbonyl C atoms in the amide linkages (C-NH-CO). These peaks confirmed the carbodiimide mediated reaction of biotin’s –NH\textsubscript{2} with acid groups to form amide linkages. An increase in peak 2 at 285.7 eV (C-O) likely resulted from C-O groups of poly(ethylene glycol) (PEG) chains contained within the biotin. An increase in the peak 3 at 283.8 eV (CH\textsubscript{2}) likely resulted from biotin CH\textsubscript{2} groups. These peak assignments were based on literature reports. [32-36]
Figure A.6 XPS high-resolution C 1s spectra for PLA-g-biotin.
The N/S ratio of a biotin modified PAA stripe on PLA was 9.2 ± 2.7, while the theoretical N/S ratio based on the biotin structure is 4.0. This indicated that the EDC/NHS activated PAA-biotin conjugation reaction conversion was only 16% (16 out of 100 activated acid groups were successfully conjugated with biotin), and the excess N concentration resulted from EDC/NHS activated acid groups that were not conjugated to biotin (Scheme A.2). This lower conversion was the same outcome for the carbodiimide based chemistry, involving poly(methacrylic acid) (PMAA) and RGD peptide conjugation, with acid-peptide conversion of only 12% as reported by others [37]. The lower EDC/NHS activated PAA-biotin conjugation conversion is thought to be a result of minimized mass transfer of biotin into the EDC/NHS activated PAA layer, due to the large acid group density (1700 acid groups/nm²) and the relatively long (23 Å [38]) spacer arm attached to biotin. Attempts were made to increase activated acid-biotin conjugation conversion by increasing the reaction time. Since this methodology significantly affected the resultant film topography (longer reaction time resulted in curled films with rough surfaces), biotin modified micropatterned films with the 16% PAA-biotin conjugation were used for the subsequent streptavidin adsorption experiments.

Streptavidin adsorption on biotin modified PAA micropatterned PLA

The biotin modified PAA micropatterns were then immersed in fluorescent streptavidin solution in PBS buffer to demonstrate their patterning efficiency. Figure
A.7a demonstrated that streptavidin adsorption was primarily confined to the biotin modified PAA regions. A control experiment was performed where a PAA micropatterned PLA surface (without biotin) was exposed to the fluorescent streptavidin solution and did not reveal any significant streptavidin adsorption on PAA stripes (Figure A.7b). This confirmed that the streptavidin adsorption resulted from the biotin modification of the PAA stripes. As shown in Figure A.8, the N concentration of biotin modified PAA micropatterned PLA film exposed to streptavidin solution was approximately the same as the N concentration of the fluorescent streptavidin solution used as received (the latter was calculated from an XPS survey scan of a gold-coated silicon wafer dipped in fluorescent streptavidin solution). XPS probes the uppermost 2-3 nm. High resolution X-ray crystallographic studies of streptavidin showed it to be approximately 5.4 nm X 5.8 nm X 4.8 nm in size with the two pairs of biotin binding sites on the opposite faces separated by the shortest dimension [39]. This implied that XPS may not detect significant biotin once streptavidin was adsorbed on it, and was confirmed by an XPS spectrum of biotin modified PAA patterns exposed to streptavidin solution, which did not reveal any S atoms (coming exclusively from biotin). The cross-sectional area of a streptavidin molecule is about 30 nm² while that of a biotin ligand is no more than 0.3 nm² [40]. Theoretically two biotin molecules bind to each streptavidin molecule. Assuming all biotin molecules were equispaced on the surface and all of them were bound to streptavidin, the surface coverage of streptavidin molecules would likely make very few unreacted activated acid groups detectable by XPS. This indicated that the N concentration of biotin modified micropatterns exposed to streptavidin resulted mainly
from the streptavidin N atoms (and not from unreacted activated acid groups or underlying biotin molecules). Hence it was inferred that even if the biotin immobilization conversion was only 16%, there was significant streptavidin adsorption on the biotin modified PAA micropatterns on PLA. This significant streptavidin adsorption could be a result of extraordinarily high streptavidin affinity for biotin.

Figure A.7 Fluorescent micrographs of (a) Alexa488-streptavidin micropatterned PLA surface and (b) PAA micropatterned PLA surface (without biotin) exposed to Alexa488-streptavidin.
Non-specific adsorption

PLA is hydrophobic with a water contact angle $\sim 80^\circ$. There is likely to be some degree of non-specific protein adsorption on PLA, some of which is evident in Figure A.7a. Control experiments were performed to investigate the extent of non-specific adsorption on PLA. In control experiment 1, neat PLA film was immersed in biotin solution for 3 h and then washed with copious amounts of ethanol to examine the extent of biotin adsorption on neat PLA. An XPS survey scan of this film did not detect the presence of N or S (elements coming exclusively from biotin), and confirmed that the biotin adsorption on neat PLA was not significant. Biotin used in this research was attached to hydrophilic PEG chains, while the neat PLA is relatively hydrophobic, resulting in insignificant biotin adsorption on neat PLA.
Figure A.8 N concentration (as calculated from XPS survey scans) of streptavidin adsorbed on biotin modified PAA stripe and neat PLA.

In control experiment 2, neat PLA film was immersed in streptavidin solution for 3 h and then washed with copious amounts of buffer to examine the extent of streptavidin adsorption on neat PLA. The N atomic concentration was monitored to examine the extent of streptavidin non-specific adsorption on PLA (Figure A.8). The N concentration of neat PLA film exposed to streptavidin solution (middle bar) was lower than that of biotin modified micropatterns exposed to streptavidin solution (left-most bar). Therefore, the streptavidin adsorption was preferentially confined to the biotin modified PAA regions. In future work, the non-specific streptavidin adsorption on the unmodified PLA
regions can be significantly reduced by grafting a non-fouling polymer like PEG to PLA prior to micropatterning.

CONCLUSIONS

Biotin was successfully covalently micropatterned on PLA using a two-step approach. Reactive PAA groups were micropatterned on PLA using photolithography in Step 1. The PAA grafted layer thickness increased with UV irradiation time. The PAA micropatterned PLA films analyzed by ATR-FTIR spectroscopy, water contact angle goniometry, and AFM indicated that “the optimum UV irradiation time” required to achieve the best pattern quality, with minimal edge defects and non-specific PAA adsorption from supernatant onto PLA, was 15 min. PAA was successfully conjugated to amine-terminated biotin using water soluble carbodiimide chemistry in Step 2. XPS analyses confirmed the amide linkages formed by reaction of biotin’s amine with acid groups. Even if the EDC/NHS activated PAA-biotin conjugation reaction conversion was only 16%, there was significant streptavidin adsorption on biotin modified micropatterns, likely due to high streptavidin affinity for biotin. The non-specific biotin adsorption on PLA was minimal and this was attributed to hydrophilic PEG chain contained within the biotin. Although streptavidin adsorption was primarily confined to biotin modified PAA regions, XPS analyses revealed some degree non-specific streptavidin adsorption on unmodified PLA.
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APPENDIX B

NOVEL TOUGHER POLY(LACTIC ACID)-POLY(ETHYLENE GLYCOL) METHACRYLATE REACTIVE BLENDS

INTRODUCTION

Poly(lactic acid) or poly(lactide) (PLA) is a renewably derived biodegradable and bioabsorbable thermoplastic polyester that has exhibited excellent biocompatibility and thermal processibility [1-8]. In addition to this, PLA is recyclable, compostable, and requires 25-55\% less energy to produce than petroleum-based polymers [9-10]. These attractive characteristics make PLA a potential replacement for many petroleum-based polymers. However, the major drawback of PLA is its poor toughness with \% elongation at break less than 10\% [11]. This limits its use in many consumer and biomedical applications.

Poly(ethylene glycol) methacrylate (PEGMA) is a thermopolymerizable macromer with excellent biocompatibility and hydrophilicity. PEGMA itself has poor mechanical properties and thermal processibility but has a potential to toughen PLA. PLA has been copolymerized with poly(ethylene glycols) (PEGs) to improve its mechanical and biomaterial properties. PLA’s drug-delivery properties have been improved by synthesizing diblock and triblock PLA-PEG copolymers. However, PLA and PEG underwent phase separation leading to poor mechanical properties of the copolymers [12]. PLA-PEG block copolymers produced by copolycondensation of PLA-
diols and PEG-diacids using carbodiimide-based wet chemistry showed better compatibility. These copolymers did not phase separate and exhibited improved mechanical properties [13].

Compared to PLA-PEG copolymerization, blending is a more simple and convenient methodology to improve PLA’s mechanical and biomaterial properties. Pillin et al. [14] have reported PEG as the most efficient for glass transition temperature reduction when compared with poly(1,3-butanediol), dibutyl sebacate, and acetyl glycerol monolaurate. PEG (Mn ~ 20 kDa)-PLA solvent cast blends (40 wt% PEG) were found to be very ductile [15]. Blend miscibility and mechanical properties are governed mainly by the composition of the constituents. Melt processed PLA-PEG bends (PEG Mn ~ 20 kDa) were found to be miscible, showed improved ductility, and reduced tensile strength for concentrations up to 50 wt% PEG. However, above 50 wt% PEG, blend crystallinity was found to increase significantly and resulted in an increased modulus and decreased ductility [16].

In this publication, we report the synthesis and mechanical properties of novel tougher PLA-PEGMA reactive blends. The mechanical properties of these reactive blends were found to be composition dependent. PLA-PEGMA blends were characterized using dynamic mechanical analysis (DMA) and tensile testing.
MATERIALS

PLA pellets (Mn ~ 110 kDa) were supplied by NatureWorks LLC. PEGMA (Mn ~ 360 Da) was obtained from Sigma and was purified by passing through a neutral alumina column to remove the monomethyl ether hydroquinone inhibitor. Chloroform was purchased from VWR. Benzoyl peroxide (BPO) was obtained from Fluka.

METHODS

PLA reactive blending: As shown in Scheme B.1, a predetermined amount of PLA was dissolved in 100 mL CHCl₃ at 100 °C followed by addition of predetermined amounts of PEGMA and BPO (10 wt% of PEGMA) predissolved in 20 mL chloroform at room temperature. The solution was allowed to stand at 100 °C for 1 h. The solution was then cooled to room temperature and poured in a glass dish. The solution was kept at room temperature overnight and then transferred to a vacuum oven at 70 °C for 24 h and cooled in the vacuum oven to remove any residual chloroform.

Scheme B.1 Reactive blending approach consisting of thermal polymerization of PEGMA.
Film Extrusion: The polymer blend was immediately transferred to an extruder after drying. A twin-screw microextruder (DSM Xplore) operating in a co-rotating mode at 190 °C was used to cast films. The tapered screws were 170 mm long and the barrel volume was 15 cm³. The polymer melt exiting the die was cooled by a stream of nitrogen gas and collected on a chill roll. The resultant films had a nominal thickness 80 ± 10 μm.

Mechanical Testing: The film samples were stored at room temperature after extrusion for 24 h before mechanical testing. The mechanical properties of the film samples (7.5 cm x 1.5 cm x 80 μm) were measured using an Applied Test System Inc. (ATS) mechanical tester according to American Society for Testing and Materials Standard (ASTM D882) specifications. A cross-head speed of 1.25 cm/min was used. The measured values averaged for five specimens with ±95% confidence intervals are reported.

RESULTS AND DISCUSSION

Scheme B.1 represents the PLA reactive blending approach consisting of thermal polymerization of PEGMA. Briefly, PEGMA was thermopolymerized in the presence of PLA using BPO thermal initiator. The resultant blend was dried and extruded using a twin-screw extruder operated in a co-rotating mode. Miscibility of the reactive blend films prepared was studied using DMA. Tan δ vs. temperature for these reactive blend films showed two well defined peaks corresponding to the constituent PLA and PEGMA
phases. This confirmed the blend constituents to be non compatible. In addition to this, PLA’s glass transition temperature decreased with an increase in PEGMA composition (i.e., at 20 wt% and 40 wt% PEGMA). However, the glass transition temperature of the PEGMA phase did not change as significantly (Figure B.1).

![Graph showing the effect of PEGMA composition on glass transition temperatures](image)

**Figure B.1** The effect of PEGMA composition on the glass transition temperatures of the reactive blend constituents.

It was observed that the reactive blends containing 10 wt% and 20 wt% PEGMA were tougher than neat PLA with the effect being more prominent for the reactive blends containing 20 wt% PEGMA (Figure B.2a). This was attributed to the increase in chain mobility as indicated by the reduction in PLA’s glass transition temperature from 59 °C for neat PLA to 53 °C for the reactive blend containing 20 wt% PEGMA. Further
increase in PEGMA concentration had only minimal toughness improvements. The observed increase in the toughness of PLA was a result of increase in % elongation at break (Figure B.2b). Although PLA was toughened successfully using this chemistry, the toughness improvements were associated with stiffness loss. It was observed that the reactive blends containing 10 wt%, 20 wt%, and 40 wt% PEGMA lost their modulus compared to neat PLA (Figure B.3). To minimize this deficiency, the PLA modification detailed in Chapter 5 was developed.
Figure B.2 (a) Toughness and (b) % Elongation at Break of neat PLA and its reactive blends. Error bars represent 95% confidence intervals.
Figure B.3 Young’s modulii of neat PLA and its reactive blends. Error bars represent 95% confidence intervals.

CONCLUSIONS

PLA was successfully toughened using a novel reactive blending technology that relies on the thermal polymerization of PEGMA in the presence of PLA. PLA bulk properties could be controlled by varying the concentrations of the blend constituents. PLA toughening (particularly the reactive blend containing 20 wt% PEGMA) was attributed to an increase in PLA chain mobility due to a rubbery PEGMA as indicated by the reduction in the glass transition temperature of the PLA phase. However, PLA
toughening was associated with stiffness loss, and further work to alleviate this problem is provided in Chapter 5.
REFERENCES


APPENDIX C

PACLITAXEL ATTACHMENT TO PLA-PHBHHx BLEND FILMS

INTRODUCTION

Poly(lactic acid) PLA is an attractive biodegradable polymer since its mechanical properties can be improved by blending with other biodegradable polymers like poly[(3-hydroxybutyrate)-co-(3-hydroxyhexanoate)] (PHBHHx) [1-2]. However, surface modification of the blend is extremely difficult due to the lack of any modifiable side chain groups on PLA or PHBHHx. Hence a sequential two-step photografting approach was used to graft poly(acrylic acid) (PAA) to blend films [3].

Paclitaxel is a widely used anti-cancer drug. It is favored due to its high efficacy against a variety of cancers including: small and non-small cell lung cancer, ovarian cancer, breast cancer, head and neck cancer, colon cancer, melanoma, and Kaposi’s sarcoma [4]. There are several drug-delivery techniques, such as poly(ethylene glycol) (PEG) based hydrogels [5-6], microspheres [7], and nanocarriers [8].

The overall objective of this small part of my research was to prepare Paclitaxel-delivering PLA-PHBHHx blend films, focusing on drug attachment to the films. In this research, Paclitaxel was covalently attached to PLA-PHBHHx blend films through an easily hydolyzable ester bond using water soluble carbodiimide chemistry. ATR-FTIR spectroscopy and thermogravimetric analyses (TGA) were used to characterize the drug-attached films.
MATERIALS

Acrylic acid (99.5% w/w) and dimethyl sulfoxide (DMSO) were obtained from Acros Organics. Benzophenone and HPLC water were obtained from Fisher Scientific. Buffer solution of pH 7.2 was obtained from Invitrogen Corporation. Paclitaxel (> 99% purity) was obtained from NATLAND International Corporation. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aldrich. All chemicals were used as received.

METHODS

The blend films (10 wt% PHBHHx) with a nominal thickness of 125 μm were extruded using a single screw extruder. The acrylic acid monomers were photografted using a previously designed sequential two-step photografting approach [3]. Briefly, a film specimen was dip coated in a 5% w/w benzophenone solution in ethanol for one minute. The film was then allowed to dry at room temperature to ensure that the ethanol was evaporated. The film was subsequently exposed to UV irradiation in an inert atmosphere for 5 min on each side. The resultant film was sonicated in ethanol to remove unreacted benzophenone. Following UV exposure and sonication, the benzophenone-grafted film was placed in a 10% v/v acrylic acid solution in water and exposed to UV irradiation for 1.5 h. The film was then sonicated in water for 5 min in order to remove
excess monomer or physisorbed polymer. To ensure attachment of PAA chains, the films were characterized using ATR-FTIR spectroscopy. The characterization was conducted using a Nicolet Avatar 360 with a horizontal, multibounce ATR attachment.

The resulting PLA-g-PAA film was stirred with a 1% w/w NHS and 7.5% EDC solution in water for 2 h. The film was then sonicated in water for 5 min to remove any unreacted chemicals. The resulting film was stirred with a 0.25% w/w Paclitaxel solution in DMSO for 3 h. The resulting film was sonicated in DMSO for 5 min to remove any extraneous Paclitaxel.

RESULTS AND DISCUSSION

A sequential two-step photografting method was successfully employed to create reactive PAA groups on film surface. These acid groups were subsequently linked to Paclitaxel –OH groups as shown in Scheme C.1. Theoretically, Paclitaxel would be attached to the film surfaces through an easily hydrolyzable ester bond.

Typical ATR-FTIR spectra of unmodified blend film, blend-g-PAA, and blend-g-Paclitaxel are shown in Figure C.1. The unmodified blend film spectrum showed a peak at 1756 cm\(^{-1}\) (spectrum C.1a) corresponding to the –C=O peak for the backbone ester in PLA. Blend-g-PAA film spectrum showed a peak corresponding to the –C=O acid stretch at 1720 cm\(^{-1}\) (spectrum C.1b). Blend-g-Paclitaxel film spectrum showed a peak at 1650 cm\(^{-1}\) corresponding to the –C=O amide (tertiary) stretch (spectrum C.1c). This confirmed the covalent attachment of Paclitaxel to the PLA film.
Scheme C.1 Reaction scheme used to attach Paclitaxel to PLA-PHBHHx blend films.
Figure C.1 Representative ATR-FTIR spectra of (a) unmodified blend film, (b) blend-g-PAA, and (c) blend-g-Paclitaxel. Spectrum (a) shows the “ester peak of PLA” at 1756 cm\(^{-1}\) (♦). Spectrum (b) shows the “acid peak of acrylic acid” at 1720 cm\(^{-1}\) (●). Spectrum (c) shows “the amide peak of Paclitaxel” at 1650 cm\(^{-1}\) (■).

One crucial step towards biocompatibility of blend-g-Paclitaxel films would be to determine the residual DMSO. TGA was employed to determine the amount of solvent that remained in the film (Figure C.2). Paclitaxel-attached blend showed residual mass around 20 wt%. This could primarily be DMSO. The presence of residual solvent is not
acceptable in terms of biocompatibility; as a result, it is essential to modify Paclitaxel attachment chemistry using more benign solvents.

![Diagram](image)

**Figure C.2** TGA curves of (a) unmodified blend film and (b) blend-g-Paclitaxel.

**CONCLUSIONS**

Reactive acid groups were successfully created on PLA-PHBHx blend films using a sequential two-step photografting method. Water soluble carbodiimide chemistry was then used to attach the Paclitaxel. Paclitaxel was successfully attached to the blend film surface; however, the chemistry used needs modification to employ more benign solvents.
REFERENCES


