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*ACS Appl. Mater. Interfaces*, **Just Accepted Manuscript** • DOI: 10.1021/acsami.6b14348 • Publication Date (Web): 20 Dec 2016

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# Increasing Stability of Biotin Functionalized Electrospun Fibers for Biosensor Applications

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*Received: / Accepted: /Published:*

## Abstract

This paper describes the effects of both solvent and co-polymer block lengths on the stability of electrospun poly(lactic acid)/poly(lactic acid)-b-poly(ethylene glycol) (PLA/PLA-b-PEG) and PLA/PLA-b-PEG-Biotin fibers in water. By tailoring the block length of copolymers PLA-b-PEG, water stability of electrospun fibers is improved over fibers reported previously. The solvent used also influenced the stability and hydrophilicity of resulting fibers. Fibers formed using 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) have greater water stability, but less PEG at the surface of fibers than fibers spun from Dimethylformamide (DMF). Attaching biotin to the end of PLA(3600)-b-PEG(2000) and spinning from DMF resulted, initially, in 7.6 % of the total biotin incorporated into the fiber, assuming every PEG terminal had one biotin attached (1.1 mg of biotin per gram of fiber) available at the fibers surface. In addition, PLA/PLA(3600)-b-PEG(2000)-Biotin spun from DMF hindered biotin migration to the aqueous phase, leaving 2 % of the incorporated biotin remaining at the surface of fibers after seven days of water exposure. The water wicking ability of DMF spun fibers also increased significantly with the biotin attachment to the PEG terminal. While HFIP spun fibers lost little biotin from fibers, there was no detectable surface available biotin, indicating biotin was at the interior. With biotin and PEG at the interior of the fibers spun from HFIP, the water wicking remained the same for PLA/PLA(3600)-b-PEG(2000) spun samples and decreased for PLA/PLA(5700)-b-PEG(1000). The dissimilarities observed in water wicking for HFIP spun samples are primarily the result of differences in fiber morphology.

**Keywords:** electrospinning; block length; poly(ethylene glycol); poly(lactic acid); biotin

## 1. Introduction

Nanofiber membranes have very high surface area to volume ratios and porous structures, making them excellent candidates for chemical detection and biosensor applications [1-4]. A nanofiber mat's ability to be implemented as a biosensor, however, is limited by the ability to control several polymer characteristics that include: (i) hydrophobicity that limits performance in aqueous biological systems, (ii) increased hydrophilicity that concurrently increases solubility and degradation in aqueous media, and (iii) specific surface functionality relevant to the individual biosensor application.

One method used to tailor fiber and membrane properties is incorporating functional polymeric and oligomeric materials directly to the processing dopes. For example, the blending of a hydrophobic polymer and a hydrophilic additive may yield a less hydrophobic membrane.[5-7] Using a modified electrospinning system with poly(lactic acid) (PLA)/PLA-b-poly(ethylene glycol) (PLA-b-PEG) Buttaro et al. found phase separation of PEG to the surface of the fibers resulting in a hydrophilic polymer.[8] In this paper, we build on this work in order to increase the surface functionality of fibers.

Biotin finds common use in biosensor applications based on rapid protein binding, and a wide variety of biotin functionalized chemistries are available for specific biomolecule capture. [4, 9-11] Li. et al. functionalized PLA nanofibers with biotin by adding biotin directly to the PLA electrospinning solution. Biotin was present and available for protein binding at the surface of the resulting PLA fibers, but the hydrophobic nature of PLA and the long term stability under aqueous conditions of biotin in the fiber were not investigated.[9] Gonzalez et al. significantly increased the amount of available biotin at the surface by adding PLA-b-PEG to the PLA/biotin system as the PLA-b-PEG aided in the migration of biotin to the surface of the fibers. After 1 to 7 days of immersion in water, however, both the biotin and the PLA-b-PEG preferentially diffuse out of the fiber into the surrounding water; the remaining fiber is no longer effective for protein binding.[12]

If biotin functionalized PLA/PLA-b-PEG fibers are to be useful as sensors in aqueous environments for long duration, we must address the water stability of PLA/PLA-b-PEG fibers by finding appropriate block lengths of PLA-b-PEG. In this work we present three approaches to

increasing stability of hydrophilic, biotin functionalized nanofibers: (i) alteration of block lengths, (ii) covalent attachment of biotin, and (iii) comparing two solvents, DMF and HFIP. Block lengths of PEG and PLA within the copolymer are optimized to both maximize hydrophilicity of the fiber and minimize diffusion of the block co-polymer into the aqueous phase. Through covalent bonding of biotin directly to PLA-b-PEG, diffusion of biotin out of the fiber is significantly reduced. Finally, the influence of the solvent used for electrospinning on the stability and functionality of the final fibers is investigated. Both DMF and HFIP have been reported as compatible solvents for PLA electrospinning. While biotin functionalization of PLA fibers spun from HFIP has not been reported, HFIP has been useful in spinning other types of biomolecules. Nuansing et al. electrospun pure protein from the fluorinated solvent HFIP and found little to no denaturing of the protein.[13]

## 2. Results and discussion

### *2.1. PLA/PLA-b-PEG Fiber Stability in Water*

Changing PLA and PEG block lengths and electrospinning solvent both significantly impacted the stability of the resulting electrospun fiber mats in water (Figure 1). While it was possible to form good nanofibers containing 26 wt% PEG from PLA(5300)-b-PEG(5000) using HFIP as the solvent, stability of these fibers in water was very poor with a 35 % loss of copolymer as determined by NMR after seven days (Figure 1). Fibers containing 26 wt% PEG from PLA/PLA(2800)-b-PEG(2000) spun from HFIP produced insufficient nanofibers and lost ~ 50 % of copolymer after seven days of water exposure. Also, due to the drastic mat differences, we do not consider any trends at 26 wt% PEG. Therefore, for the remainder of this article we will focus on fibers containing 12 wt% PEG.

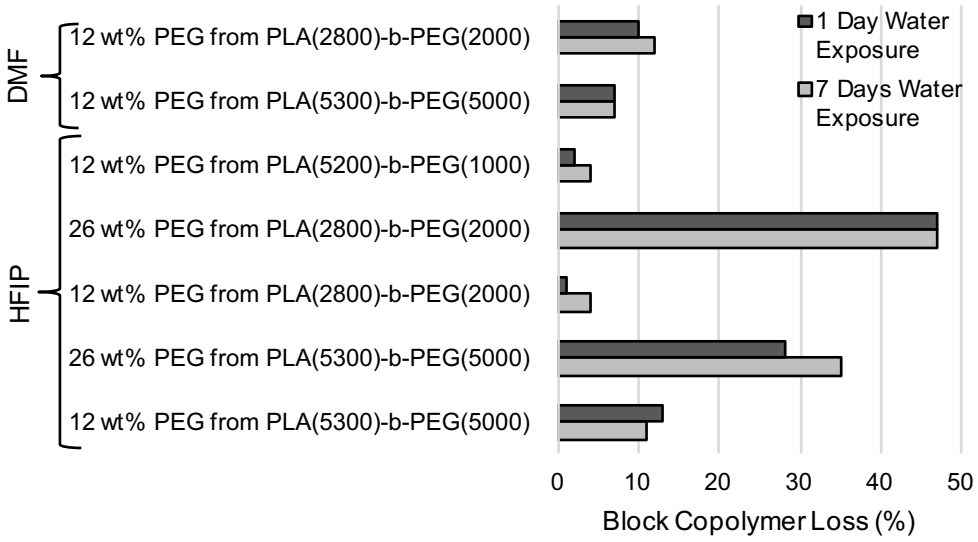


**Figure 1.** Block copolymer lost after one day and seven days of water exposure as determined by NMR.

Electrospun fibers containing 12 wt% PEG from PLA(5300)-b-PEG(5000), and PLA(2800)-b-PEG(2000) spun from DMF and HFIP were studied for copolymer loss by a change of composition in NMR spectra (Figure 1). As reported previously, solutions with 12 wt% PEG from PLA(5200)-b-PEG(1000) in DMF were not spinnable,[8] however fibers could be formed and studied for copolymer loss when spun from HFIP. Considering just copolymers spun from DMF, by only altering the block lengths, we are able to significantly decrease the loss of copolymer from 64 % for PLA(730)-b-PEG(5000) reported by Gonzalez et al.[12] to less than 10 % after seven days of water exposure. We further increased the stability of the copolymer when we used HFIP as the electrospinning solvent.

Interestingly, block copolymer stability within fibers spun from DMF slightly improves for longer block lengths of PEG, which also have increasing block length of PLA; being spun from a homogeneous solution, the longer block lengths of PLA are likely to cause greater chain entanglements with the high molecular weight PLA of the fiber. HFIP spun samples, however, are more stable with shorter PEG block lengths (at 12 wt% PEG fiber loadings). Differences in both the spinning dopes and the resulting fiber morphologies contribute to the difference observed in water stability. The HFIP spinning dope is spun from a suspension that is likely to contain micelles and their aggregates and is not homogenous like the DMF dopes. We imaged an HFIP

suspension with copolymer that has sediment (Supporting Figure S1) and therefore assume micelle aggregates have formed as the PLA is soluble in HFIP; however, under the conditions of this study the PEG was not. By changing the solvent of the spinning dopes from DMF to HFIP, over seven days of water exposure we decreased the copolymer loss of PLA(2800)-b-PEG(2000) (for 12 wt% PEG in the final fiber) from 12 % to 4 % (Figure 1). Further analysis presented below details the differences in the PEG phase formation within the fiber and fiber structure.

### *2.2. PLA/PLA-b-PEG Fiber Morphology*

Compared to pure PLA fibers, fibers spun from DMF decrease in fiber diameter when copolymers are added to the dope, however, fibers spun from HFIP increase in fiber diameter when copolymers are added. HFIP and DMF spun fibers both increase in fiber diameter for increasing PEG block length (Figure 2). HFIP fibers containing PLA(5200)-b-PEG(1000) have the largest diameter; this increase is associated with the melding of fibers as a result of landing wet with residual solvent. Since fibers land wet, they take on a ribbon shape rather than a circular or oval shape. As a result, when we measure fiber diameters, larger diameters are observed. While the cross section perpendicular to the fiber's axis has a larger diameter, the cross section along the fiber axis would have a much smaller diameter since it is not circular. In addition, the copolymers themselves differed in texture, which may have further contributed to this observance; the higher molecular weight copolymers form powders while the lower molecular weights form a tacky viscous substance (Supporting Figure S2).

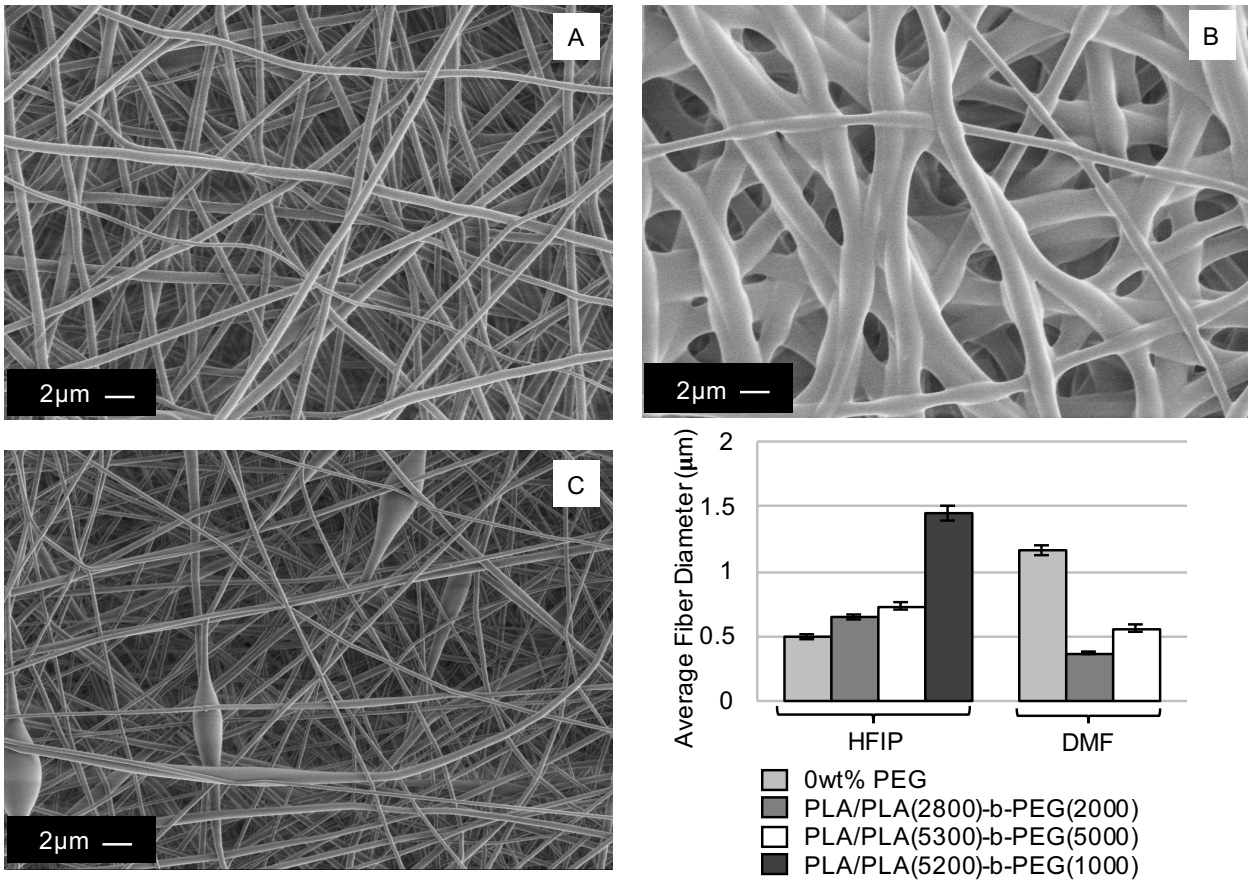


**Figure 2.** SEM images of (A) 12 wt% PEG from PLA(2800)-b-PEG(2000) spun from HFIP, (B) 12 wt% PEG from PLA(5200)-b-PEG(1000) spun from HFIP, and (C) 12 wt% PEG from PLA(2800)-b-PEG(2000) spun from DMF. Average fiber diameters are represented in the graph for control PLA fibers and fibers containing 12 wt% PEG from PLA-b-PEG copolymers. Error bars represent standard error.

### *2.3. PLA/PLA-b-PEG Fiber Water Wicking*

Fibers containing copolymers with PEG block lengths of 2,000 and 5,000 do not wick as much water per their weight when spun from HFIP as compared to DMF spun samples (Figure 3). This indicates DMF promotes PEG to the fiber surface more effectively than HFIP. DMF samples spun from PLA/PLA(5300)-b-PEG(5000) wicked approximately 1,100 % its own fiber weight in water. Fibers of PLA/PLA(2800)-b-PEG(2000) spun from DMF were also able to wick a similar amount. Interestingly, fibers of PLA/PLA(5200)-b-PEG(1000) spun from HFIP had an increase in water wicking, which is associated with the flat ribbon-like fiber morphology previously mentioned.

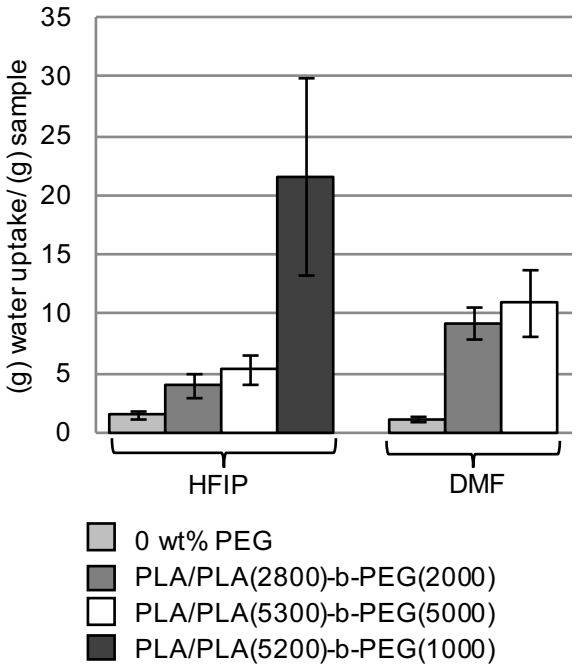


**Figure 3.** Water wicking data for control PLA fibers and fibers containing 12 wt% PEG in the final fiber from PLA-b-PEG. Error bars represent standard error.

We considered the amount of copolymer lost and water wicking data of the fiber mats to be the most important parameters in deciding what copolymers to synthesize with biotin attached. For this reason, we synthesized PLA(5700)-b-PEG(1000)-Biotin. As mentioned earlier PLA(5200)-b-PEG(1000) was not able to be spun from DMF, for this reason we also synthesized PLA(3600)-b-PEG(2000)-Biotin in order to be able to compare it to the fibers spun from HFIP. We extensively study both copolymers in the following sections. Although DMF spun samples had the lowest copolymer loss with PLA(5300)-b-PEG(5000), we chose not to investigate it further; if we maintain 12 wt% PEG in the final fiber we are limited to the amount of biotin we can incorporate, as with longer block lengths of PEG, the amount of biotin able to be incorporated decreases. We aimed at creating copolymers with identical molecular weights to the ones studied in the previous sections, but with biotin attached. Due to the nature of solution polymerization, however, we were able to create similar but not identical block lengths of PLA.

### *2.4. PLA/PLA-b-PEG-Biotin Fiber Stability in Water*

We use NMR to determine copolymer lost after 1 and 7 days of water exposure for fibers spun from HFIP and DMF (Figure 4). As with the polymers synthesized without biotin attached, we

observe some loss of PLA-b-PEG-Biotin copolymers with exposure to water. The fiber spun from DMF lost the most PLA-b-PEG-Biotin, up to 16 % of the total copolymer put into the fiber after 7 days. This loss is the result of the phase separated copolymer being present at the surface of fibers and preferentially migrating to the aqueous media. Fibers spun from HFIP lost far less PLA-b-PEG-Biotin compared to the DMF spun fibers, which was also previously shown in our study of PLA/PLA-b-PEG spun fibers.

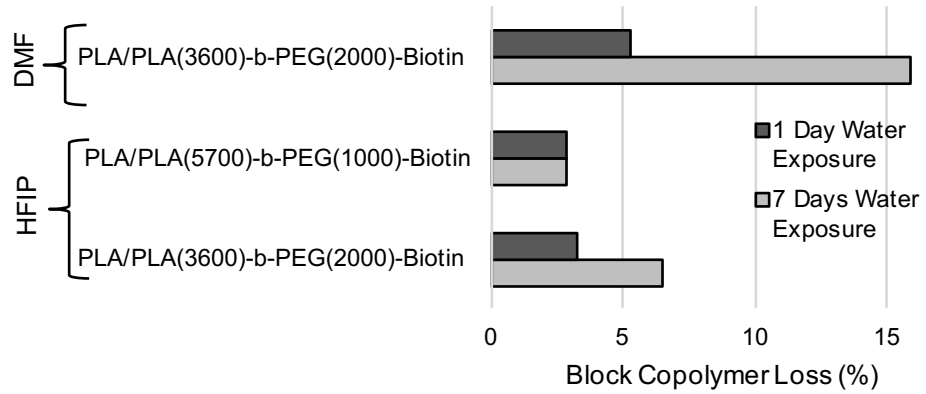


**Figure 4.** PLA-b-PEG-Biotin block copolymer lost after one day and seven days of water exposure as determined by NMR.

### *2.5. PLA/PLA-b-PEG-Biotin Fiber Morphology*

To be complete, we again look at fiber morphology and diameter size (Figure 5). With the exception of PLA/PLA(5700)-b-PEG(1000)-Biotin, fiber diameter decreases when biotin is attached. When PLA/PLA(5700)-b-PEG(1000)-Biotin is spun from HFIP, the fibers take on a larger diameter and a more spherical shape as compared to when biotin was not attached to the copolymer, PLA(5200)-b-PEG(1000). This change in morphology is a function of both biotin being attached and because the PLA block length is slightly longer, we no longer have flattening of the fibers by residual solvent.

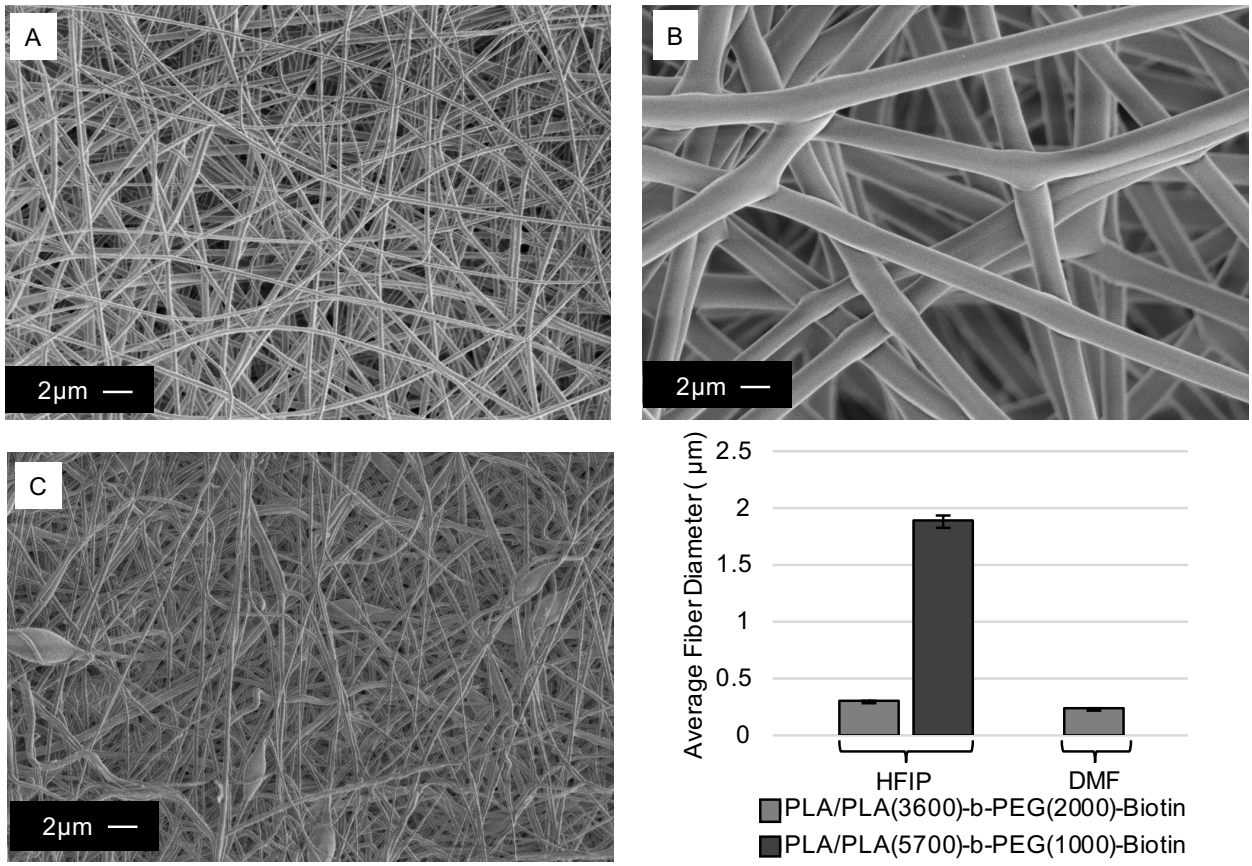


**Figure 5.** SEM images of (A) 12 wt% PEG from PLA(3600)-b-PEG(2000)-Biotin spun from HFIP, (B) 12 wt% PEG from PLA(5700)-b-PEG(1000)-Biotin spun from HFIP, and (C) 12 wt% PEG from PLA(3600)-b-PEG(2000)-Biotin spun from DMF. Average fiber diameters are represented in the graph for fibers containing 12 wt% PEG from PLA-b-PEG-Biotin. Error bars represent standard error.

### *2.6. PLA/PLA-b-PEG-Biotin Fiber Water Wicking*

While the fibers spun from HFIP lost less copolymer, the low water wicking provides evidence of smaller quantities of PEG and biotin present at a fiber's surface compared to fibers spun from DMF (Figure 6). Interestingly, the hydrophilic nature of the fibers spun from DMF increases with the incorporation of biotin, while the fibers spun from HFIP remain relatively unchanged or decrease in hydrophilicity. The fibers spun from HFIP containing PLA(5700)-b-PEG(1000)-Biotin show a drastic loss in hydrophilicity compared to when the copolymer PLA(5200)-b-PEG(1000) was electrospun. This change is explained by fiber diameter being larger and the

circular morphology, rather than ribbon and oval shaped. As a result of the water wicking data, DMF electrospun samples would be more useful as biosensors in aqueous environments than the less hydrophilic samples spun using HFIP.

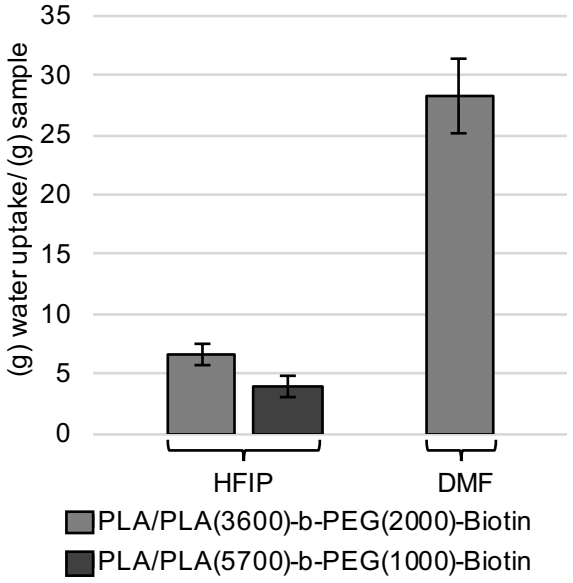


**Figure 6.** Water wicking data for fibers containing 12 wt% PEG from PLA-b-PEG-Biotin. Error bars represent standard error.

### *2.7. PLA/PLA-b-PEG-Biotin Surface Available Biotin*

The available biotin at the fiber's surface is significantly impacted by the solvent system used. The use of HFIP dopes with either PLA(3600)-b-PEG(2000)-Biotin or PLA(5700)-b-PEG(1000)-Biotin have little to no biotin at the surface of fibers (Figure 7), and with exposure to DI water for one and seven days there is virtually no increase in biotin at the surface. This phenomenon is again explained by the insolubility of PEG in HFIP that is likely producing micelle aggregates in the spinning dopes, preventing the phase separation of PEG-Biotin to the fiber's surface. On the contrary, with dopes of DMF where phase separation is known to be aided by the modified electrospinning process used in this study[8], we are able to increase the amount of surface available biotin to a far greater degree than when Li et al. added biotin freely to the electrospinning dope. With ~15 mg of biotin per gram of fiber, we detect on average 7.6 % of available biotin at the surface of the fibers (assuming every PEG has one biotin attached), resulting in 1.1 mg of biotin per gram of fiber (Figure 7); Li et al. who incorporated 10 wt% biotin (100 mg of biotin per gram of fiber) was only able to detect 1.2 mg of biotin per gram of fiber, resulting in detection of only

1.2 % of the biotin incorporated into the fiber.[9] Consequently, by attaching biotin directly to the copolymers we are able to add 85 % less biotin, but still achieve the same relative amount of biotin at the fiber's surface.

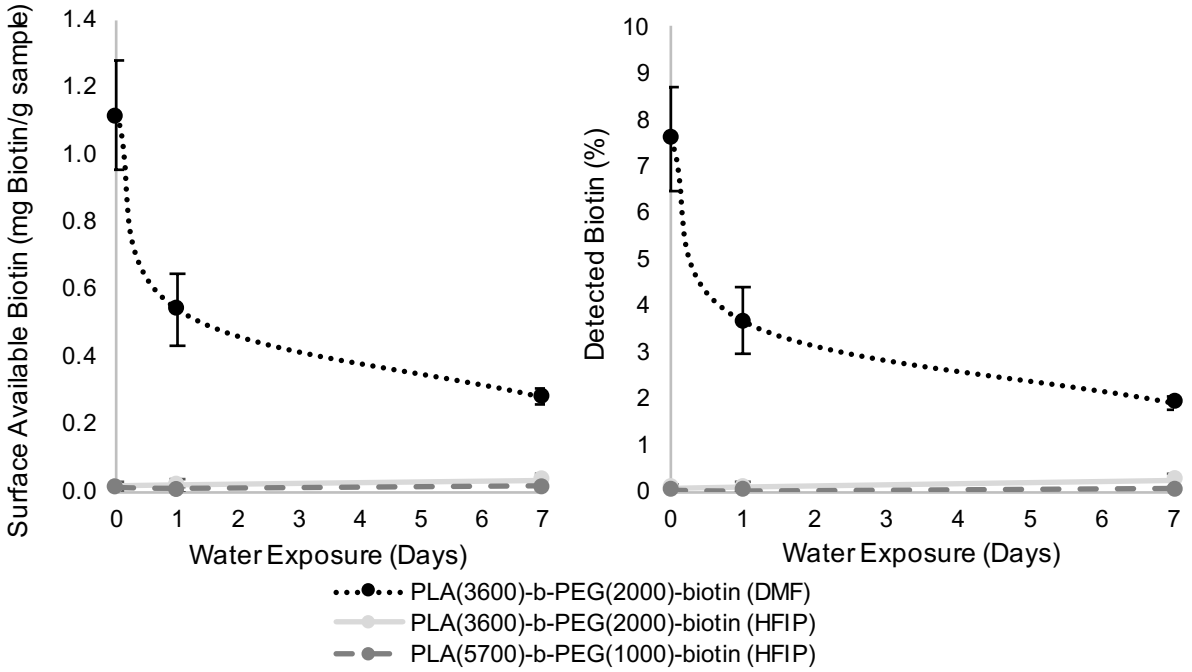


**Figure 7.** (Left) Surface available biotin and (Right) Percent biotin detected in relation to days exposed to water. Error bars represent standard error.

At this concentration of surface available biotin, the fibers made by Li et al. were sufficient for reliable detection[9], however, they were not studied for stability in aqueous solution. With similar amounts of initial biotin available, we expect the fibers produced in this study to also be viable for adequate detection. Our fibers spun from DMF with PLA(3600)-b-PEG(2000)-Biotin saw less biotin loss than that observed by Gonzalez et al., whose biotin when incorporated freely with PLA-PEG copolymers, after only one day of water exposure show no presence of biotin at the fiber's surface. With exposure to water over seven days, our fibers spun from DMF have 2 % biotin remaining at the fiber surface available for use in chemical detection.

## 3. Conclusions

In this study we have shown tailoring the block lengths of PLA-b-PEG copolymers can lead to increased stability of electrospun fibers of PLA/PLA-b-PEG and PLA/PLA-b-PEG-Biotin in

water. While HFIP dopes provided electrospun samples that were more water stable than DMF dopes, the HFIP spun samples had no biotin present at the surface of fibers for detection. Electrspun samples of PLA(3600)-b-PEG(2000)-Biotin spun from DMF allowed for 1.1 mg of biotin per gram of fiber to be detected at the surface of fibers, with the incorporation of 85 % less biotin than previous studies.

With low hydrophilicity and no biotin detected from HFIP electrospun samples, it is necessary to continue future research with DMF as the solvent. It will be necessary to overcome the heat and organic solvent denaturing of proteins we seek to incorporate. Therefore, instead of attaching our proteins directly to PEG and electrospinning them, we will create sensors by utilizing the biotin functionalized fibers spun from DMF. From this study, we have shown Avidin protein binding to biotin is achievable after the fiber mats are formed; this would allow sandwich type sensor assays to be developed for detection of specific contaminants.

## 4. Experimental Procedures

### *4.1. Materials*

PLA-b-PEG block copolymers were synthesized by ring opening polymerization[14] of lactide (Sigma-Aldrich) in the presence of poly(ethylene glycol) methyl ether (Number average molecular weights, $M_n$ : 1000, 2000, and 5000 g/mol) using catalyst stannous octate (Sigma- Aldrich). The copolymers produced were PLA(5200)-b-PEG(1000), PLA(2800)-b-PEG(2000), and PLA(5300)-b-PEG(5000). To produced biotin functionalized polymer PLA(3600)-b-PEG(2000)-Biotin and PLA(5700)-b-PEG(1000)-Biotin, biotin-PEG with $M_n$ of 1000 and 2000 g/mol were purchased from Creative PEGWorks. PLA 4043D (MW= 153,315 g/mol, PDI=1.81) was purchased from NatureWorks LLC. Solvents 1,1,1,3,3,3,3-Hexafluoro-2-propanol and 99.8 % anhydrous N,N-Dimethylformamide were purchased from Sigma-Aldrich. 25-gauge x 1 needles were purchased from Small Parts, Inc., and needle deflect point 20-gauge x 2inch were purchased from Fisher Scientific Company LLC. For colorimetric assays we used Pierce™ biotin quantification kits purchased from ThermoFisher Scientific.

*4.2. Electrospinning*

All spinning dopes in this study are made of high molecular weight PLA and PLA-b-PEG copolymers. The amount of block copolymer was adjusted to obtain 12 wt% or 26 wt% of PEG in the final fibers. All DMF solutions had 12 wt% PEG, as previous work showed that the greatest water wicking occurred at this concentration. [8] For comparison, we also investigated 12 wt% PEG with HFIP. After characterization of the block lengths of PLA-b-PEG (Figure 8A) that promote the greatest water wicking and demonstrate excellent water stability, we synthesized PLA-b-PEG with biotin at the other terminal end of the PEG blocks (Figure 8B). The maximum biotin loading was limited by the PLA-b-PEG block length as the PEG loadings were fixed throughout these studies.

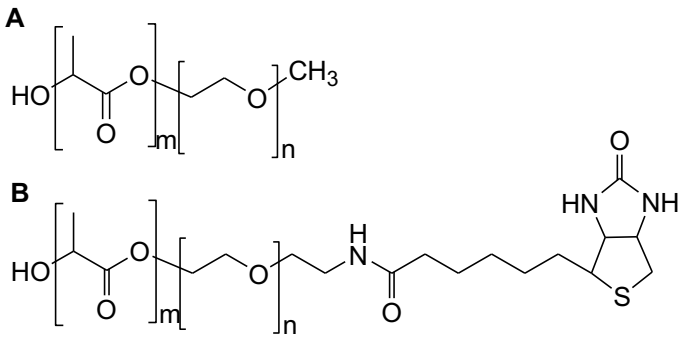


**Figure 8.** Molecular structures of polymers synthesized by solution polymerization (A) PLA-b-PEG, and (B) PLA-b-PEG-Biotin.

Using HFIP as the solvent for PLA in the electrospinning dope resulted in the formation of a suspension when we added PLA-b-PEG or PLA-b-PEG-Biotin (note PLA is completely soluble in HFIP). This suspension does settle over time, so we agitated them prior to use. The spinning dopes contained 3.6 wt% PLA (high molecular weight homopolymer) and were placed on a wrist shaker for at least 16 hours prior to electrospinning to form the spinning dopes. We used a standard electrospinning system at a feed rate of 1.5mL/hr (PHS Ultra syringe pump, Harvard Apparatus) to form fibers from the PLA-b-PEG and PLA-b-PEG-Biotin suspensions. We drove fiber formation by applying 15 kV (Gamma High Voltage Research Inc., FL) between a 25 g needle tip and grounded collector consisting a copper sheet wrapped in aluminum ~ 22 cm away (68 kV $m^{-1}$ electric field).

For comparison, we also used DMF as a solvent because, with heating, it can dissolve homopolymer PLA and copolymers of PLA (22 wt%)[8, 12] without forming a suspension. We used a modified electrospinning apparatus[8] to keep the syringe and needle at 70˚C ± 5˚C and applied a voltage of 15 kV (Gamma High Voltage Research Inc., FL) between the metal needle and the grounded collector (copper sheet wrapped in aluminum) placed ~10 cm away (150 kV $m^{-1}$ electric field). We maintained a solution feed rate of 10 μL/min using a programmable PHS Ultra syringe pump (Harvard Apparatus).

We stored all samples in a desiccator prior to water wicking, stability, DSC, and SEM studies. PLA fibers spun from DMF were prepared previously and stored at room temperature prior to experiments.

### *4.3. Water Stability*

To study the stability of the nanofiber membranes in water, we cut ~ 4 x 1/2 cm rectangles of the deposited mats. Cut samples were placed in 15mL of DI water for either 24 hrs or 7 days. After we removed them from the DI water, we placed them in a vacuum oven set to 25°C (oven temperature ranged from 24 to 29°C) for 24 hrs. Note: fiber mat samples of PLA/PLA(5700)-b-PEG(1000)-Biotin shrunk a couple of centimeters prior to experiments.

### *4.4. Nuclear Magnetic Resonance Spectroscopy (NMR)*

We analyzed the NMR spectra on the control and dried samples (after water immersion) to accurately measure copolymer dissolution. $^{1}$H-NMR experiments were recorded at room temperature with an INOVA 400 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 400 MHz. Deuterated chloroform (CDCl3) was used to dissolve fibers mats and tetramethylsilane (TMS) was used as an internal reference. The PLA/PEG ratio of the samples was determined from the integration of $^{1}$H-NMR resonances belonging to the PEG blocks at 3.6 ppm (-O-$CH_2$-$CH_2$-singlet) and to the PLA blocks at 5.2 ppm (-CH quartet).[15-17] The difference in the PLA/PEG ratio between control samples and the samples immersed in water was considered to be caused by the dissolution of the PLA-b-PEG block copolymer into the water phase.

### *4.5. Field Emission Scanning Electron Microscopy (FE-SEM)*

By using an FE-SEM, we observed the microstructure of the fiber mats in high contrast. We sputter coat samples in gold-palladium for 30s prior to imaging them with a LEO 1550 FESEM using a 1kV accelerating voltage and 50:50 mixture of SE2 and InLens detectors. Using ImageJ™ software on the recorded SEM images, we calculated the average diameters from 50 measurements gathered from two images.

### *4.6. Water Wicking*

Each electrospun sample was cut into 3 x ½ cm fabrics. We weighed the samples and then put a fishhook through the nanofiber mat to ensure the membrane penetrated the meniscus.[8] Wettability testing was done using the KSV Sigma 701 and DI water. Note: fiber mat samples of PLA/PLA(5700)-b-PEG(1000)-Biotin shrunk a couple of centimeters prior to experiments.

### *4.7. Biotin/Avidin Binding*

We are able to compare the effect of solvent on biotin reaching the fiber surface by using a quantifiable competitive colorimetric assay, whose change in absorbance at 500 nm signifies the Avidin removal from a 4'-hydroxyazobenzene-2-carboxylic acid (HABA)/Avidin solution to the fiber surface containing biotin. A Lambda 35 UV/Vis spectrophotometer from Perkin Elmer is used to measure the change in absorbance. We reconstituted HABA/Avidin solutions by the manufacturer's protocol, and took its absorbance in PBS Buffer at 500nm. We weighed the nanofiber fabrics prior to placing them into a cuvette and shaking for three minutes; afterwards, we removed the fiber mat and measured the absorbance. For each sample we perform at least three replicates. Equation 1 is used to calculate the available biotin at the surface of fibers.[12]

$$Surface\ Available\ Biotin\ \left(\frac{mg\ Biotin}{g\ fiber}\right) = \left(A^0_{500} - A_{500}\right)\left(\frac{Mw_{biotin}V}{\varepsilon bW}\right) x\ 10^3 \qquad \text{Equation 1}$$

Where $A^o_{500}$ is the absorbance of the solution prior to the addition of nanofiber, $A_{500}$ is the absorbance of the solution after reaction with nanofiber, $MW_{biotin}$ is the molecular weight of the biotin (244.3 g/mol), $V$ is the volume of the solution (L), $b$ is the cuvette path length (1 cm) and $\varepsilon$ is the extinction coefficient of the HABA/Avidin complex at 500 nm ($3.4x10^4$ L/(mol·cm)).

**Supporting Information**
Settling of copolymer in HFIP suspensions and textural appearance of PLA-b-PEG block copolymers

## 5. Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, Hatch NC1194 project #3297816. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the National Institute of Food and Agriculture (NIFA) or the United States Department of Agriculture(USDA). This work was also in part funded by a grant from the Department of Fiber Science & Apparel Design Graduate Student Research Awards Fund. Heating element apparatus used in electrospinning was provided by Dr. Daehwan Cho. This research made use of the Cornell Center for Materials Research Shared Facilities which are supported through the NSF MRSEC program (DMR-1120296). NSF-MRI (CHE-1531632 - PI: Aye) is acknowledged for NMR instrumentation support at Cornell University

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