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Availability of biotin incorporated in electrospun PLA fibers for streptavidin binding

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Abstract

Distribution of biotin at a depth of 3–10 nm from the surface of electrospun polylactic acid (PLA) fibers has been assessed by X-ray photoelectron spectroscopy (XPS) and compared to the distribution predicted by bulk calculations. Biotin concentration in the outer 3–10 nm of the fibers is greater than the predicted if biotin was distributed uniformly within the fiber. Availability of biotin for streptavidin binding at the surface of the fibers has been determined via a competitive colorimetric assay. Availability of biotin at the fiber surface was also determined to be greater than predicted by calculations assuming uniform biotin distribution. Additionally, the segregation of biotin to the exterior of the fiber increases disproportionately with increasing overall biotin concentration in the fibers. Confocal microscopy has been used to confirm capture of streptavidin, primary antibodies and fluorescence labeled secondary antibodies on PLA/biotin fibers.

Keywords: Polylactic acid (PLA); Biotin; Streptavidin binding

1. Introduction

The strong, rapid and specific binding between biotin and streptavidin has been used to develop a wide range of biosensors [1,2]. The system has been shown to be sensitive and accurate in a wide variety of formats including surface plasmon resonance (SPR) devices [3–8], monolithic membranes [2,9–12], and microfluidic lab-on-a-chip devices [13–15]. Incorporation of biotin into fiber based membranes as a platform for streptavidin binding has been explored in our laboratory [16,17]. Preliminary results indicated that biotin could be successfully incorporated into electrospun fibers via suspension or co-dissolution in an electrospinning dope with polylactic acid (PLA) and that some of the incorporated biotin was available for streptavidin binding. For incorporated biotin to be useful in a fiber based system it must be at or near enough to the fiber surface to bind with streptavidin and the fiber based membrane must not inhibit subsequent capture and detection of biological molecules. The work reported here focuses on determining the location of biotin within the electrospun fibers, the amount of incorporated biotin available for streptavidin binding and the ability of the electrospun membranes to capture and detect biological molecules.

Several researchers have hypothesized that the electrical charge traveling along the exterior of the jet during fiber spinning will drive polarizable species to the surface of the electrospun fiber [18–20]. Sun et al. applied this concept to electrospinning of a PEO-*co*-peptide copolymer and successfully prepared polypeptide surface-enriched electrospun fibers, thus developing a method of biofunctionalization of polymer fibers via electrospinning [18]. Sun et al. concluded that the high electric field applied during the electrospinning process promoted the migration of the more polarizable peptide segment towards fiber surfaces, leading to formation of a nanofiber surface with desired biofunction. Royen et al. explored the

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application of static secondary ion mass spectrometry (S-SIMS) to determine of the partitioning of a polar additive in electrospun nanofibers [19,20]. Using cetyltrimethylammonium bromide (CTAB) as probe (i.e. the polar component) and poly(ε-caprolactone) (PCL) as the model polymer to prepare electrospinning dopes, they observed surface enrichment of CTAB in the nanofibers spun from PCL–CTAB solutions of 1–12 mol% CTAB concentration, which was driven by the high electric force applied in the electrospinning process.

Characterization of the dispersion of additives incorporated into electrospun fibers has presented challenges based on the small size both the fibers and the additives. When relatively large materials such as nanoclays or carbon nanotubes have been included in electrospun fibers, microscopy techniques such as TEM of cross-sections or longitudinal-sections have been useful in confirming that particles are dispersed and aligned in the fiber direction [21–23]. When the additive material contains a distinct atomic nucleus from the electrospun fiber, spectroscopic techniques become relevant. Bulk distribution of biotin in particular through out fibers has been imaged and measured via electron probe micro-analysis [16,17] although the precise location of biotin within the cross-section of the fiber could not be assessed by this technique. X-ray photospectroscopy has been used to measure trace impurities in electrospun fibers [24] and has a very specific depth of sensitivity within a solid substrate. In this study, the XPS technique [25] has been utilized to probe the shell between 3 and 10 nm from the surface of the electrospun PLA fibers. This technique is sensitive to bonding states of individual atoms and can provide identification of the chemical species present and establish the abundance of biotin near the membrane surface.

The general morphology of the PLA fibers and the non-woven membranes comprised of these fibers studied in this report has been well characterized [16,37]. Fiber diameters average 280 nm independent of biotin loading. Pore sizes between fibers in non-woven membranes average 0.82 μm [37]. Additionally, no change in surface morphology is evident with increase in biotin loading as biotin content increases from 0% to 18% by weight [16].

Three different assessments of the biotin distribution in PLA electrospun fibers are presented. First a bulk distribution was calculated based on the overall biotin loading in the samples. Second, an assessment of the biotin in the outer 3–10 nm of the fiber is measured as the sulfur to carbon ratio by XPS. Finally, a direct measurement of the amount of biotin available to bind with streptavidin protein is measured via a competitive colorimetric assay.

2. Materials and methods

2.1. Materials

Polylactic acid (PLA) polymer ($M_w = 211{,}332$ Da, $M_n = 109{,}337$ Da, density $= 1.25$ g/cm^3) was supplied by Cargill Dow (Minnetonka, MN). *N*,*N*-Dimethyl formamide (DMF) and HPLC grade water were purchased from Mallinckrodt Laboratory Chemicals (Phillipsburg, NJ). Phosphate buffered saline (PBS), Tween 20, and 4-hydroxyazobenzene-2′-carboxylic acid (HABA, 99.5%) were purchased from Sigma–Aldrich (St. Louis, MO). Streptavidin was purchased from Zymed Laboratories (South San Francisco, CA) and reconstituted with PBS. Biotin and streptavidin-Alexa 488 conjugate were purchased from Pierce Biotechnology, Inc. (Rockford, IL). All the reagents were used without further purification.

2.2. Preparation of electrospinning solutions and nanofiber membranes

Preparation of the electrospinning dopes and electrospun nanofiber membranes followed the procedures reported previously [16]. In brief, PLA/biotin/DMF solutions were prepared by first dissolving biotin in DMF at 70–100 °C while stirring on a hot plate (VWR Scientific, West Chester, PA). Desired amount of PLA pellets was then added to the resulting solution. Heating and stirring continued until the polymer was completely dissolved to form a homogeneous viscous solution. For preparation of the PLA/DMF solution, dissolution of biotin was omitted.

Electrospinning was conducted using a programmable syringe pump (Harvard Apparatus, MA) and at 15 kV voltage supplied by a high-voltage supply (Gamma High Voltage Research Inc., FL). Other electrospinning conditions included 10 μL/min feed rate, 12 cm collection distance, 0.41 mm (ID) needle size, and aluminum foil collector. Electrospinning solutions were heated to 70 °C to maintain uniform dissolution during the electrospinning process [26]. Membranes were removed from the aluminum foil collectors and air-dried overnight before testing.

2.3. X-ray photoelectron spectroscopy (XPS)

XPS measurements were carried out with a Surface Science SSX-100 XPS instrument. The samples were analyzed with a monochromatic AlKα radiation source (1486.6 eV) operating at a base pressure of 3×10^{-9} torr. Wide survey spectra were recorded at a pass energy of 150 eV in order to determine the surface chemical compositions. Spot size had a 1 mm diameter with incident and take-off angles of 55 degrees. High-resolution spectra were obtained at a pass energy of 20 eV in order to determine the chemical state of the examined elements. The binding energy (BE) values were calculated relative to the C (1s) photoelectron peak at 285.0 eV. The surface analyses were obtained at four different locations on each sample in order to ensure reproducibility.

2.4. Colorimetric assay

To quantify the amount of biotin available for streptavidin binding at the surface of the PLA fibers, a colorimetric assay was developed based on the protocol of Green [27,28]. The method measures change in absorbance at 500 nm as streptavidin is removed from a standard solution, i.e. bound to the fiber surface.

2.4.1. Preparation of solution

A stock solution containing 8.46 mmol/L HABA in 0.012 M NaOH was prepared. For the colorimetric assays, 59 μL of the stock HABA solution and 100 μL, 100 μmol/L streptavidin in PBS buffer were added to 841 μL PBS buffer. The final test solution contained 10 nmol/mL streptavidin, 50 nmol/mL HABA, and 0.01 mol/L PBS buffer. After vortexing, the mixture was orange and had pH between 7.0 and 7.2.

Standard curve: A Perkin Elmer Lambda 35 UV–vis spectrophotometer (Wellesley, MA) was used to perform the colorimetric assay. A standard absorbance curve was prepared by measuring absorbance of the stock solution before and after addition of known amounts of biotin. As biotin is added to the solution, streptavidin is removed from the HABA–streptavidin complex and forms a biotin–streptavidin complex with no absorbance at 500 nm. Absorbance at 500 nm of 900 μL of the HABA–streptavidin test solution in a 1 mL cuvette was measured and used as the reference value A^0_{500}. Biotin was added to the cuvette as repeated 10 μL aliquots of 102 μmol/L biotin solution. After each addition, the cuvette was vortexed and absorbance at 500 nm was measured. When the absorbance value was stable for at least 15 s the value was recorded as A^n_{500}. The superscript n corresponds to the number of biotin aliquots added [29]. The predicted biotin concentrations (μmol/mL) were calculated based on the decrease in A_{500} for each addition of biotin standard solution using Eq. (2). These measured concentration values were plotted against the theoretical biotin concentrations to generate the standard curve.

$$[\text{Biotin}] = \frac{\frac{v_i}{v_i+v}A^{\circ}_{500} - A^n_{500}}{\varepsilon \cdot b}. \quad (1)$$

where:

ε: the extinction coefficient of HABA–streptavidin complex at 500 nm = 35,000 L/(mol cm),
b: the cuvette path length = 1 cm,
v_i: the initial volume of HABA–streptavidin complex solution used for each assay,
v: the cumulative volume of biotin standard solution added.

2.5. Determination of surface-available biotin

A piece of PLA/biotin membrane was weighed and pre-wet with 50–100 μL 0.01 M PBS/Tween 20 solution and mixed with 900 μL of the standard HABA–streptavidin complex solution. The mixture was vortexed and the absorbance of the solution at 500 nm measured. Vortexing and absorbance measurements were repeated until the absorbance reading was stable. Additional pieces of PLA/biotin membrane were added to the cuvette and absorbance values and cumulative weight of membrane added were recorded. Surface-availability of biotin (mg biotin/g membrane) was calculated based on the decrease in absorbance at 500 nm as:

$$\text{available\_biotin} = \left[A^{\circ}_{500} - A^n_{500}\right] \times \frac{M_{\text{W,biotin}} \times v}{\varepsilon \cdot b \times \sum_{i=1}^{n} w_i} \quad (2)$$

where

$M_{\text{W, biotin}}$: the molecular weight of biotin (244.3 g/mol),
v: the volume of HABA–streptavidin complex solution used,
ε: the extinction coefficient of HABA–streptavidin complex at 500 nm = 35,000 L/(mol cm),
b: the cuvette path length,
w_i: the weight of the ith PLA/biotin membrane added, and
n: the total number of PLA/biotin membrane pieces added.

As control, a piece of PLA membrane was treated using the same procedures mentioned above. The HABA–streptavidin complex solution before and after the addition of PLA membrane was measured and no decrease in absorbance at 500 nm was measured.

2.6. Confocal microscopy

A Leica TCS SP2 laser confocal scanning microscope was used to detect binding of streptavidin to biotin incorporated in PLA membranes by imaging the fluorescence of Rabbit IgG antibody (labeled with fluorescein isothiocyanate (FITC)) attached nanofiber membranes according to the method of Baeumner et al. [30,31]. To prepare samples for confocal analysis, the nanofiber membrane (0.4 × 0.4 cm) was wet with PBS/Tween 20 (PBST), dried and then treated with 50 μL, 1 μM streptavidin/PBS buffer (PBS). After washing with PBS, the membrane was blocked with a blocking reagent for 30 min [30,31]. Extra solution was removed and the membrane was washed again, and then dried in a vacuum oven at 37 °C for 3 h. The membrane was then treated with 50 μL, 1 μM *Escherichia coli* polyclonal antibody (biotin conjugated) at room temperature for 30 min. After repeated washing with PBST, the membrane was dried and then treated with 50 μL rabbit IgG antibody. Extra solution was removed and the membrane was washed again and then dried the membrane in air for 30 min. Repeated washing steps insured that any fluorescence in the samples reflected specific binding between biotin incorporated in PLA membranes, streptaviding, *E. coli* polyclonal antibody (biotin conjugated) and FITC-labeled Rabbit IgG antibody.

3. Results and discussion

XPS spectra taken in broad survey mode to detect major atomic species and in targeted scans to detect sulfur contributed by biotin confirmed the chemical composition of the PLA and PLA/biotin fibers. XPS peaks correspond to specific energy states of electrons in the s or p orbitals of their respective atoms. The XPS survey spectrum of PLA membrane, Fig. 1, showed the major photoelectron peaks located at binding energy values of 531 and 285 eV corresponding to the O (1s) and C (1s) signal intensities, respectively. The C (1s) XPS spectrum of PLA membrane indicated that the major species were C–H/C–C, C–O and O–C=O, with the binding energies values located at 285.0, 286.9 and 291.0 eV,

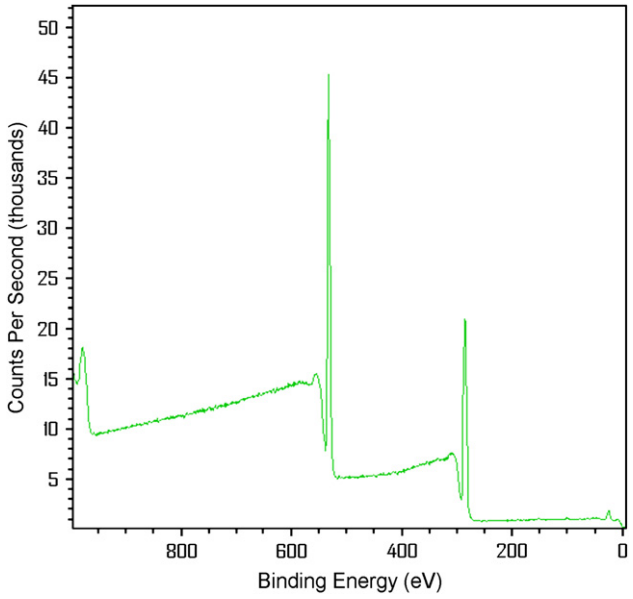

Fig. 1. XPS survey spectrum of PLA fibers. Peaks represent carbon (285 eV) and oxygen (531 eV) binding states.

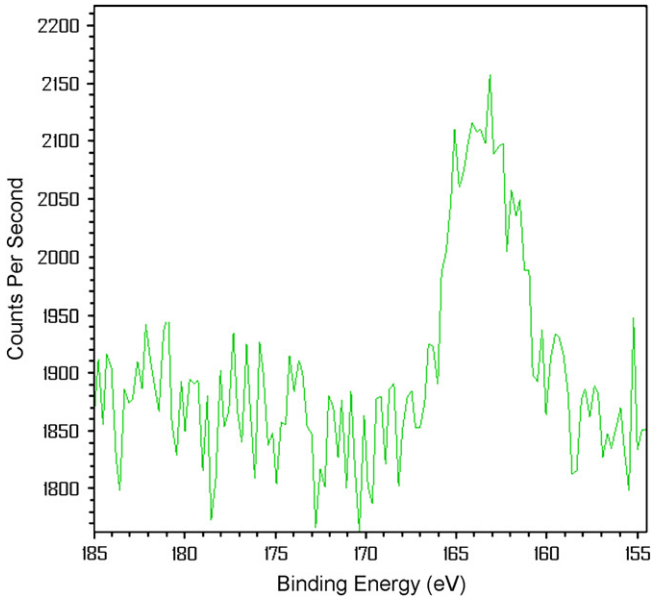

Fig. 2. Biotin (w/w PLA) 10% samples show measurable sulfur peak.

respectively, which is in good agreement with the literature and with the structure of the PLA polymer [25,32].

To assess the presence of sulfur in the samples, high-resolution measurements in the region below 200 eV were taken. Although the biotin molecule contains two nitrogen atoms and only one sulfur atom, the heavier sulfur produces a stronger XPS signal. Nitrogen spectra were also taken, but had poor signal to noise ratio in all samples. The S (2p) XPS spectrum of PLA membrane had a poor signal to noise ratio, with no sulfur species detected in the layer between 3 and 10 nm depth from electrospun PLA fiber surface. These results are consistent with previously reported Electron probe micro-analysis data [16,] confirming that the PLA does not contain measurable quantities of sulfur. Examination of the S (2p) spectrum of 3% biotin (w/w PLA) membrane, indicated the presence of sulfur species (0.7 at.%) located at 164 eV consistent with the S^{2+} form sulfur binding within the biotin molecule. Increasing the concentration of biotin in the spinning solution (10–18%) led to an increase in the sulfur concentration observed (2.3–3.4 at.%), Figs. 2 and 3, respectively. The amount of biotin contained in the shell between 3 and 10 nm from the fiber surface can be determined by comparing the ratio of sulfur counts to carbon counts measured by XPS. Since only biotin contains sulfur in this system, the ratio of sulfur to carbon (*S*/*C*) directly reflects the ratio of biotin to PLA near the surface of the fiber. *S*/*C* ratios determined from XPS measurements are presented in Table 1.

As a starting point for assessing the distribution of biotin in the PLA/biotin fibers, the overall sulfur to carbon mass ratio (*S*/*C*) for each sample was calculated. Calculations were based on the initial composition of the spinning dope assuming complete or at least proportionate incorporation of biotin and PLA in the resulting fibers and complete removal of the DMF solvent during the electrospinning or subsequent drying processes. Previously reported Electron Probe Micro Analysis (EPMA) [16,17] and XPS data reported here confirm that the PLA used in these experiments does not contain a measurable quantity of sulfur. Based on the molecular structure of PLA (Fig. 4) and biotin (Fig. 5), the *S*/*C* ratio was calculated as:

$$S/C = \frac{S_{\mathrm{PLA}} + S_{\mathrm{biotin}}}{C_{\mathrm{PLA}} + C_{\mathrm{biotin}}} \tag{3}$$

$$S_{\mathrm{PLA}} = 0 \tag{4}$$

$$S_{\mathrm{biotin}} = [\mathrm{B}]\frac{M_{\mathrm{S,biotin}}}{M_{\mathrm{biotin}}} = [\mathrm{B}]\cdot\frac{32}{244.3} \tag{5}$$

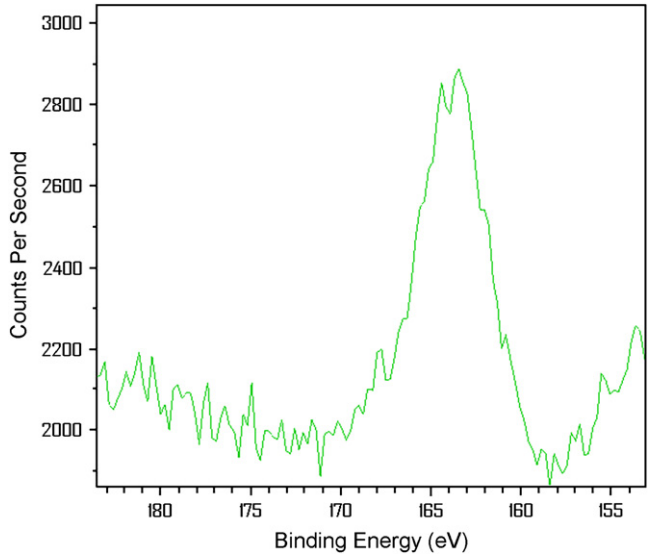

Fig. 3. XPS spectra of 18% biotin (w/w PLA).

Table 1
Calculated and measured sulfur (S) to carbon (C) ratio in PLA biotin fibers

Biotin (wt%, relative to PLA)	*S/C*	
	Calculated	XPS
0	0	0
3	0.008	0.011
10	0.024	0.038
18	0.040	0.056

$$C_{PLA} = \frac{M_{C,PLA}}{M_{PLA}} = 0.5 \quad (6)$$

$$C_{biotin} = [B]\frac{M_{C,biotin}}{M_{biotin}} = [B]\cdot\frac{120}{244.3} \quad (7)$$

where:

S = total mass of sulfur,
C = total mass of carbon,
[B] = biotin concentration (weight of biotin/weight of PLA),
$M_{S,PLA}$ = mass of sulfur in PLA,
$M_{S,biotin}$ = mass of sulfur in biotin,
$M_{C,PLA}$ = mass of carbon in PLA,
$M_{C,biotin}$ = mass of carbon in biotin

The *S/C* ratios predicted from calculations assuming uniform dispersion and measured via XPS analysis are presented in Table 1. The surface *S/C* ratio obtained by XPS analysis was 35–58% higher than the calculated ratio. The relatively high amounts of sulfur in the layer 3–10 nm from PLA–biotin fiber surface provide the first indication that biotin is partitioning to the exterior of the PLA fibers. Two possible explanations can be presented for the partitioning of biotin to the surface of electrospun fibers as evidenced by XPS measurements. First, as several researchers have proposed [18–20] a dipole moment in the biotin molecule could cause migration towards the charged jet surface during the electrospinning process. Secondly, the typical 'blooming' phenomenon of additives in polymer substrates could lead to phase separation in the fiber systems [33,34]. Because the entropy of mixing is extremely small in polymeric systems, equilibrium tends towards separation rather than blending of small molecule additives.

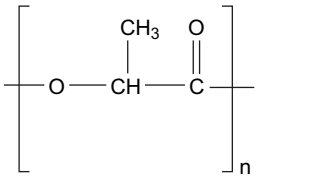

Fig. 4. Molecular structure of PLA.

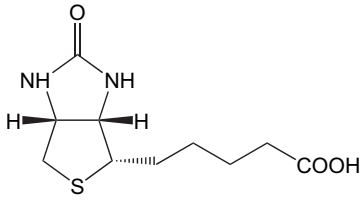

Fig. 5. Molecular structure of biotin.

3.1. Validation of color change assay

Ultimately, for biotin incorporated in PLA fibers to be useful, it must be available to interact with other biological molecules. To measure this property directly, the HABA–streptavidin color change assay was adapted and run on the membrane samples. A standard curve was developed using solutions of known biotin concentration based on the method developed by Green, which is based on the decrease in absorbance at 500 nm, the characteristic absorbance of HABA–streptavidin complex (A_{500}) [28]. Fig. 6 shows the three plots from three separate duplicate assays. The results are very reproducible and give a slope of 0.88 between the actual and calculated values. This slope has been attributed to aggregation of streptavidin [35] and is consistent with other reported values for direct vs. HABA assays for biotin–streptavidin binding [36]. The factor of 0.88 will be incorporated in analysis of biotin availability in PLA fiber membranes.

The schematic diagram in Fig. 7 describes the process for available biotin detection on the electrospun PLA fiber membranes. When the membrane is added to a solution containing HABA–streptavidin complex, streptavidin is removed from the complex and binds preferentially with available biotin. Any biotin that is not on or near the surface of a PLA fiber will be sterically prevented from binding with streptavidin and will not be detected in this assay. The images in Fig. 8 show the actual color changes that occur on formation and destruction of the HABA–streptavidin complex. The color change from the initial yellow solution to orange/red on addition of streptavidin confirms that the HABA–streptavidin

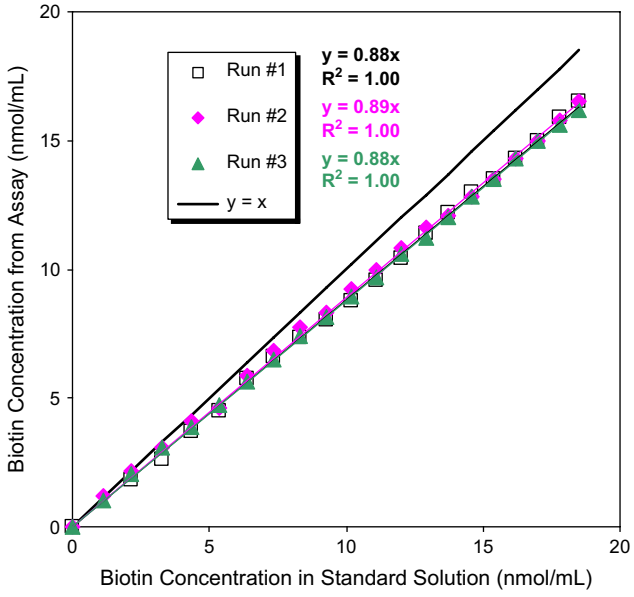

Fig. 6. Biotin concentration calculated from Eq. (2) vs. known biotin concentration.

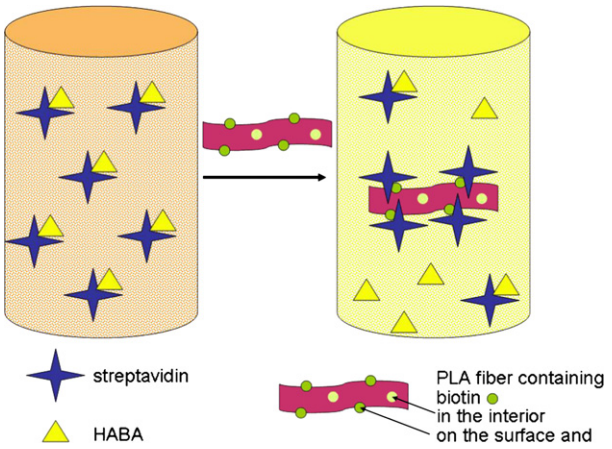

Fig. 7. Biotin on PLA fiber surface binds with streptavidin, removing streptavidin from the streptavidin–HABA complex and changing the absorbance of the solution at 500 nm.

complex formed. Upon addition of PLA/biotin membrane, the solution color shifts rapidly (seconds) towards yellow indicating that some streptavidin has been removed from the HABA–streptavidin complex.

Using the sequential addition method, biotin surface-availability of each of the of PLA/biotin membrane samples was determined. Fig. 9 shows the change of surface biotin availability as a function of the biotin concentration in the initial spinning dopes. The control sample, containing no biotin, did not remove streptavidin from the HABA–streptavidin complex. No binding of streptaviding on the sample containing 3% biotin (w/w PLA) was measured by this technique. Based on the sensitivity exhibited in the standard curves, biotin concentration on the surface of the membranes containing 3% biotin (w/w PLA) is vanishingly small. Increasing the concentration of biotin in the spinning dope increases the biotin surface-availability in a non-linear manner. When 10% biotin (w/w PLA is incorporated in the spinning dope, 1.2 mg biotin/g membrane are available for streptavidin binding at the fiber surface. When 18% biotin (w/w PLA) is incorporated,

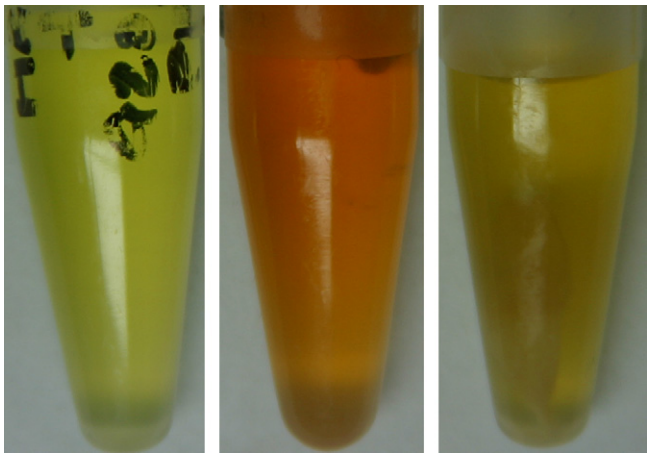

Fig. 8. HABA dye solution before addition of streptavidin, after addition of streptavidin and after addition of membrane samples. Color change was measured by absorbance at 500 nm.

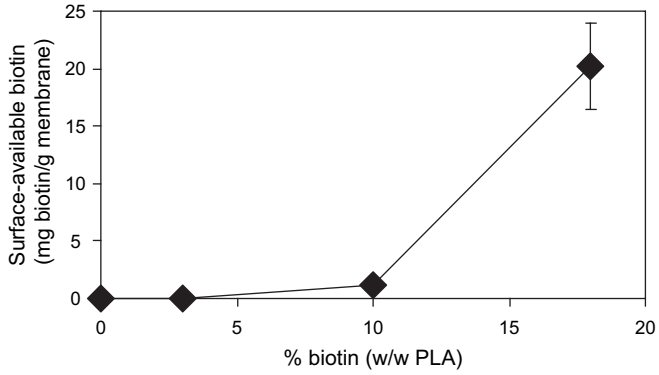

Fig. 9. Increased loading of biotin in spinning dopes leads to increased surface biotin availability on the spun membranes.

however, the available quantity jumps to 20 mg biotin/g membrane. The non-linear jump in biotin availability at the fiber surface does not match the more proportional enrichment of biotin in the shell between 3 and 10 nm beneath the fiber surface observed in XPS measurements. As a basis for comparison, of the total amount of biotin added to the membranes, 1% is available at the surface of the 10% biotin (w/w PLA) membrane, while 9% is available at the surface of the 18% biotin (w/w PLA) membrane.

Phase separation is known to occur in this system during the electrospinning process. Biotin has poor solubility in DMF at room temperature, but soluble at elevated temperatures. The PLA/biotin/DMF spinning solutions were homogeneous at 70 °C in the electrospinning spinneret. During the spinning process, the temperature of the jet decreased to room temperature and DMF evaporated from the system. As an additional driving force to segregate biotin to the surface, the applied electrical charge can attract the highly polar ureido group on the biotin molecule [38]. PLA, however, has very low conductivity. Conductivity values on the order of 10^{-8} S/cm have been reported for PLA membranes [39].

3.2. Biosensor assay

To assess the ability of the PLA fibers containing biotin to capture and detect biological molecules a simple assay was performed to create a sandwich-style detection with streptavidin protein bound to the PLA/biotin membrane, a biotinylated primary antibody and a fluorescence labeled secondary antibody. A schematic diagram of the assay procedure is presented in Fig. 10. Confocal microscopy was used to detect fluorescence of the membranes. Items on the schematic diagram in Fig. 10 labeled 'A', 'B' and 'C' correspond with the images in Fig. 11. Fig. 11A is a confocal image of a 10% biotin (w/w PLA) membrane presented as a control to confirm that the membranes themselves are not fluorescent. Slight green color at the top of the membrane is attributed to reflection off the cover glass used in mounting the sample. As a second control, a 10% biotin (w/w PLA) membrane was treated with streptavidin followed by the secondary, fluorescence labeled antibody. Streptavidin will bind with available biotin on the

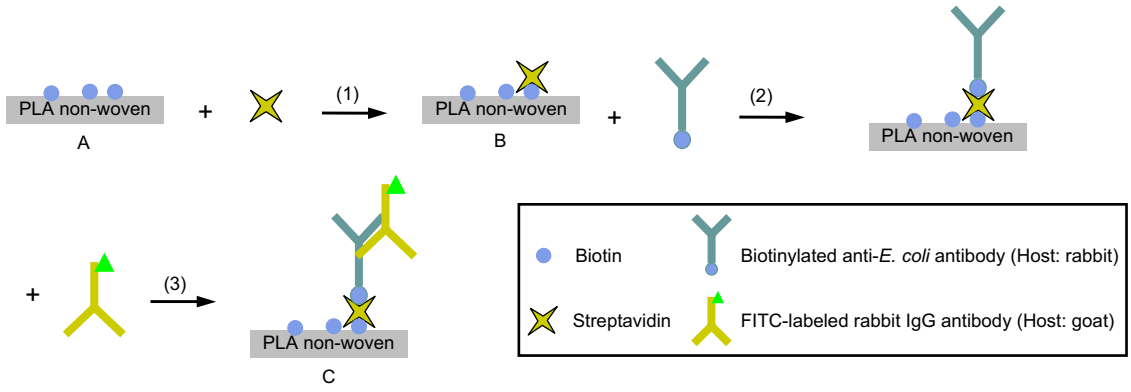

Fig. 10. Schematic diagram of biosensor assay experiment. Confocal microscope images of samples labeled A, B (after treatment with FITC-labeled rabbit IgG antibody) and C are presented in Fig. 11.

surface of fibers contained in the membrane as confirmed by the colorimetric assay. Since the secondary antibody is not biotinylated, it should not bind with streptavidin on the fiber surface. Fig. 11B confirms that no additional fluorescence is registered and no measurable non-specific binding between the secondary antibody and the membrane occurs. Finally, Fig. 11C contains the confocal microscopy image of the membrane upon completion of the biosensor assay. The 10% biotin (w/w PLA) membrane has been treated with streptavidin, followed by the biotinylated primary antibody which serves as a capture probe for the fluorescence labeled secondary antibody. Significant fluorescence is observed to a depth of 10 μm into the non-woven membrane sample. Diminishing fluorescence with depth in the sample may have resulted from capture of all available fluorescence labeled antibodies prior to diffusion to that depth in the membrane. Although only 1% of the total amount of biotin added to the membranes is available at the fiber surface according to the colorimetric assay, significant capture of streptavidin, biotinylated primary antibody and fluorescence labeled secondary antibody are evident in Fig. 11C.

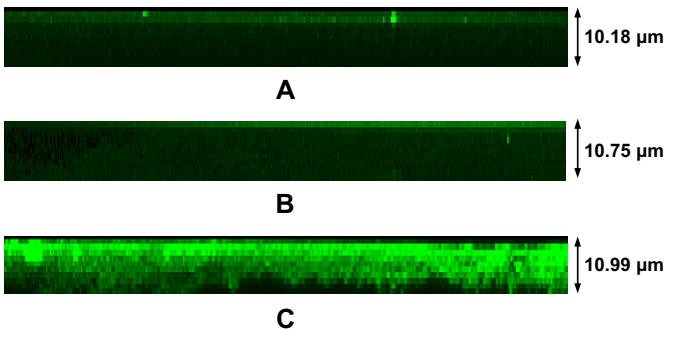

Fig. 11. A, B and C confocal microscope images indicating fluorescence of 10% biotin (w/w PLA) membrane only where streptavidin, a biotinylated primary antibody and a fluorescence labeled secondary antibody have been captured. (A) Confocal image of PLA/biotin membrane (10% biotin w/w PLA). (B) Confocal image of PLA/biotin membrane (10% biotin w/w PLA) treated with streptavidin followed by FITC-labeled rabbit IgG antibody. (C) Confocal image of PLA/biotin membrane (10% biotin w/w PLA) treated with streptavidin and biotinylated anti-*Escherichia coli* antibody followed by FITC-labeled rabbit IgG antibody.

4. Conclusions

Distribution of biotin incorporated in PLA fibers during electrospinning near the fiber surface has been assessed by XPS measurement of the sulfur and carbon abundance in the layer between 3 and 10 nm from the fiber surface. Abundance of sulfur, associated with biotin was 37–58% greater than the amount predicted assuming uniform distribution within the fibers. The amount of biotin available to bind streptavidin protein at the fiber surface was measured directly using a colorimetric assay. Samples containing larger overall concentration of biotin were able to bind disproportionately larger quantities of streptavidin. The segregation of a small molecule towards the surface of the fibers is expected based both on the nature of charge concentration on the electrospinning jet skin during the spinning process and the lack of entropy of mixing in polymer systems tend to favor phase separation rather than intimate mixing as the equilibrium condition.

A biosensor assay confirms that the electrospun PLA membranes containing biotin can successfully capture and be used for detection of biological molecules. With surface biotin availability as low as 1.2 mg/g membrane significant capture of streptavidin, a biotinylated antibody and a fluorescence labeled secondary antibody were observed.

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