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Abstract Mechanically robust, non-toxic polymer fiber mats are promising materials for a range of biomedical applications; however, further research into enhancing polymer selection is needed. In this study, poly(allylamine) (PAH), an amine-containing polyelectrolyte, was successfully electrospun from aqueous solutions into continuous, cylindrical fibers with a mean diameter of 150 ± 41 nm. A one-step crosslinking method using glutaraldehyde provides insight into the chemical and morphological changes that result from altering the molar ratio of amine to aldehyde groups, whereas a two-step crosslinking method yielded chemically and mechanically robust mats. These results indicate PAH fibrous mats synthesized from aqueous solutions could potentially be applied in biomedical applications.

Introduction

Electrospinning has emerged as a widely implemented fabrication technique for creating fibrous mats for

biomedical applications including controlled-release drug delivery, tissue engineering scaffolds, implants, and biocatalysis supports [1]. This inexpensive, scalable method yields flexible mats, which exhibit enhanced specific surface-to-volume ratios, improved mechanical properties, and smaller pores than fibers produced using traditional methods.

Numerous electrospun synthetic polymers: poly(lactic-co-glycolic acid) (PLGA), poly(ethylene oxide) (PEO), and poly(ϵ -caprolactone) (PCL) have demonstrated potential for tissue regeneration [2–6]. Additionally, further tailoring of the mats can elicit specific biological responses, for example, encapsulation of antibiotics for sustained release or fiber alignment to direct neurite growth [6, 7]. Despite these recent advances with electrospun fibers, synthetic polymer selection and mats fabricated using green solvents still remain a key challenge toward advancing mat applicability in tissue engineering applications [5, 8–10].

Poly(allylamine) (PAH, Fig. 1) is an amine-containing ($\sim \text{pK}_a$ 8–9) [11–16] cationic polyelectrolyte in aqueous solutions. Commercially, PAH is available in its salt form, poly(allylamine) hydrochloride (PAH-HCl). PAH-HCl in aqueous solutions is acidic and cationic with a Cl^- counterion next to its protonated amine groups [17]. Most studies on PAH-HCl or PAH focus on its layer-by-layer assembly [18–21] or capsule applications [22, 23]. Moreover, recent studies have shown its improved biocompatibility after chemical modification, suggesting the promising use of the polymer in biomedical fields for various applications, such as in vitro gene transfer vectors [24–26] or insulin delivery [27].

Previously, incorporation of PAH-HCl with PEO and polyethylene terephthalate (PET) increased the charge density of the electrospinning solution to facilitate fiber formation [28–33]. Chunder et al. [34] electrospun

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Fig. 1 Chemical structure and protonation of PAH in the presence of an acid (HA)

PAH-HCl (70000 Da) by pairing it in a one-to-one ratio with the weak anionic polyelectrolyte, poly(acrylic acid) (PAA, 90000 Da). In this work, we focused on facilitating mat fabrication using the charge carrying PAH. To date, investigations into the electrospinning of PAH without additional polymer have yet to be reported.

Additionally, we have determined the conditions to electrospin PAH fiber mats at various pHs. Solely aqueous solutions are needed to spin pH 11 PAH solutions. To prevent dissolution of the fibrous mats, chemical crosslinking of the as-spun mats utilizing the organic molecule glutaraldehyde (GA), a known amine crosslinking agent, was confirmed using Fourier transform infrared spectroscopy (FTIR). Tensile mechanical tests were conducted, and scanning electron microscopy (SEM) was used to confirm fibrous morphology.

Experimental

Materials

Poly(allylamine) (PAH, 20 wt% solution in water, $M_w = 65000$ Da), trifluoroacetic acid (TFA, 99 % ReagentPlus[®]), and glutaraldehyde (GA, 50 wt% in water) were used as received from Sigma-Aldrich (St. Louis, MO).

Electrospinning of PAH (at pH 2 and 11) and one-step crosslinked PAH-GA mats

PAH (20 wt% in water) solutions with a pH of 11 were electrospun as received. To electrospin PAH solutions having a pH of 2, a 1:1 PAH/TFA volume ratio was mixed for 10 min on an Arma-Rotator A-1 (Bethesda, MD). One-step crosslinked mats were electrospun from PAH/TFA/GA solutions wherein the previously mentioned PAH/TFA solution was prepared, followed by the addition of GA 5 s prior to electrospinning [35]. Three different amine/aldehyde molar ratios (1:1, 2:1, and 1:3) were investigated. One-step crosslinked mats electrospun from PAH/TFA/GA solutions are referred to as PAH-GA mats.

Approximately, 2 mL of each solution was loaded into a 5 mL Luer-Lok Tip syringe (Becton–Dickinson and Co, Franklin Lakes, NJ), and a Precision Glide 21-gauge needle (Becton–Dickinson and Co) was attached. Using an alligator clip, the positive electrode of a high voltage supply

(Gamma High Voltage Research Inc., Ormond Beach, FL) was directly connected to the needle. The syringe was then placed on an advancement pump (Harvard Apparatus, Plymouth Meeting, PA) at a fixed distance (10 cm) from the negative electrode that was clipped to a copper plate wrapped in wax paper, which facilitated the removal of mats post-electrospinning. A voltage (10–15 kV) was then applied between the positive and the negative anodes as the solution was advanced at a constant rate of 1.40 mL h^{-1} for 5 h. During electrospinning, both the temperature ($^{\circ}\text{C}$) and the relative humidity in percent (% RH) were monitored using a digital thermohygrometer (Fisher Scientific, Pittsburgh, PA).

Fabrication of two-step crosslinked PAH-GA mats

As-spun PAH fiber mats were placed in a $11.43 \text{ cm} \times 7.62 \text{ cm} \times 5.08 \text{ cm}$ vapor chamber (VWR Scientific Products, Bridgeport, NJ) as previously described [35]. GA liquid (5 mL) was added to the bottom of the chamber and allowed to vaporize at room temperature (23°C) to crosslink the sample for 24 h.

Characterization of electrospun PAH mats

Micrographs of the electrospun fiber mats were acquired using a Zeiss Supra 50VP field-emission scanning electron microscope (FESEM) (Carl Zeiss NTS, LLC, North America). A Denton Vacuum Desk II (Moorestown, NJ) sputter machine was utilized to coat the samples for 10 s at a current of 40 mA, depositing a layer ($\sim 3 \text{ nm}$) of platinum/palladium. Mats exposed to 65 % RH were prepared in the humidity chamber and stored in a sealed bag before imaging. Mean fiber diameters ($N = 150$) were measured using ImageJ 1.41o software (National Institute of Health, USA). All statistical differences were determined using an unpaired t test with values of $p \leq 0.05$ considered to be statistically significant. Spectra of all fiber mats were acquired utilizing an attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR, Varian Excalibur FTS-3000, Varian, Inc., Palo Alto, CA). Spectra were acquired in the wavenumber range of $4000\text{--}500 \text{ cm}^{-1}$ by accumulating 64 scans with a resolution of 4 cm^{-1} . All samples were kept at a maximum relative humidity of 40 % RH.

The Young's modulus, E , and ultimate tensile strength, σ_{UTS} , of the as-spun PAH mats were determined using an Instron 5500R (Model 1125, Instron, Norwood, MA). For testing, each mat was cut into three $5 \text{ mm} \times 35 \text{ mm}$ strips and mounted with adhesive tape between two square paper frames. The frames had an opening ($25 \text{ mm} \times 25 \text{ mm}$) that prevented the sample from loading before testing. The sample holder frames were mounted in the Instron using

pneumatic grips, and their vertical sides cut prior to testing. Tensile tests were carried out with a 500 g load cell at a strain rate of 0.02 s^{-1} . The mat properties were determined at constant temperature (21°C) and for three different relative humidities (RH): low (40 % RH), intermediate, and high (65 % RH). Stress and strain were calculated using an effective cross-sectional area of the fiber mats and a gauge length of 25 mm determined at 40 % RH. The effective cross-sectional area for each sample was determined from the weight per unit length of the strip at 40 % RH, assuming a dry polymer density of $1,200 \text{ kg/m}^3$. The modulus of the fiber mats was calculated from the initial linear slope of the stress–strain curve; the tensile strength is the maximum tensile stress supported by the mat. The two-step PAH–GA mats were tested with a Kawabata Evaluation System tabletop uniaxial tensile testing system (KES-G1, Kato Tech Co, Ltd., Kyoto, Japan). The strips were prepared and evaluated using the same parameters as the as-spun PAH mats; however, the humidity was maintained at 40 % RH.

Results and discussion

Characteristics of as-spun poly(allylamine) (PAH) fiber mats

PAH mats were successfully electrospun from aqueous solutions (Fig. 2a) and were composed of smooth, continuous cylindrical fibers. The polymer solution (20 wt%) was electrospun utilizing an applied voltage of 15 kV, as well

as a fixed separation distance (10 cm) between the needle and collector plate. Mean fiber diameter ($150 \pm 41 \text{ nm}$) is displayed in Fig. 3. The mean diameter was smaller than that reported (300 nm) for fibers electrospun from a 1:1 ratio of PAH–HCl/PAA [34].

Figure 4 displays the spectra obtained for as-spun PAH mats. The vertical solid or dotted lines and the upward arrows correspond to the peaks assigned for the functional groups of interest. The as-spun PAH mats (Fig. 4a) displayed peaks characteristic of PAH: $3356\text{--}3174 \text{ cm}^{-1}$ (m, N–H), 2900 cm^{-1} (s, C–H), 1578 cm^{-1} (s, N–H), and 1311 cm^{-1} (m, C–N). The free amines on the mat are attractive for various chemical reactions such as functionalization or crosslinking procedures to further tailor the mats toward biomedical applications.

Characteristics of one-step crosslinked PAH–GA fiber mats

In order to improve the chemical and mechanical stability of as-spun PAH mats, two different routes toward crosslinking utilizing GA were investigated (Fig. 5). PAH has one amine group, which acts as the site for crosslinking with GA [36]. Each GA molecule has two crosslinking sites. The successful crosslinking between a primary amine (Fig. 5b) and an aldehyde (Fig. 5a) via Schiff base reaction yields imine (<C=N-) functionalities (Fig. 5d) at ambient conditions. More stable products are yielded if the reaction occurs under extreme pHs ($\text{pH} > 9$) [35, 37].

To minimize processing steps, an in situ crosslinking technique featuring GA was optimized. The direct

Fig. 2 Micrographs of as-spun **a** PAH and **c** PAH–TFA mats. These mats are not crosslinked. Adding TFA into the aqueous PAH solution lowered the pH of the electrospinning solution and enabled in situ crosslinking of PAH mats. Micrographs **(d–f)** display one-step

crosslinked PAH–GA fiber mats containing a **c** 1:1, **d** 2:1, and **e** 1:3 molar ratio of amine/aldehyde groups. Increased branching was observed for **b** PAH–GA (two-step) crosslinked mats

incorporation of GA into the basic (pH 11) aqueous PAH solution yielded an instant gel thus inhibiting electrospinning. Literature states that at low pH values, 4 and below, almost all of the amine groups ($\sim pK_a$ 8–9) of PAH are protonated [13, 15, 38] (Fig. 1) thus reducing its ability to react with the aldehyde (Fig. 5a) of GA [36]. It is known that at low pH, the carbinolamine (Fig. 5c) is formed when an amine reacts with an aldehyde, which proceeds to form an iminium cation (Fig. 5e) before the formation of the imine crosslink (Fig. 5d). Hence, a strong acid, trifluoroacetic acid (TFA), was added to the aqueous PAH solution until the solution had a pH of 2. Mats (Fig. 2c) with a mean fiber diameter of 743 ± 346 nm were electrospun from the

pH 2 (1:1 PAH:TFA v:v) solution. This mean is statistically ($p \leq 0.05$) larger than the mean of fibers synthesized from the aqueous PAH solution.

GA addition into the pH 2 PAH solution (containing TFA) exhibited a delayed gelation in comparison with aqueous PAH (pH 11) solutions. The PAH–TFA solutions featuring GA could be successfully electrospun for up to 2.5 h, at which point the solution gelled. All mats electrospun from TFA-containing solutions contained smooth, spherical, and continuous fibers; no beading was observed. Solutions featuring 1:1 and 2:1 amine/aldehyde molar ratios yielded mats (Fig. 2d–f) with mean fiber diameters that were statistically the same (148 ± 46 nm) and statistically larger (202 ± 82 nm) (Fig. 3) than as-spun PAH mats (150 ± 41 nm). FTIR confirmed that crosslinking occurred as indicated by the formation of the imine peak at 1627 cm^{-1} (Fig. 4b–d, C=N). As expected, unreacted amines (Fig. 4b–c, shifted N–H arrows) and aldehyde groups (Fig. 4b–c, HC=O) were observed at 1525 and 1660 cm^{-1} , respectively. Residual TFA (Fig. 4b–d, C=O and C–O peaks) was detected in the PAH–GA mats. The presence of residual TFA ions may have (1) prevented the deprotonation or (2) caused screening/blocking of the amines to occur. Thus, amines were available in the PAH–GA (1:1) mats (Fig. 4b), and aldehydes were unreacted in the PAH–GA (2:1) mats (Fig. 4c).

The addition of a threefold excess of aldehyde groups in the PAH–GA (1:3) mat yielded a statistically larger mean fiber diameter, 1075 ± 959 nm, than the as-spun PAH, as well as PAH–GA mats featuring a 1:1 and a 2:1 amine/aldehyde ratios. Fiber branching was observed (Fig. 2f). Most likely, the morphological change is due to extensive crosslinking [39]. Another approach to increasing the fiber diameter, which is common to the electrospinning literature relates increasing the polymer chain interactions via increasing polymer concentration [40–42]. This hypothesis correlates well with observations in the FTIR spectra. Mats

Fig. 3 Fiber diameter distribution of all PAH-based electrospun mats. Box heights represent the 25–75 % range with the asterisk on both ends corresponding to maximum and minimum diameter. Vertical whiskers indicate the standard deviation range, while the open square inside each box indicate the mean ($N = 150$). From left to right, the mean fiber diameter of as-spun PAH is 150 ± 41 nm, PAH–GA (two-step crosslinked) is 190 ± 63 nm, PAH–TFA is 743 ± 346 nm, PAH–GA (1:1) is 148 ± 46 nm, PAH–GA (2:1) is 202 ± 82 nm, and PAH–GA (1:3) is 1075 ± 959 nm

Fig. 4 FTIR transmission spectra of **a** as-spun PAH, **b** PAH–GA (1:1), **c** PAH–GA (2:1), **d** PAH–GA (1:3), and **e** PAH–GA (two-step) mats. IR (ATR) cm^{-1} : 3356–3174 (m, N–H), 2900 (s, C–H), 1770 (w, C=O of acid), 1660 (s, HCO of GA), 1627 (sh, C=N, imine), 1578 or 1525 (m, N–H), 1311 (m, C–N), 1130 (s, C–O of acid), and 1197–1128 (m, C–O–C from GA)

Fig. 5 Schematic showing the crosslinking mechanism of PAH with GA [36] under basic or acidic aqueous conditions: **a** aldehyde group from GA, **b** deprotonated amine from PAH, **c** unstable carbinolamine, **d** imine functionality where the C=O of GA is replaced with C=N, and **e** iminium cation

spun from the 1:3 PAH–GA solution display the formation of an imine at 1627 cm^{-1} , and the corresponding loss of all the primary amine peaks at $3356\text{--}3174\text{ cm}^{-1}$ and 1525 cm^{-1} (Fig. 4e).

Characteristics of two-step crosslinked PAH–GA fiber mats

One-step crosslinked mats could be electrospun for 2.5 h until solution gelation occurred; however, spinning for that duration of time was not long enough to enable the

fabrication of mats thick enough to yield reliable tensile testing data. Previously, a two-step method featuring GA-vapor was demonstrated to crosslink electrospun chitosan via a Schiff base imine functionality [35]. Employing this technique to as-spun PAH mats yielded two-step crosslinked PAH fibers (Fig. 2b). Increased fiber swelling and branching were evident after the PAH fibers interacted with the GA-vapor (50 wt% in water). The mean fiber diameter $190 \pm 63\text{ nm}$ of two-step crosslinked PAH–GA mats was statistically larger than those reported for the as-spun PAH mats (Fig. 3). Similar to the one-step crosslinked PAH–GA mats, the FTIR spectra (Fig. 4e) displayed imine peaks (shifted C=N) along with the expected presence of strong peaks indicative of unreacted aldehyde (HC=O) and amine (N–H and C–N) moieties. The strong aldehyde and amine may be attributed to GA reacting slightly reversibly with the amino groups present at $\text{pH} > 3$ [43]. As-spun PAH mats are at this pH since they are electrospun from aqueous solutions (pH 11). Numerous studies and a review by Migneault et al. [37] have reported that GA undergoes aldol condensation in neutral to basic pHs, i.e., 7–13. This yields mixtures of monomeric dialdehydes, as well as dimers or trimers, or poly-glutaraldehydes mostly having C–O–C functionalities. Our FTIR spectra support these findings; strong peaks are present at $1197\text{--}1128\text{ cm}^{-1}$ (Fig. 4e). Also, it is notable that Stachewicz et al. [44] have reported that surface functionality is influenced by the applied voltage polarity. Their findings suggest that the cationic amine groups would be pulled toward the surface of the fibers since the electrospinning setup used in this study features a connection between the positive electrode

Fig. 6 Micrographs of **a** PAH post-tensile testing at 65 % RH and **b** PAH–TFA at 65 % RH. Micrographs of **c** PAH–GA (1:3) at 65 % RH and **d** two-step crosslinked PAH–GA post-tensile testing at 40 % RH

Fig. 7 Representative **a** stress–strain curve and **b** micrograph of the fractured surface post-tensile testing at 40 % RH of two-step crosslinked PAH–GA mats

and the needle. This improves the interaction of the amine functional groups with the surrounding media for cellular interactions or functional group manipulation.

Mechanical properties and humidity effects of PAH fiber mats

Micrographs revealed that after storage at 65 % RH, the as-spun PAH (micrograph not shown) and PAH–TFA mats lost their fiber structure (Fig. 6). This ‘filming-out’ behavior is likely due to the hygroscopic characteristic of PAH and is consistent with previous reports on the polyelectrolyte PAA, which was observed to swell at a higher relative humidity [32]. Both crosslinking methods improved the morphological stability of the PAH mats (Fig. 6c, d) and at elevated percent relative humidity, their fiber structure was retained.

The Young’s modulus and ultimate tensile strength of as-spun PAH mats seemed to increase with increasing percent relative humidity. However, this was due to the morphological changes observed in as-spun mats at 65 % RH. Thus, stress calculations based on the cross-sectional area determined in the unswollen state were no longer relevant. Attention was, therefore, focused on determining the properties of three different mat compositions at a constant RH of 40 %, ensuring that a fibrous structure was maintained in each case.

Figure 7 displays a representative stress–strain curve and brittle break observed post-tensile testing of the two-step crosslinked PAH–GA mats. Tensile tests determined that the modulus of the as-spun PAH mats initially were 0.17 MPa and increased to approximately 2.3 MPa and 1.4 MPa in the case of TFA addition and two-step crosslinking, respectively. Tensile strength increased to 1.0 MPa and 1.2 MPa from 0.16 MPa after the addition of TFA and GA, respectively. We suggest that our increased mechanical properties stem from the increase in charge density that occurs at low pHs; the as-spun PAH precursor solutions have a pH of 11, whereas the pH decreases to 2 for PAH–TFA and PAH–GA spinning. This is consistent with the effect that pH had on the mechanical properties of

polyelectrolyte multilayer microcapsules [45], as well as the effect that molecular level orientation [46] had on polymer fibers.

Conclusion

We have successfully electrospun poly(allylamine) (PAH) into continuous cylindrical fibers from basic and acidic aqueous solutions with no required additional polymers. Glutaraldehyde (GA) was utilized to controllably crosslink, in a one- and a two-step process, the electrospun PAH mats. PAH–GA (two-step) mats had a mean fiber diameter of 190 ± 63 nm, which was statistically larger than the as-spun PAH (150 ± 41 nm) mats. At 40 % RH, the mechanical and morphological properties of PAH mats are retained. However, PAH mats at 65 % RH display hygroscopic ‘filming-out’ behavior. crosslinking and understanding the storage limitations of PAH fiber mats is an imperative step for biomedical applications that require a mat that is chemically and mechanically robust.

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