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Removal of Retained Electrospinning Solvent Prolongs Drug Release from Electrospun PLLA Fibers

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Abstract

A major challenge in developing drug-releasing electrospun nanofibers is obtaining long-term drug release over many weeks with no burst release of drug. Here, we present new methods capable of prolonging the diffusive release of small molecule drugs from electrospun poly-L-lactic acid (PLLA) nanofibers. The methods focus on removal of retained electrospinning solvent through fiber heating, maintaining fibers in a laboratory setting, or a combination of these methods. These post-fabrication methods altered the release characteristics of a model small molecule drug, 6-aminonicotinamide (6AN), from PLLA fibers. Specifically, untreated fibers released 6AN over 9 days, and fibers that underwent a combined treatment of maintenance in a laboratory setting and heating released 6AN over 44 days. The unique and simple method presented here prolongs diffusive release of a small molecule drug from electrospun fibers and has potential to assist in lengthening small molecule drug release from a variety of polymeric nanomaterials.

Graphical Abstract

Keywords

Drug delivery; biomaterials; electrospun fibers

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1. Introduction

Electrospun fibers are created for applications including filtration,[1–3] battery fabrication, [4–6] tissue engineering[7–11] and drug or protein delivery.[12–16] For cellular applications, electrospinning creates polymer nanofibers with diameters similar to those of fibrous proteins found in the extracellular matrix. Due to the fibers resembling the structure of the extracellular matrix, aligned, electrospun fibers are used as tissue engineering scaffolds to guide directional tissue regeneration. In recent years, however, electrospun fiber scaffolds are developed to deliver drug locally. By incorporating a drug within the polymer matrix of electrospun fibers, these scaffolds can provide physical cues that facilitate fast tissue regeneration while also delivering drugs to an injury site. Different approaches can modify the rate of drug release, including changing the amount of drug loaded into fibers, [13,17,18] altering fiber diameter,[19,20] selecting different polymers or utilizing different polymer concentrations,[13,18,19] or selecting specific solvents[19] to name a few. These fiber modifications are necessary since electrospun fibers frequently release drugs in a burst fashion within hours or days. However, a burst release of drug is not beneficial for situations requiring long-term drug delivery.

Among studies that utilize electrospun Poly-L-lactic acid (PLLA) fibers as drug delivery vehicles there are large differences in the release rates of drugs from fibers.[13,17–19,21] A study by Kenawy and colleagues shows an abbreviated release of tetracycline HCl from electrospun PLLA fibers occurring instantaneously upon submersion in release buffer.[18] In contrast, Roman et al. show prolonged release of paclitaxel from electrospun PLLA fibers that lasted for 84 days.[17] A study by Zeng and colleagues discusses the effects of drug solubility and compatibility in a drug/polymer/solvent system which potentially explains the drastic differences in release kinetics observed from these various fiber scaffolds. This study showed that selecting a drug that is more compatible within a drug/polymer/solvent system will result in better encapsulation of drug within fibers and thus prolong the drug release from fibers.[22] However, for scenarios where a drug or polymer within a drug/polymer/solvent system cannot be altered, it is necessary to develop procedures to alter drug release kinetics from these fibers.

In this study we developed a new method to obtain sustained release of the hydrophobic drug, 6-aminonicotinamide (6AN), from slowly degrading, hydrophobic, electrospun PLLA nanofibers. To prolong the release of 6AN, our new method focused on removing retained solvent in fibers following electrospinning. A recent study conducted by D'Amato et al. revealed large amounts of the electrospinning solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) being retained in electrospun PLLA fibers after fabrication.[23] Retained solvent plasticizes the polymer and creates free volume between polymer chains. Furthermore, retained solvent can potentially complex with drug and shuttle drug out of the fiber quickly when the fiber is immersed in an aqueous environment. Therefore, we hypothesized that removing retained solvent would prolong 6AN release from PLLA fibers by decreasing the free volume within the polymer matrix and eliminating the potential of the solvent to shuttle drug out the fiber quickly. To our knowledge, removing retained solvent from drug-containing electrospun fibers to alter drug release kinetics is a new approach not before examined in the literature.

2. Materials and Methods

2.1 Materials

PLLA (NatureWorks; grade 6201D) was purchased from Cargill Dow LLC (Minnetonka, MN). HFP (Product No. 105228) was purchased from Sigma-Aldrich Corp (St. Louis, MO). 6AN (Product No. L06692) was purchased from Alfa Aesar (Haverhill, MA). Glass cover slips (15x15 mm) were purchased from Knittel Glass (Brausenweig, Germany). Syringes (5 mL) and needles (22 ½ gauge) were purchased through Becton Dickinson (BD, Franklin Lakes, NJ). Hermetic DSC pans (TA instruments, Product No. A40548-04) and lids (TA instruments, Product No. A39817-2) were purchased from DSC Consumables Inc. (Austin, MN).

2.2 Description of Electrospinning Apparatus

The Gilbert lab electrospinner is completely housed in a 35 in. x 36 in. dissipative PVC glove box (Terra Universal, Fullerton, CA). This closed environment allows for accurate humidity control through the use of a humidifier and dry air supply. The electrospinner is described in detail in previously published literature from the Gilbert laboratory. Briefly, electrospinning solutions were pumped out of a 5 mL syringe through a 22 ½ gauge needle that was attached to a Gamma High Voltage Power Supply (Model No. ES50P-10W, State College, PA). Fibers were collected on 15 x 15 mm glass cover slips that were attached to a grounded, spinning aluminum disk (22 cm diameter, 1 cm thick).

2.3 Electrospun Fiber Fabrication and Treatment

6AN-releasing electrospun PLLA fibers were electrospun from a polymer solution containing 12% w/w PLLA in HFP with 10% w 6AN/w PLLA added to the solution. All solutions were electrospun with a relative humidity of 33% ± 3% inside of the electrospinning environment with a rotational wheel speed of 1000 rpm, and an applied voltage of 10 kV. Fibers were electrospun for 15 minutes.

After electrospinning, fibers were exposed to various solvent removal treatments. A previous study by D'Amato et al. showed that ~15.5% w/w of HFP in PLLA remains in fibers after electrospinning.[23] Briefly, that study showed that leaving fibers in a laboratory environment for 14 days reduced the amount of retained HFP in fibers to ~9% w/w, and heating fibers in an incubator reduced the amount of retained HFP in fibers to ~6% w/w after a single, four hour treatment. The previous study by D'Amato and colleagues revealed the ability of these two techniques to remove residual solvent from electrospun fibers. This motivated us to study how residual solvent and its removal may effect drug release from fibers. Importantly, drug was not included in the fibers in this previous study. Since 6AN has affinity for HFP, it is possible that following electrospinning, more solvent was retained in the current study then was previously reported.

Fiber scaffolds in the current study were either left in the laboratory at ambient conditions for 28 days, placed in a cell culture incubator for 4 hours, or left in ambient conditions for 28 days followed by 4 hour incubation. The cell culture incubator was maintained at 37 °C, 5 % CO₂, and 90% – 95% relative humidity. The 28 day time point was selected based on a

previous study that showed that HFP elutes slowly out of electrospun fibers over the course of weeks following fabrication.[23] Fibers that were characterized immediately after electrospinning without further treatment served as a control. The treatment groups analyzed in this study were chosen after an initial set of experiments where fibers were heated in the incubator or on a hot plate to 40 °C or 100 °C for four hours. These treatments were performed either immediately after electrospinning or 28 days after electrospinning as previously described. The incubated fiber groups showed the most interesting data by releasing an increased amount of 6AN over a significantly increased period of time. Thus, these groups were studied in further detail in the current study. All 6AN release data from fibers heated to other temperatures is included in Supplemental Figure 1.

2.4 Morphological Characterization of Electrospun Fibers

Since drug release from electrospun fibers can be affected by fiber diameter, scanning electron microscopy (SEM) was performed on all fiber scaffolds to characterize fiber diameter. In preparation for SEM, all electrospun fiber scaffolds were sputter coated with a 5 Å thick layer of platinum using a Technics Hummer V sputter coater (Anatech Ltd., Denver, NC). SEM was conducted using a FEI Versa 3D DualBeam SEM (Hillsboro, OR). Low accelerating voltages (2 – 5 kV) were used during SEM to prevent melting the polymer fibers while imaging. Fiber SEM images were analyzed using NIH ImageJ software (Bethesda, MD). Briefly, fiber cross-sectional diameters were measured in ImageJ, and the pixel length of this diameter was compared to the pixel length of the accompanying image scale bar to obtain a fiber diameter value. Three independently fabricated electrospun fiber scaffolds were imaged for each fiber group and 100 fiber diameters were analyzed in each fiber scaffold. Thus, a total of 300 fiber diameters were measured for each fiber treatment group.

2.5 6AN Release and Loading Characterization

2.5.1 6AN Standard Curve—To create a standard curve for 6AN concentrations, 10 mg of 6AN was dissolved in 10 mL of PBS. A 1:10 dilution was performed to obtain a 6AN concentration of 100 µg/mL, followed by a serial 1:2 dilution to a final 6AN concentration of 0.2 µg/mL. The standard curve was created by reading UV absorbance at 267 nm (A_{267}) over the range of 6AN concentrations in a Tecan Infinite M200 plate reader (Morrisville, NC). A_{267} values were plotted against concentrations to obtain a standard curve. This procedure was performed in triplicate using three independently fabricated 6AN stock solutions.

2.5.2 6AN Release Characterization—6AN-releasing scaffolds were submerged in 1 mL of PBS 24 hours after either electrospinning or fiber treatment. Every 24 hours, 100 µL of PBS was removed and A_{267} was assessed using a plate reader. A_{267} values were converted to 6AN concentrations using the previously obtained standard curve. After UV analysis, the remaining PBS on each fiber scaffold was removed and replaced with 1 mL of fresh PBS. After 14 days, 6AN concentration was assessed every 48 hours instead of every 24 hours to increase A_{267} values since 6AN release at later time points was lower than earlier time points.

2.5.3 6AN Loading Capacity—After fabrication, 6AN-loaded scaffolds were dissolved in 1 mL of HFP. A_{267} of the solution was assessed using a plate reader to determine the amount of 6AN incorporated into each fiber scaffold. A_{267} values were converted to 6AN concentrations using a standard curve of 6AN concentrations in HFP. This standard curve was prepared independently of the standard curve used to characterize 6AN release in PBS.

2.6 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was performed to analyze changes in the glass transition temperatures (T_g) and % crystallinities of electrospun fiber scaffolds after the various treatments. Fibers were removed from the collection wheel after electrospinning and 5 mg samples were collected and placed into hermetic DSC pans. A hermetic DSC lid was crimped onto the pan to seal the sample inside of the pan. DSC was then performed on three independently fabricated samples for each experimental fiber group. A thermal cycle from 20 °C to 180 °C and back to 20 °C was used with a 10 °C/min heating and cooling ramp. DSC curves were then used to determine T_g , and enthalpy changes during polymer crystallization (ΔH_C) and melting (ΔH_M) using TA Universal Analysis software. Fiber % crystallinity was calculated using equation 1.

$$\% Crystallinity = 100 * \frac{\Delta H_M - \Delta H_C}{\Delta H_C} \quad (1)$$

2.7 Statistical Analysis

All statistical analysis was performed using JMP IN software (Release 8.0.1; SAS, Cary, NC). A one-way ANOVA was used to analyze statistical differences in fiber diameter, T_g , and duration and amount of 6AN release from fibers. A post-hoc Tukey's HSD test was used to compare values directly between differentially treated fibers. Statistical differences with $p < 0.005$ were considered statistically significant.

3. Results

6AN release from electrospun PLLA fibers is summarized in Table 1. Fibers loaded with 6AN that were untreated (fibers with a high degree of retained solvent) and submerged in PBS immediately after fabrication released 6AN in a burst fashion over 4 days (Figure 1A). This initial burst release was followed by minimal release that ceased after 9 days. Fibers placed in the incubator for four hours after fabrication (Figure 1B) released 6AN in a similar manner. These fibers, however, released more 6AN than the control fibers (Table 1), and the burst release was delayed by two days. Release from incubator-treated fibers also ceased after 9 days. Fibers that were left in ambient lab conditions for 28 days following fabrication (Figure 1C) released drug differently than the previous two scaffolds. These fibers initially released drug in a similar burst fashion, however, this burst was followed by sustained 6AN release that persisted until day 32. These fibers released more than twice the amount of 6AN released from control fibers (Table 1). The final scaffold that consisted of fibers that were left in ambient lab conditions for 28 days prior to incubator heating released 6AN most uniquely (Figure 1D). These fibers received combined treatment and released 6AN in a

linear fashion (linear fit $R^2=0.9$) with no burst release over the course of 44 days (~5-fold increased release duration compared to control fibers). These fibers also released more than twice the amount of 6AN that control fibers released. The increased amounts of 6AN released from treated fibers were not statistically significant compared to the amount of 6AN released from control fibers using Tukey's HSD. The increases in drug release duration, however, were statistically significant for both sets of fibers that were maintained in the lab for 28 days prior to treatment or release ($p<0.005$).

After observing that treated fibers released more 6AN than untreated controls, we analyzed the amount of 6AN loaded into the fibrous scaffolds during fabrication. These additional experiments were conducted to determine if all of the 6AN was being released after treatment or if drug remained within the fibers after release had ceased. Prior to release or treatment, fiber scaffolds contained 101.1 ± 12.4 μg of 6AN. The fiber scaffold that released the most 6AN (fibers that were incubated after 28 days in the lab) released only 17.9 ± 6.0 % of the 6AN that was incorporated into the fibers (Table 1).

To determine the mechanism by which the varying fiber treatments affected drug release characteristics from electrospun fibers, we first explored potential changes in fiber morphology. Representative SEM micrographs of each fiber scaffold are presented in Figure 2A–D. Immediately after electrospinning, untreated fibers had a diameter of 798 ± 55 nm. All three solvent removal techniques resulted in statistically significant changes in fiber diameter compared to untreated controls (Figure 2E). Specifically, the average diameter of electrospun fibers that were placed in the incubator immediately after fabrication increased significantly to 839 ± 61 nm. The average diameter of electrospun fibers that were maintained in laboratory conditions for 28 days had a decreased average diameter of 761 ± 67 nm, and fibers that were incubated after 28 day maintenance in lab conditions had an increased diameter of 809 ± 70 nm. Because there were no observable trends relating drug release kinetics and fiber diameter, we explored a second potential mechanism by which drug release kinetics were prolonged through fiber treatment.

The second mechanism examined the changes in polymer free volume in the fibers that occurred during solvent removal treatments. To analyze polymer free volume we used differential scanning calorimetry (DSC) to analyze the glass transition temperatures (T_g) of all four fiber scaffolds (Figure 3A). An increase in polymer T_g occurs when the free volume in a polymer decreases. Freshly prepared, unheated fibers had a T_g of 37.1 ± 1.2 °C. After incubation, the freshly prepared fibers had an increased T_g of 51.4 ± 0.5 °C. Fibers that were left in lab conditions for 28 days had a T_g of 49.5 ± 0.6 °C, and fibers that were heated in the incubator after 28 days of laboratory maintenance had a T_g of 55.5 ± 0.5 °C (Figure 3B).

To substantiate our analysis of T_g we also determined % crystallinity of all electrospun fiber scaffolds (Figure 3C). Using DSC, the % crystallinity of fibers were 16.78 ± 2.29 % for freshly prepared, unheated fibers, 23.44 ± 2.37 % for freshly prepared, incubator heated fibers, 21.93 ± 1.54 % for fibers that were held in lab conditions for 28 days, and 23.59 ± 3.03 % for fibers that were held in lab conditions for 28 days prior to incubator heating. Although the differences in % crystallinity among fiber groups were not statistically significant, the trend in crystallinity mimicked the observed trend in T_g nearly identically.

4. Discussion

The current study focused on four distinct electrospun fiber scaffolds that were each exposed to a different solvent removal treatment regimen. The major findings of this study were: 1) Long term solvent removal (28 days) from electrospun PLLA fibers followed by heating in an incubator increased the duration of 6AN release from fibers by a factor of ~5 compared to untreated controls. 2) All solvent removal treatments increased the amount of 6AN released from electrospun fibers. 3) Changes in drug release characteristics between differentially treated electrospun fibers was not caused by a change in fiber morphology that occurred during treatment. 4) DSC results suggest that the changes in drug release characteristics observed in fibers are caused by a decrease in solvent-induced polymer free volume or an increase in polymer crystallization occurring during solvent removal. The increase in amount and duration of drug release from electrospun fibers observed in this study using procedures to remove retained solvent has not previously been reported in the literature.

It is well established from the literature that drug burst release from electrospun fibers results from different mechanisms. A large contributor to drug burst release from fibers is the high surface area to volume ratio (SA:V) of nanofibers. The high SA:V enables greater exposure of the polymer to the surrounding release buffer (such as PBS). Theoretically, an increase in fiber diameter will result in a decrease in SA:V and consequently a decrease in the rate of diffusion from the fibers. A study by Chen et al. analyzed the effects of changing fiber diameter on drug release kinetics from electrospun PLLA fibers.[19] In that study, the researchers concluded that larger diameter fibers were able to release drug for longer periods of time. Since we observed prolonged 6AN release from both scaffolds that were maintained in the lab for 28 days, we hypothesized that this fiber treatment (alone or coupled with incubator heating) was increasing fiber diameter and consequently decreasing the SA:V of these scaffolds. All four scaffolds studied here were fabricated using identical fabrication materials and methods. Prior to treatment, all scaffolds had the same fiber diameter. We rejected our hypothesis that 28 day maintenance in the lab was increasing fiber diameter and causing the prolonged 6AN release from these fibers. Of the two scaffolds that released 6AN over extended periods of time, one had fibers with decreased diameters (28 day maintenance in the lab) and one had fibers with increased diameters (28 day maintenance followed by incubation). Thus, we concluded that the observed changes in fiber diameter were likely not responsible for the dramatic differences that we observed in drug release kinetics. It is important to note, however, that both fiber scaffolds that received incubator treatment had fibers with significantly increased diameters. This change in diameter may be the result of pore formation within the fibers during solvent evaporation, particularly in the presence of a non-solvent of PLLA. In this scenario, the non-solvent was water present in the humid environment of the incubator (90–95% relative humidity). Solvent evaporation in the presence of a non-solvent can lead to vapor induced phase separations which results in pore formation in polymer materials.[24,25] Although pore formation was not analyzed in this study, it may have contributed to the mechanism by which more 6AN was available for release from treated fibers by allowing PBS diffusion deeper within the fibers.

To determine the mechanism by which solvent removal affected drug release, we also analyzed polymer free volume within fibers via T_g analysis using DSC. To our knowledge,

no study has analyzed the effects of solvent-induced polymer free volume on the drug release kinetics from electrospun fibers. We hypothesized that solvent removal during treatment reduced polymer free volume within fibers. A reduction in polymer free volume would hinder 6AN diffusion from the fibers, and likely slow release buffer penetration into the polymer matrix. Thus, we expected the scaffolds with a prolonged 6AN release to have higher values for T_g which correspond with decreased amounts of polymer free volume. All fiber scaffolds that underwent treatment had a significantly higher T_g and thus less free volume inside of the polymer fibers compared to untreated fibers (Figure 3B). Fibers undergoing combined treatment had the greatest change in polymer free volume and these fibers were able to release the most 6AN for the longest duration. Thus, our second hypothesis (fiber treatment decreases polymer free volume) was validated. However, in analyzing polymer free volume using DSC, fibers that were left in ambient lab conditions for 28 days had T_g values that were not statistically different from T_g values of fibers undergoing incubator treatment immediately after electrospinning. These scaffolds, however, exhibited drastically different 6AN release characteristics. Thus, solvent removal rate had a stronger impact on 6AN release characteristics than solvent removal in general.

Analyzing fiber diameter and T_g provided insight towards potential mechanisms by which 6AN release was prolonged. However, these analyses likely do not explain the ability of each treatment to increase the amount of 6AN release. Since the high molecular weight PLLA used in this study is hydrophobic, the decreased amount of 6AN released from the unheated fiber controls immediately after electrospinning may have resulted from 6AN entrapment in the polymer core. Due to the hydrophobicity of PLLA, the release buffer cannot penetrate deeply into polymer fibers. Thus, drug near the fiber core does not diffuse out of the fibers. Further, when fibers are treated, 6AN, which is highly soluble in HFP, likely travels with the evaporating HFP towards the periphery of the fibers. While it seems likely that more 6AN available for diffusion near the fiber surface may lead to a strong burst release, this burst was not evident in fibers that underwent long-term solvent removal. It is possible that burst release of 6AN from these scaffolds was prevented by the decrease in polymer free volume that accompanied drug mobility during solvent removal from fibers. Thus, once fibers are submerged in release buffer, more drug is available at the surface of the fiber for diffusion. A schematic summarizing this description is displayed in Figure 4. Figure 4A presents a theoretical image of a fiber immediately after electrospinning that contains a greater amount of retained solvent. In this image, drug is uniformly distributed throughout the fiber, and drug near the fiber core is inaccessible by the surrounding release buffer. Figure 4B presents a theoretical image of a fiber after solvent removal, where drug initially near the fiber core is now closer to the surface of the fiber. The fiber depicted in Figure 4B presents a plausible explanation for how 6AN release amount is increased in treated fibers. This theory may explain why all three treated scaffolds released more 6AN than control fibers, and future studies will attempt to verify the mechanism displayed in Figure 4. Although the three treated scaffolds released increased amounts of 6AN, only the two scaffolds undergoing long-term solvent removal (28 day maintenance in lab) released 6AN over an increased period of time. This result corroborates our earlier claim that solvent removal rate influenced drug release characteristics more so than solvent removal in general.

A study by Miyajima et al. observed drug release from extruded PLLA rods (1 mm diameter) occurring in two distinct stages.[26] The first stage consisted of diffusive drug release. A second stage occurred upon polymer crystallization which occurred as the polymer matrix was hydrated by the release buffer. Polymer matrix hydration allowed for drug in different regions of the fibers to be released. The release kinetics observed in the study by Miyajima and colleagues differ from the study described in this manuscript due to differences in polymer fiber diameter, drug selection, and drug-polymer interactions. However, the discussion of polymer crystallization and drug release in that study provides insight to better understand the governing mechanisms behind the current study's results. The decreased amount of 6AN release observed in Figure 1A is likely the result of a portion of the incorporated 6AN being inaccessible by the release buffer as described in Figure 4A and substantiated by Miyajima's findings.[26] A second study by Miyajima et al. showed that pre-crystallization of PLLA rods negated the two-phase release profile of drug and instead resulted in linear release kinetics.[21] Although the rods were three orders of magnitude larger in diameter, the findings in that study corroborate our data. In Figure 3C we show that fibers that released 6AN for the longest duration in a nearly linear fashion also had the highest degree of crystallinity. Thus, we concluded that solvent removal from fibers presents a new approach to increase the amount and duration of drug release from electrospun fibers by altering polymer free volume and % crystallinity. At this time, however, it is unclear whether a change in polymer free volume or crystallinity within fibers is the prevalent mechanism by which drug release kinetics are altered.

5. Conclusions

Future work will focus on validating our hypothesis of treatment increasing drug mobility within fibers which in turn increases the amount of 6AN released from fibers. Further experiments will also include kinetic modelling of 6AN release data to determine if solvent removal treatments alter the mode of 6AN release from fibers. In the interim, this study details simple fiber treatment methods to significantly lengthen and increase small molecule release from PLLA nanofibers. In closing, it is important to compare findings presented here to other studies also releasing drug from electrospun fibers. While other studies report prolonged drug release from electrospun fibers,[17,20,27] those findings, along with results presented in this manuscript, are specific to the drug/polymer/solvent system that is being studied. For drug/polymer/solvent systems that inherently release drug in a linear fashion, these treatment methods are not necessary to prolong drug release. These methods are impactful, however, in scenarios where a drug or polymer selection may not be altered. Electrospun PLLA nanofibers release 6AN by means of a burst release. Unreported in the field, the burst release is changed through the use of simple post-fabrication placement in ambient laboratory conditions or through heating. Herein lies the impact of this study: a simple, repeatable method to dramatically increase the amount and duration of small molecule drug release from electrospun PLLA nanofibers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Solvent removal from electrospun PLLA fibers prolonged release of the drug 6AN ~5-fold.
- Solvent removal treatment increased amount of 6AN released ~2-fold.
- Different 6AN release kinetics due to changes in solvent-induced free volume/crystallinity.

Figure 1.

Release profiles of 6AN from electrospun nanofiber scaffolds. (A) Scaffold submerged in PBS immediately after electrospinning. (B) Scaffold placed in incubator for four hours immediately after electrospinning, then submerged in PBS. (C) Scaffold left out in laboratory for 28 days prior to submersion in PBS. (D) Scaffold left out in laboratory for 28 days prior to being placed in the incubator for four hours and then in PBS. (n=3, data represented are mean values $\pm$ standard error of means)

Figure 2.

Electrospun fiber SEM and characterization. (A–D) SEM micrographs of electrospun fibers that were (A) untreated, (B) placed in cell culture incubator immediately after electrospinning, (C) maintained in laboratory conditions for 28 days after electrospinning, or (D) placed in the incubator 28 days after electrospinning. (E) Fiber diameter was analyzed using FIJI software. (n=3 for fiber diameter analysis, all data reported are mean values $\pm$ std. error of means, *p<0.005 using Tukey's HSD)

Figure 3.

Electrospun fiber DSC and analysis. (A) Representative DSC curves for all treated fiber samples. (B) Glass transition temperatures (T_g), and (C) calculated % crystallinities for all fiber samples. (n=3 for all DSC analyses, all data reported are mean values $\pm$ std. error of means, *p<0.005 using Tukey's HSD)

Figure 4. Schematic of the effects of solvent removal on the 6AN distribution inside of an individual electrospun nanofiber. (A) A single PLLA fiber immediately after electrospinning with uniformly distributed 6AN. (B) A single PLLA fiber after solvent removal shuttled 6AN from inaccessible fiber core towards the fiber surface.

Table 1

Summary of 6AN release from electrospun fibers

Time of treatment	Treatment	Total 6AN released (μg)	Percentage of Incorporated 6AN Released (%)	Release Duration (days)
0 days post	Unheated (●)	8.2 ± 4.2	8.1 ± 4.2	9
fabrication	Incubator (▲)	11.8 ± 4.0	11.6 ± 3.9	9
28 days post	Unheated (●)	17.8 ± 4.0	17.6 ± 4.0	32
fabrication	Incubator (▲)	18.1 ± 6.1	17.9 ± 6.0	44