Effect of the Electrospinning Process on Polymer Crystallization Chain Conformation in Nylon-6 and Nylon-12

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ABSTRACT: Electrospinning of biologically significant polymers (natural and synthetic polypeptides) has increased since electrospun membranes were identified as candidates for tissue engineering constructs. These materials have a specific secondary structure, which influences their properties. The effect of electrospinning on the secondary structure of nylon-6 and nylon-12 is examined using Raman spectroscopy in order to identify and quantify any conformational changes that occur due to processing. Nylon-6 and nylon-12 were chosen because they possess a specific chain conformation and have a backbone chemical structure similar to the amino acid sequence in polypeptides. Results indicate that a change in the chain conformation due to electrospinning occurs, implying that a high stress is induced on the electrospinning jet as the fibers are being formed, and this stress alters the chain conformation of the nylon backbone.

Introduction

As technological advances continue in the area of polymeric biomedical devices for tissue engineering applications, the ability to assess the effect of materials processing on the polymer chain conformation is important in determining and understanding materials properties. The chain conformation (secondary structure) of biocompatible, bioinspired, and biodervied polymers, which are generally the materials of choice for the above applications, directly impacts their physical and mechanical properties as well as their biological function. For example, not only is the chemical architecture of collagen (Gly−X−Y repeat) important, but its ability to form a triple helix, which promotes cellular activity, is also critical.[1] This is seen in dragline spider silk as well, where the amino acid sequence allows the chain to assemble into the β-sheet and α-helical/disordered regions providing the hard crystalline and elastic segments, respectively, providing the silk's toughness (strength and elasticity).[2]

Tissue engineering devices are commonly a matrix or scaffold onto which the cells are seeded. These matrices are three-dimensional (3-D) interconnected porous networks with large void volumes and high surface-to-volume ratios that allows for nutrient supply/transport while providing adequate space for cell migration and attachment within the structure. Several techniques have been utilized to construct 3-D interconnected porous matrices, with the most popular techniques being fiber bonding, particulate leaching, and melt molding.[3−5] Recently, electrospinning has been identified as a viable candidate for tissue engineering applications due to the inherent 3-D interconnected porous nature of the membranes produced. In addition, a wide variety of materials that are desirable for biomedical applications can be processed via the electrospinning technique.[6−11] However, the effect of the electrospinning process on chain conformation and crystal structure has yet to be explored.

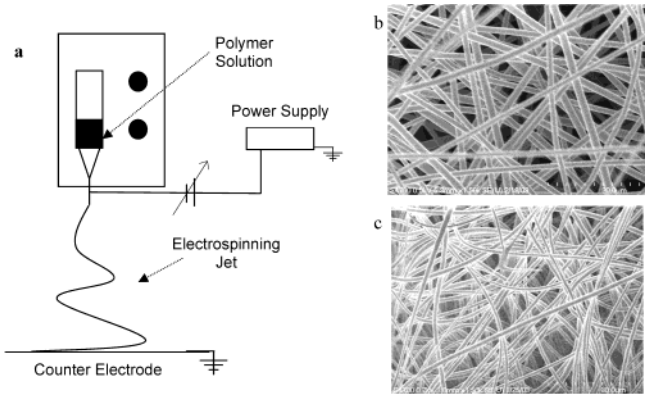

Figure 1. Schematic of electrospinning setup (a) and SEM micrographs on electrospun nylon-6 (b) and nylon-12 (c). The nylon-6 has an average fiber diameter of 1.25 μm, and the nylon-12 has an average fiber diameter of 750 nm. The scale bar on both micrographs is 30 μm.

Electrospinning is a fiber formation technique that creates micro- and nanodiameter fibers from a wide variety of polymer/solvent systems and polymer melts.[12] The solution or melt is placed in a pipet or a syringe (with a needle attached to the end), and a droplet of solution is suspended from the tip of the needle. An electrode from a high-voltage power supply is placed in contact with the solution, and an electric potential is applied. The electric charge overcomes the surface tension of the droplet, and a charged jet is emitted from the droplet. The jet travels a set distance and is collected on a counter electrode in the form of a nonwoven membrane as shown in Figure 1. There are several attractive features of electrospun materials and the electrospinning process; first the fibers produced are generally less than 1 μm in diameter, whereas conventional fiber formation techniques (e.g., melt spinning, solution spinning) produce fibers 10 μm or greater in diameter. Second, only a small amount of polymer, as little as 50 mg, is needed to create fibers, in comparison

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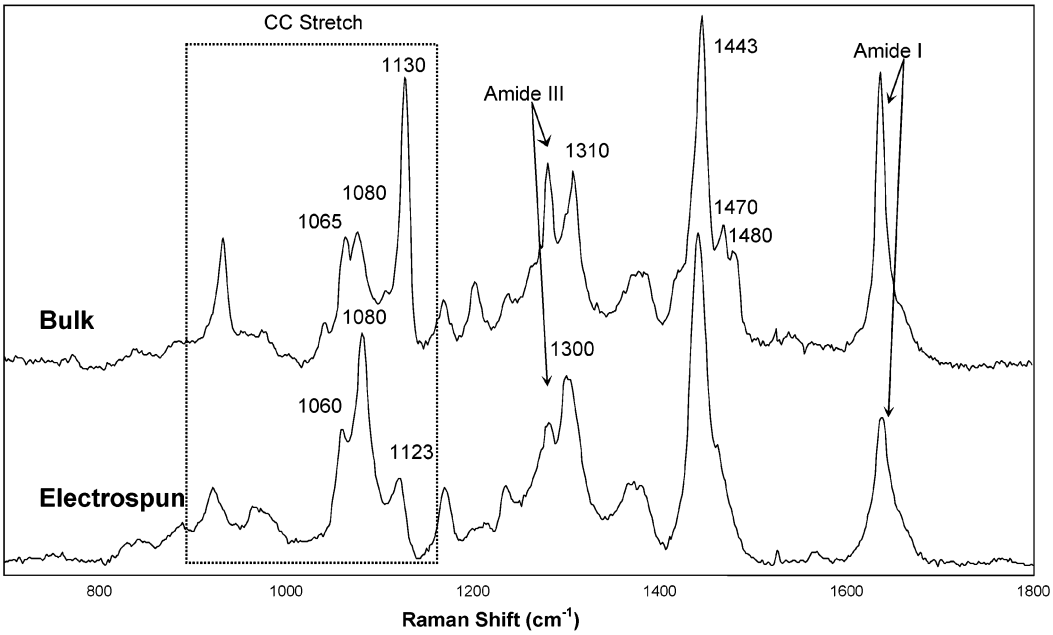

Figure 2. Nylon-6, comparison of Raman spectra of the bulk (top) and the electrospun membrane (bottom). The two spectra are not in agreement, indicating a change in the chain conformation due to electrospinning. The increase of the 1080 cm^{-1} (gauche CC stretch) and the loss of the peaks at 1470 and 1480 cm^{-1} (trans amide) in the spectrum of the electrospun membrane indicates the transition from a trans conformation (extended planar zigzag) of the bulk to a gauche conformation (pleated sheet) in the electrospun membrane.

to conventional methods, which require tens of kilograms.[13]

In this work we report the results of an investigation of the effect of electrospinning on the chain conformation of biomimetic polymers, nylon-6 ($-(CH_2)_5C(=O)N(H)-$) and nylon-12 ($-(CH_2)_{11}C(=O)N(H)-$). The nylons were chosen since they are a well-studied system that offers a simple model for protein-based polymers because their backbone structure is similar to the amino acid sequences found in polypeptides.[14] Raman spectroscopy has been used as a characterization technique to compare spectra between the pre-electrospun and post-electrospun samples to investigate the effect of the process on polymer backbone degradation and changes in chain conformation. Raman was chosen because it is sensitive to secondary structure and it is nondestructive and noninvasive; therefore, the materials can be analyzed as processed with no further sample preparation. In addition, this study extends our efforts to elucidate structure/property/process relationships of the electrospinning process and further the knowledge of microstructure development that occurs during this fiber formation method.

Experimental Section

Materials. The nylon-6 (molecular weight (M_w): 43 300; polydispersity index (PDI): 1.75) and nylon-12 (M_w: 32 000; PDI: 1.52) were both laboratory samples. The molecular weight (MW) and molecular weight distribution of each sample were verified using gel permeation chromatography (GPC). The solvent used was 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma). All sample preparations were carried out under ambient conditions.

Electrospinning Setup. All fibers were electrospun from a 15 wt % concentration (nylon/HFIP) solutions. The polymer solution was placed in a 2 mL syringe with a 17 gauge needle (Hamilton), which was mounted in a syringe pump (Orion SAGE). The syringe pump was used to provide a constant droplet of solution at the tip of the needle. The potential (12 kV) was applied to the needle by a high-voltage power supply (Glassman High Voltage, 0−30 kV). An aluminum (Al) screen, placed 25 cm from the tip of the needle, was grounded and used as the counter electrode. All fiber spinning was done under ambient conditions. A schematic of the electrospinning apparatus and micrographs of the electrospun nylon-6 and nylon-12 are shown in Figure 1.

Raman. A Kaiser HoloPro spectrograph was used to collect the Raman spectra. A polarized fiber-optic probe head (Kaiser Holoprobe) in 180° backscattering geometry was used, with a collection lens (*f*/1.8) working distance of 2.5 in. (6.35 cm). All Raman spectra were parallel-polarized, meaning that the polarization of the incident photons from the laser and the polarization of the scattered photons were parallel. The excitation source was a diode-pump solid-state Nd:YAG laser (Coherent Verdi, 532 nm, 0.5 W) that contains a frequency-doubling crystal to bring the excitation frequency into the visible (532 nm). A liquid nitrogen cooled (−120 °C) CCD detector (Princeton Instruments, EHRB-1024 × 256) was used with the Raman system. To compare the prespun and postspun nylon-6 and nylon-12 spectra, the spectra were normalized by matching intensities of the 1440 cm^{-1} (CH_2 bending) vibration.

FE-SEM. A high-resolution Hitachi S 4700 field emission scanning electron microscope (FE-SEM) was used to record electron micrographs of the electrospun membranes. The imaging conditions were 0.7 kV and 10 μA, and the samples were imaged as spun.

Results and Discussion

Nylon-6. In comparing the Raman spectra of the nylon-6 bulk and the nylon-6 electrospun membrane, there are obvious differences in the CC stretch region (900−1150 cm^{-1}), the CNH bending region (1310−1350, 1440−1490 cm^{-1}), and to a lesser extent in the intensity of the amide I (1638 cm^{-1}) and amide III (1283 cm^{-1}) regions (Figure 2). The amide I (1638 cm^{-1}) mode is primarily attributed to the C═O stretch, while the amide III mode is a combination of the CN stretch and the C═O in-plane bending of the amide group ($-C(=O)-NHC-$). There is an amide II (1550 cm^{-1}) (NH stretching and C═O in-plane bending), but it is very weak in the Raman.[15,16] The CC stretching region is composed of three primary peaks, 1065, 1080, and 1130

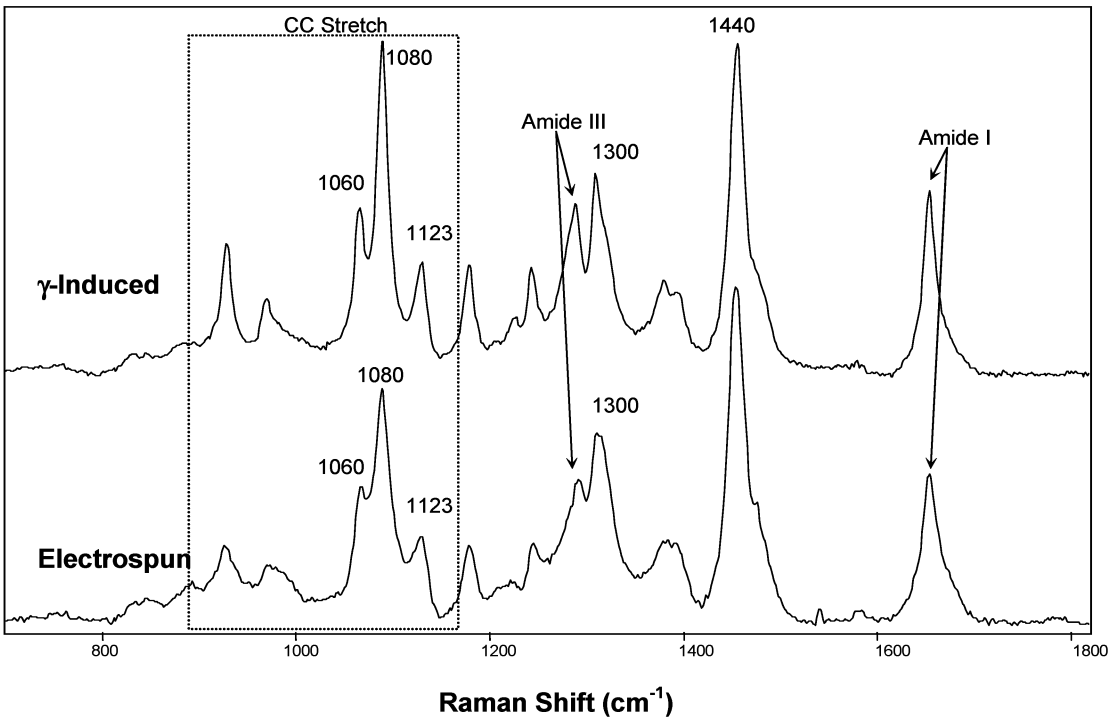

Figure 3. Nylon-6, comparison of Raman spectra of the KI/I_2 γ-induced (top) and the electrospun membrane (bottom). The spectra are in good agreement with only minor intensity variations due to differences in crystallization times, showing that the electrospun membrane is in the γ-form.

cm^{-1}. The 1065 and 1130 cm^{-1} peaks are indicative of an all-trans CC backbone conformation while the 1080 cm^{-1} peak is attributed to the presence of gauche bonds.[14] The CNH bending region of the Raman spectrum is also sensitive to the conformation (planar or nonplanar) of the amide group. Bands observed in the regions of 1310−1350 and 1440−1490 cm^{-1} are indicative of a trans amide group.[17]

To assess the differences described above, the effect of solvent on the nylon-6 bulk was investigated. Nylon-6 bulk/HFIP solution, made at the same concentration as that used for electrospinning, was cast into films and allowed to vapor dry. The spectrum of the cast film and the nylon-6 bulk are identical, showing that the solvent is not the cause for the change between the bulk and the electrospun sample. Next the electrospun membrane was investigated to determine whether the electrospinning process is "denaturing" or causing degradation of the chain architecture. To assess this, the electrospun membrane was dissolved in HFIP and cast into a film and allowed to vapor dry, and the spectrum was then compared to the nylon-6 bulk. These two spectra are also in excellent agreement, showing that the electrospinning process is not destroying the chemical architecture of the nylon-6, but rather causing some alteration in the conformation. GPC was also done to investigate chain degradation as a result of electrospinning. The GPC data show that the M_w was within the accuracy of the technique for the prespun sample as compared to the postspun sample; therefore, electrospinning is not causing chain degradation. In the electrospun sample the intensity of the 1080 cm^{-1} peak has increased and the 1130 cm^{-1} peak, which appeared in the bulk, has decreased in intensity and shifted to a lower wavenumber (1123 cm^{-1}). Also, the nylon-6 bulk has peaks at 1310, 1470, and 1480 cm^{-1}, which are indicative of a trans amide group. These bands are not readily observed in the electrospun sample and, hence, strongly suggest that there is a change in the backbone structure of the nylon-6 as a result of electrospinning.

It is well-known that nylon-6 is a polymorphic material, having more then one energetically favorable crystalline structure (and/or chain conformation).[18,19] The most common structure, which is found in most commercially available nylon-6, is the α-form, where hydrogen bonding occurs between antiparallel chains resulting in a fully extended planar zigzag conformation.[20] Comparison of the Raman spectrum of nylon-6 bulk with that of α-form nylon-6 films found in the literature indicates that the bulk and cast films, in this study, are in the α-form.[14] The other crystalline structure of nylon-6 is the γ-form, which has hydrogen bonding between parallel chains resulting in a mismatch of hydrogen-bonding sites. To compensate for this mismatch, the chain twists at equal angles (~60°) in opposite directions at the $C(=O)-CH_2$ and $NH-CH_2$ bonds to align the hydrogen-bonding sites. This results in a pleated sheet conformation.[21,22] Recently, X-ray studies have shown that electrospinning of nylon-6 results in the γ-form of the polymer.[23,24] The γ-form has also been observed in melt spinning studies of fibers obtained at high take-up speeds.[25]

To follow the rationale that electrospinning will create the γ-form of nylon-6, the spectrum of an electrospun nylon-6 membrane was compared to the spectrum of a γ-form nylon-6 as shown in Figure 3.[19,26] The γ-form was induced in a cast film by treatment in aqueous potassium iodide/iodine (KI/I_2).[27,28] The spectra obtained are in excellent agreement, showing that only minor intensity differences occur. This most likely is a result of differences in crystallization times between the samples. The KI/I_2 sample was crystallized in the γ-form over a period of several days unlike the electrospun sample where the chain conformation and crystal structure are locked in place in a matter of milliseconds. This shows that the electrospinning process is not degrading the

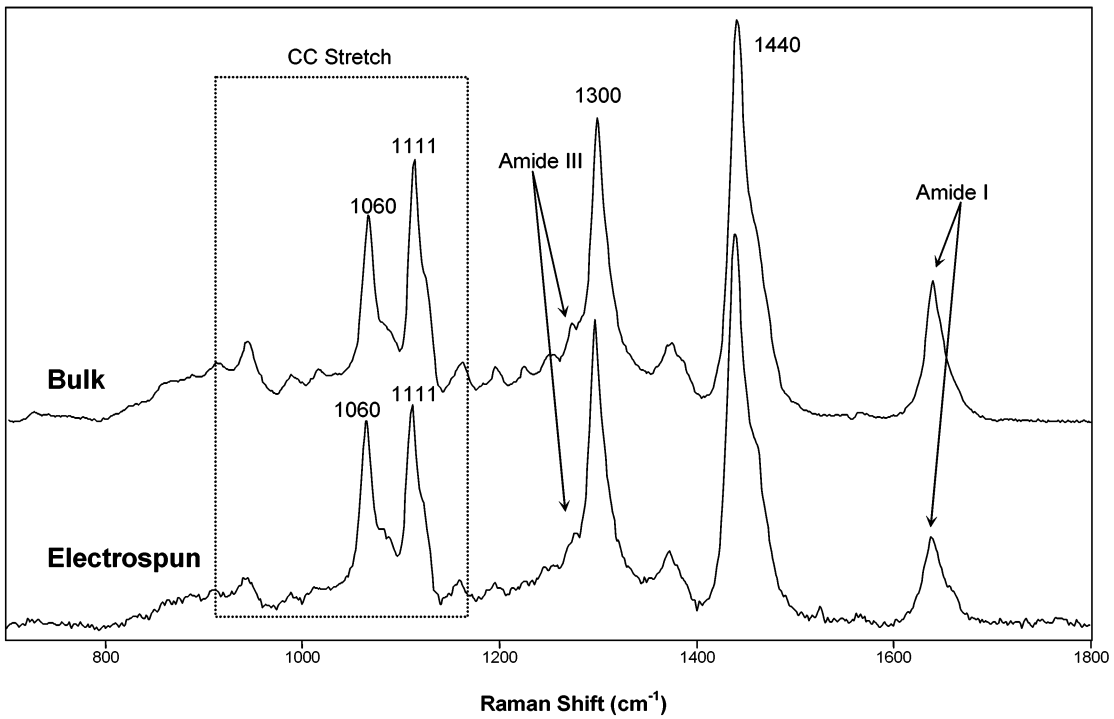

Figure 4. Nylon-12, comparison of the Raman spectra of the bulk (top) and the electrospun membrane (bottom). The spectra are identical, revealing that the electrospinning has not altered the chain conformation.

nylon-6, but rather transforming it from the bulk conformation (α-form) to the γ-form. Our own X-ray diffraction experiments also confirm the α → γ transformation of nylon-6 observed in the samples above.

From the changes in the CNH bend (1310−1350, 1440−1490 cm^{-1}) and the CC stretch (900−1150 cm^{-1}) regions observed in the spectrum of the electrospun nylon-6 membrane as compared to the bulk, it is clear that the conformation of the polymer backbone has been altered (Figure 2). The twist in the γ-form to align the hydrogen-bonding sites accounts for the loss of the peak at 1310, 1470, and 1480 cm^{-1} identifying the transition from a trans amide to a gauche amide. Also, the increase in the 1080 cm^{-1} peak indicates gauche bond formation in the CC region as a result of the twist in the amide region. This gauche band (1080 cm^{-1}) is not readily observed in highly crystalline polyethylene (PE)[29,30] but is seen in short chain hydrocarbons, such as tetradecane ($C_{14}H_{30}$), which is a liquid at room temperature.[31] Interestingly, the 1080 cm^{-1} peak in the short chain hydrocarbon is considerably broader than that seen in the γ-form of nylon-6. Comparing the values at full width at half-maximum (FWHM), the $C_{14}H_{30}$ 1080 cm^{-1} band is >35 cm^{-1} while the γ-form nylon-6 is <20 cm^{-1} in fwhm, indicating that the gauche in the γ-form nylon-6 is not randomly located but is found at a specific location within the chain.

The γ-conformation obtained as a result of electrospinning provides an insight into the stress applied during fiber formation in the electrospinning process. In melt spinning of nylon-6, the γ-form is obtained when high take-up speeds (above 250 m/min (mpm)) are used, resulting in high stress on the fibers. The high stress does not allow for the necessary time for crystallization of the α-form to develop. This indicates that during the electrospinning process polymer chains also experience a high stress during the fiber formation process and kinetically locks in the γ-form.

Now that the chain conformations have been determined for the bulk and electrospun nylon-6 samples, it is assumed that the conformation is being altered for this polymer because it is polymorphic. An important question to ask is, would there be a change in chain conformation for a polymer that only has one energetically preferred conformational structure? To further assess the effect of electrospinning on chain conformation, nylon-12 was investigated because it exhibits only one energetically preferred chain conformation and crystal structure, γ-form.[32−34]

Nylon-12. In comparing the Raman spectra of the nylon-12 bulk and the nylon-12 electrospun membrane, it is observed that there is no change in the spectra, indicating that they have the same chain conformation (Figure 4).[35] This result suggests that the electrospinning process does not alter the chain conformation in a material that only has one preferred crystalline structure. GPC was also preformed on the nylon-12 to verify that electrospinning does not affect the M_w of the polymer. For the nylon-12 a small change in the M_w, slightly larger than the accuracy of GPC, was found.

There are, however, differences in the spectra of the γ-form of nylon-6 and nylon-12. As described above, the γ-form of nylon exhibits hydrogen bonding between parallel chains, resulting in a twist at the C(=O)−CH_2 and NH−CH_2 bonds to allow the hydrogen-bonding sites to align. In nylon-6 this twist not only affects the amide region but also results in a gauche formation in the hydrocarbon segment of the backbone. In the nylon-12, the peaks attributed to a trans amide are not clearly visible in the CNH bending region (1310−1350, 1440−1490 cm^{-1}), indicating that the amide region is in a gauche conformation. The gauche formation in the hydrocarbon region (gauche CC stretch, 1080 cm^{-1}) is not readily apparent in the nylon-12, as it is in the γ-form of nylon-6 spectrum. The longer hydrocarbon region in the nylon-12, as compared to nylon-6, $(CH_2)_{11}$

vs $(CH_2)_5$, respectively, is not perturbed by the twist in the amide segment and therefore does not form a gauche in the $(CH_2)_{11}$ segment. However, there is a spectral perturbation in this region, which is manifested by the movement of the trans CC symmetric stretch from 1130 cm^{-1} in nylon-6 bulk (α-form) to 1123 cm^{-1} in the electrospun nylon-6 (γ-form) to 1111 cm^{-1} in nylon-12 (γ-form). Therefore, the increased length of the hydrocarbon region in the nylon-12 gives rises to the difference seen in the spectra of nylon-6 and nylon-12.[16] Also, the shift in the 1130 cm^{-1} peak to 1123 and 1111 cm^{-1} may be attributed to a new conformation, perhaps a helix, found in the hydrocarbon region of the nylon-12 γ-form, since a slight twist will cause a "shortening" of the chains allowing the hydrogen bonds between parallel chains to form without the introduction of a gauche bond as has been observed in the γ-form of nylon-6. Usually a helix does not require a gauche, therefore ruling out the formation of a helix in the hydrocarbon region of the nylon-6 γ-form.

Conclusions

In this study it has been shown that Raman spectroscopy can be used to investigate the effects of electrospinning on the chain conformation of polymers by comparing the pre-electrospun and post-electrospun fibers. In the case of nylon-6, the polymer crystalline structure was altered from an α-form to a γ-form when electrospun since nylon-6 has more than one energetically stable crystalline structure. This however is not a permanent conformational change and can be converted back to the α-form by solvent casting a film from the electrospun membrane. The ability of the electrospinning process to produce the γ-form implies that the fibers are under high stress when they are being formed. For a polymer that only has one preferred conformation, nylon-12, the chain conformation is conserved after processing. The GPC results support our contention that the electrospinning process does not significantly degrade the MW of polymers. The spectra of the γ-forms of nylon-6 and nylon-12 provide an insight into the conformation of the hydrocarbon region of the backbone as a result of the twist in the amide regions to align the hydrogen-bonding sites. Because of the short hydrocarbon sequence between the amide groups in the nylon-6, a gauche CC bond forms to accommodate the mismatch of hydrogen-bonding sites. The longer the CH_2 sequence in nylon-12, no gauche bond results, and instead a new nonplanar conformation, perhaps a helix, results. The goal of this investigation has been to build a basis to study more complicated conformational structures in bioderived and bioinspired polymers in order to better understand the effect of the electrospinning process on structural conformation and ultimately understand this effect with respect to biological response and polymer properties in tissue engineering devices.

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