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Raman microscopic imaging of electrospun fibers made from a polycaprolactone and polyethylene oxide blend

Geoffrey P.S. Smith[a], Andrew W. McLaughlin[b], Andrew N. Clarkson[c], Keith C. Gordon[a,*], Greg F. Walker[b]

[a] Dodd-Walls Centre for Photonic and Quantum Technologies, Department of Chemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[b] School of Pharmacy, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[c] Department of Anatomy, University of Otago, PO Box 56, Dunedin 9054, New Zealand

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ABSTRACT

Raman spectroscopy is a useful technique for providing compositional information about samples. By combining this technique with microscopy, Raman data can be collected from domain sizes smaller than one micron in diameter. This research aims to utilize this technique to examine the submicron component distribution in electrospun nanofibers. Nanofibers containing a 50:50 blend of polyethylene oxide (PEO) and polycaprolactone (PCL) were analyzed using Raman microscopy and scanning electron microscopy (SEM). This was performed before and after the samples were incubated in Milli-Q water to observe the effects of PEO dissolution on the nanofiber structure. Raman results indicate that both polymers are distributed evenly throughout individual nanofibers, but that some nanofibers may contain more of one component than the other, and the technique provided direct evidence for PEO dissolution after submersion in water. The dissolution of PEO observed by Raman microscopy correlated with results of mass loss analysis. SEM results also provided evidence for component dissolution and suggest that electrospinning may result in the formation nanofibers made up of PEO and PCL nanofibrils.

1. Introduction

Nanofibers have several characteristics which make them useful in a variety of applications. These characteristics include a large surface area to volume ratio, they can be used as porous materials with variable pore shapes and sizes, and they can be used to create strong, rigid structures [1]. Electrospinning is a technique whereby nanofiber mats can be made relatively simply by passing a polymer solution through a thin capillary tube, applying an electric field, and collecting the resulting nanofibers on a charged collection plate [2]. In order to make the technique more useful on an industrial scale, this technique has been modified in various ways in the literature [3]. This has been accomplished through use of a larger number of capillary tubes [4], using electrostatic and rotational forces to generate nanofibers [5,6], or other methods which forgo the use of a capillary tube for generating the electrostatically charged jets of polymer solution [7–9].

Nanofibers are now being investigated for their uses in the pharmaceutical industry as it is possible to produce drug/polymer and polymer/polymer nanofiber composites through the process of electrospinning [5,10–17]. There are many potential benefits to using such composites for medical treatments, especially for use in drug delivery [13]. These benefits include: lack of potentially harmful solvent; improved dissolution rate due to increased surface area; improved dissolution rate due to drug amorphization; improved drug solubility due to reduced particle size; improved stability of amorphous drug; and due to the improved stability of a supersaturated solution [13]. Many of these benefits are also useful when constructing nanofiber mats used for wound dressings or tissue scaffolds [18–20]. In such systems, the choice of which polymer or polymer blend can be very important.

Polycaprolactone (PCL) is a synthetic polyester which has been found to have several medical applications due to the low cost of the material, its biocompatibility, its strength and due to its low degradation rate in the body [21–26]. However, this material has two disadvantages with respect to being used as a scaffold for guiding tissue regeneration. Firstly, the hydrophobicity of PCL hinders cell adhesion and proliferation; and secondly, PCL membranes take too long to be resorbed *in vivo* [21]. Polymer blends are created in order to mitigate these disadvantages, and create a material with moderated properties. In general, naturally

* Corresponding author.
E-mail address: keith.gordon@otago.ac.nz (K.C. Gordon).

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occurring polymers tend to be better suited to incorporation into biological systems but lack mechanical strength and often degrade too quickly, whereas synthetic polymers often have the strength necessary, but degrade too slowly and are less compatible with biological systems [21,23]. In the literature this has been achieved using blends of PCL with gelatin [18], or chitosan [20,27]. Despite being a synthetic polymer, polyethylene oxides (PEOs) have also been blended with PCL as it has some properties which are similar to natural polymers, namely biocompatibility, hydrophilicity, and fast degradation in the body [28–32]. These properties have allowed these polymers to be used in relatively short-term drug delivery systems such as nanoparticles, nanofibers, hydrogels, and liposomes [29,30,33].

Raman spectroscopy has been extensively used in the analysis of electrospun fibers. Indeed Stephens et al. [34] showed that it is possible to characterize spun fibers virtually in real time as a way of providing the bulk (not single fibers) properties. Since then Raman spectroscopy studies of electrospun fibers may be categorized as follows:

(i) Studies which examine bulk molecular properties of fibers, such as polymer conformation. In a study on electrospun silk nanofibers it has been shown that the index of crystallinity for the fibers may be determined using the band intensity ratio amide I/amide III [35]. It may also be used to indicate the presence of two materials in a fiber mix and the removal of one of these via processes such as heating. Wang et al. [36] have shown that graphene oxide/polyvinyl alcohol (PVA) nanofibers show Raman signatures from each component with the PVA signal disappearing when the material is heated above 150 °C. In combination with SEM and other results they conclude that the graphene oxide is a nanofiller within the PVA matrix;

(ii) Studies in which time dependent changes of the bulk molecular properties are observed. In a study of silk fibroins (some with acrylate grating) and their interaction with aqueous methanol, Raman spectroscopy showed that the β-sheet conformation of the silk was altered by grafting of the 2-hydroxyethyl methacrylate (HEMA) and 4-hydroxybutyl acrylate (HBA) [37]. Treatment with methanolic solution did increase crystallization to the β-sheet conformation as shown by the changes in the bandwidth of the amide I and band ratio changes – this recrystallization was inhibited by grafting. The dynamic behavior of isotactic poly(1-butene) electrospun membranes has been studied in which Raman spectroscopy was used to show that initially spun fibers were form I and II polymorphs but with aging a conversion to the thermodynamically stable form I occurred [38];

(iii) Studies in which mapping is used to determine compositional makeup. Nanofibers made from hyaluronic acid (HA) and either PCL or PEO have been investigated using Raman spectroscopic mapping [39]. Single value decomposition (SVD) was used to give an image of composition. By calculating the SVD coefficient for each spectrum and plotting it against the "spectrum number", the relative concentration of drug was able to be compared for each mapped point. This analytical method showed that the nanofibers made from PEO and hyaluronic acid were homogeneous with regards to their component distribution, but nanofibers made from PCL and hyaluronic acid were not. This was attributed to PEO and hyaluronic acid having high water solubilities, but PCL having relatively low water solubility [39]. Therefore it is evident that a homogeneous distribution of components within electrospun nanofibers cannot be assumed.

Nagy et al. [13] used Raman microscopy to image a nanofiber matrix of spironolactone and Soluplus® in a 1200 × 1200 μm area using a 10 × objective [13]. Results showed that spironolactone distribution could be imaged effectively and that electrospinning appears to be the more effective technique for attaining homogeneous drug/polymer mixtures than extrusion or physical mixing processes. Sóti et al. [11] performed similar comparative experiments using Raman microscopy to image caffeine distribution on spray dried and electrospun nanofiber drug delivery systems. The domains imaged in these experiments were approximately 50 × 50 μm and contained 41 × 41 spectra, resulting in a step size of approximately 1 μm. The results of this study indicated that electrospinning provided better drug homogeneity than spray drying. Raman imaging may also be used to visualize the efficiacy of interaction between a polymer (polycaprolactone) and molecule (cyclodextrin or amine functionalized cyclodextrin). The analysis of the Raman maps with hierarchical cluster analysis suggests that amine functionalized cyclodextrin coats the fiber more effectively than the non-functionalised;

One study has combined mapping with a chemical change [40]. In a study of molecularly imprinted polymer microspheres within a polymer membrane, Raman microscopy (with lateral resolution of 270 nm) was used to distinguish between the microspheres (made up of methacrylic acid and divinylbenzene) within a polyacrylonitrile nanofiber matrix and the same sample loaded with a target drug (–)-cinchonidine [40].

Nanofibers may be characterized using scanning electron microscopy (SEM) [14–16,27,41–44]. SEM provides excellent spatial resolution and is therefore informative with respect to the fiber structure and morphology. High spatial resolution Raman microscopy could potentially be an effective complementary technique for imaging the distribution of components within a nanofiber as the technique is non-destructive and has the ability to provide detailed structural and compositional information. Because Raman spectroscopy is an optical technique, a spatial resolution of 300 nm may be achieved but higher spatial resolutions would require the use of other spectroscopic techniques such as tip enhanced Raman spectroscopy (TERS) [45].

This research aimed to determine whether Raman microscopy could provide structural and compositional information about electrospun nanofibers on smaller length scales between 300 and 400 nm and the changes in these fibres with process effects, such as dissolution. While it may not be possible to resolve exact component distributions in smaller nanofibers, the signals from smaller nanofibers will still be detectable, and will therefore still be useful for providing information on the general fiber contents. Fibers in this investigation were made from synthetic polymers, PCL and PEO, and were analyzed before and after dissolution in water. This system was studied because the dissolution properties of PEO are well known for the nanofibers [46–49]. Following this analysis, scanning electron microscopy was performed on the same samples to provide the higher resolution morphological information. A further goal of this study was to determine whether any structural information can be gathered about individual nanofibers. Ultimately, this high resolution data may prove useful for understanding the mechanisms through which electrospun nanofibers made from polymer blends function in drug delivery and tissue scaffolds.

2. Experimental

2.1. Preparation of electrospinning solutions

PCL (MW, 70–90 kDa), PEO (200 kDa) and formic acid were obtained from Sigma Aldrich Co. (St. Louis, Mo, USA). Glacial acetic acid was purchased from Merck (Darmstadt, Germany). Acetic acid and formic acid (AA/FA) was the solvent system used for all electrospun solutions at a ratio of 90:10 v/v. PCL was prepared by dissolving 0.2 g in 2 mL of AA/FA to give a 10% (w/v) solution. PEO was prepared by dissolving 0.2 g in 1.33 mL of milli-Q water to give a 15% (w/v) solution. PCL/PEO solutions were prepared by dissolving 0.1 g of PCL and 0.1 g PEO in 1 mL of AA/FA to give a