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Nanometer depth resolution in 3D topographic analysis of drug-loaded nanofibrous mats without sample preparation

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ABSTRACT

We showed that scanning white light interferometry (SWLI) can provide nanometer depth resolution in 3D topographic analysis of electrospun drug-loaded nanofibrous mats without sample preparation. The method permits rapidly investigating geometric properties (e.g. fiber diameter, orientation and morphology) and surface topography of drug-loaded nanofibers and nanomats. Electrospun nanofibers of a model drug, piroxicam (PRX), and hydroxypropyl methylcellulose (HPMC) were imaged. Scanning electron microscopy (SEM) served as a reference method. SWLI 3D images featuring 29 nm by 29 nm active pixel size were obtained of a 55 μm × 40 μm area. The thickness of the drug-loaded non-woven nanomats was uniform, ranging from 2.0 μm to 3.0 μm (SWLI), and independent of the ratio between HPMC and PRX. The average diameters ($n = 100$, SEM) for drug-loaded nanofibers were 387 ± 125 nm (HPMC and PRX 1:1), 407 ± 144 nm (HPMC and PRX 1:2), and 290 ± 100 nm (HPMC and PRX 1:4). We found advantages and limitations in both techniques. SWLI permits rapid non-contacting and non-destructive characterization of layer orientation, layer thickness, porosity, and surface morphology of electrospun drug-loaded nanofibers and nanomats. Such analysis is important because the surface topography affects the performance of nanomats in pharmaceutical and biomedical applications.

1. Introduction

Polymeric nanofibrous mats charged with drug substance(s) have found use in many pharmaceutical and biomedical applications such as topical/transdermal drug delivery systems, wound dressings, tissue engineering scaffolding, tissue templates and medical protheses (Agarwal et al., 2008; Huang et al., 2003; Quaglia, 2008; Zhang et al., 2012). Electrospinning is a simple and versatile technique to fabricate polymeric nanofibers with a diameter ranging from a few nanometers to several micrometers, with a tunable porosity, and with a large surface area to volume ratio (Agarwal et al., 2008; Brewster et al., 2004; Li and Xia, 2004; Lu et al., 2009; Teo and Ramakrishna, 2006). In electrospinning, a polymer solution is generated from a capillary toward a grounded metal collector plate by applying high voltage between the capillary and the plate (Naraghi and Chasiotis, 2007). Several material and process parameters influence the transformation of polymer solutions into nanofibers. The morphology and diameter of electrospun nanofibers depend on the intrinsic properties of the solution, type of polymer, conformation of polymer chain, viscosity, elasticity, electric conductivity, as well as on the polarity and surface tension of the solvent (Agarwal et al., 2008; Chronakis, 2005; Haghi and Akbari, 2007; Huang et al., 2003; Park et al., 2008; Teo and Ramakrishna, 2006; Zong et al., 2002). Among current fabrication methods, electrospinning is considered the only process with potential for mass production (Jayaraman et al., 2004).

To identify use and therapeutic efficiency of pharmaceutical and biomedical nanofibers, their geometric properties (diameter, diameter distribution, fiber orientation and morphology) and porosity (of both fibers and mats) need to be known. For example, fiber diameter and corresponding specific surface area have been shown

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to determine cellular attachment and proliferation in electrospun tissue engineering scaffolds (Chen et al., 2007) and to affect drug release and sustained release duration in multilayered nanofiber meshes (Chen et al., 2012; Huang et al., 2012). In addition, the surface porosity of the fibers affects the way nanoparticles, drugs, or enzymes attached to their surface or are incorporated into their inner volume (Casper et al., 2004; Ziabari et al., 2008).

It is difficult to determine the geometric properties of single nanofibers and the porosity of nanofibrous mats because of the small size and random orientation of the individual fibers. Especially, polymer nanofibers are challenging to characterize with respect to their mechanical and geometric properties since mechanical contact or thermal mismatch may result in permanent deformation of the fibers (Naraghi and Chasiotis, 2007; Jayaraman et al., 2004; Tan and Lim, 2006). To date, most measurements of the geometric properties of nanofibers have been performed with different microscopy techniques and instruments such as scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Chung et al., 2009; Ding et al., 2002; Huang et al., 2003; Li et al., 2002; Megelski et al., 2002; Pelipenko et al., 2013; Taepaiboon et al., 2006; Tan and Lim, 2005). Ziabari et al. (2007, 2008) introduced a novel image analysis method for measuring the diameter of nanofibers and the pore characteristics of nanofibrous mats. The core idea of this method is to use a distance transform algorithm and image analysis. Its main strength is fast and accurate measurement whereas its main weakness is sensitivity to noise. Recently, Janković et al. (2013) evaluated successfully the morphology and mechanical properties of single electrospun nanofibers by means of AFM for the specific tissue scaffold applications. New techniques are still needed to characterize individual fibers and nanofibrous mats intended for pharmaceutical and biomedical applications. For instance to develop fabrication methods one needs a technique that rapidly provides high depth resolution and that does not require sample preparation or modification.

Scanning white light interferometry (SWLI) is a non-destructive, non-contacting, method employed, e.g. in microelectronics and optics to examine miniature elements, micro-fluidic devices, and micro-electro mechanical systems (Coupland and Lobera, 2010; Kassamakov et al., 2007, 2009; Madani-Grasset et al., 2008; O'Mahony et al., 2003). Few studies report on applying SWLI and white light interferometry to characterize pharmaceutical and biomedical systems. Holme et al. (2005) measured the etching of dental enamel, and Shekhawatyz et al. (2009) measured the surface topography of viable articular cartilage. Hanhijärvi et al. (2010) and more recently Genina et al. (2012) measured surface roughness (topography) of pharmaceutical thin films and flexographic coated controlled-release systems, respectively. To our knowledge, SWLI has not been applied to determine geometric properties and surface topography of polymeric nanofibers and nanofibrous mats intended for pharmaceutical or biomedical applications.

We therefore investigated SWLI as a technique for determining geometry (i.e. fiber diameter, diameter distribution, alignment, and morphology) and 3D surface topography of pharmaceutical polymer nanofibers and non-woven nanofibrous mats. The nanofibers of a poorly water-soluble piroxicam (PRX) and hydroxypropyl methylcellulose (HPMC) were fabricated by electrospinning – a simple and continuous method to prepare nanofibrous mats. To show the robustness of the SWLI method, two additional electrospun nanofibrous mats loaded with another active substance, β-sitosterol, and with two different carrier polymers were prepared and analyzed. A high-resolution SEM equipped with a customized measurement program served as a reference method.
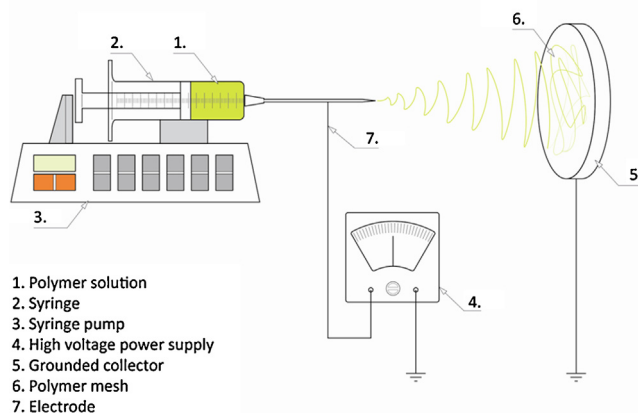

1. Polymer solution
2. Syringe
3. Syringe pump
4. High voltage power supply
5. Grounded collector
6. Polymer mesh
7. Electrode

Fig. 1. Laboratory-scale electrospinning equipment employed to fabricate nanofibers and nanomats.

2. Materials and methods

2.1. Materials

Piroxicam (anhydrous PRX pure form I, PRXAH I, Letco Medical, Inc., USA) and β-sitosterol (Calbiochem, France) were used as poorly water-soluble model drugs when fabricating the electrospun nanofibers and non-woven nanomats. Hydroxypropyl methylcellulose, HPMC (Methocel K100M premium CR, Colorcon Ltd., U.K.), Soluplus® (polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer, BASF SE Pharma Ingredients & Services, Ludwigshafen, Germany) and chitosan (Primex Ingredients ASA, Norway) were used as carrier polymers in the nanofibers. The solvents applied in the electrospinning studies were 1,1,1,3,3,3-hexa-fluoro-2-propanol (HFIP) (≥99.0%) and acetone (Sigma–Aldrich Co., Belgium).

2.2. Preparation of nanofibers and nanofibrous mats

The electrospinning set-up used to prepare the nanofibrous systems is shown in Fig. 1. The automatic syringe pump was a KdScientific, Model No: KDS-250-CE (Geneq Inc., USA) whereas the high-voltage power supply was a Gamma High Voltage Research, Model No. ES3OP-10W (DAM, USA). The electrospinning rate of the solutions was 0.5 ml/h (chitosan-β-sitosterol), 1 ml/h (HPMC-PRX), 2.0 ml/h (Soluplus®-PRX). The voltage applied in the experiments ranged from 7 kV (HPMC-PRX) to 12 kV (chitosan-β-sitosterol). The distance between the spinneret and the fiber collector was 8–10 cm. The ratio of Soluplus® to PRX used to produce the nanofibers was 13:1 (w/w) thus the total polymer concentration in the acetone was 33% (w/v). All experiments were carried out at room temperature (21 ± 2 °C).

2.3. Scanning white light interferometry

The geometric properties and surface topography of the fibers and mats were characterized by SWLI. In SWLI, a light beam passes through an interferometric objective (Nikon, Michelson type, magnification 120×) containing a beam splitter that reflects half the incident beam onto a reference surface and that passes the other half onto the test surface (Fig. 2). Light reflected from the test and reference surfaces recombines and interferes, forming an interferogram. The objective is translated horizontally (scanning) and several interferograms are sequentially imaged. For each camera pixel, the modulation signal is extracted from the intensity