Polymer 54 (2013) 6702–6708

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Three-dimensional coating of nanofibers on surfaces of poorly conductive objects

Phillip McClellan, William J. Landis*

Department of Polymer Science, The University of Akron, Akron, OH 44325-3909, USA

A R T I C L E I N F O

Article history:
Received 3 July 2013
Received in revised form
25 September 2013
Accepted 6 October 2013
Available online 11 October 2013

Keywords:
Electrospinning
Three-dimensional coating
Polycaprolactone

A B S T R A C T

Rectangular-shaped, poorly conductive synthetic polymer scaffolds composed of a mixture of polycaprolactone and poly-L-lactic acid (PCL/PLLA, 75:25) were coated directly with nanofibers composed of PLLA using an electrospinning technique having a modified design for the electrically grounded collector. The design modification consisted of mounting each scaffold onto a fine-point needle which was attached directly to the ground electrode of the electrospinning unit. Nanofibers were collected on all six surfaces of each scaffold. The coated scaffolds were then dried at ambient temperature overnight before sterilization by immersion in 100% ethanol to assess and ensure adherence between the scaffold and nanofibers. Photomicrographs from scanning electron microscopy illustrate nanofiber coverage over all six surfaces of the polymer scaffold. The design in this manner for three-dimensional coating of poorly conductive objects advances electrospinning capability for numerous new applications.

1. Introduction

Electrospun nanofibers have been utilized in the field of tissue engineering for over a decade and the technology of electrospinning and the nanofibers themselves are advantageous for several reasons: Nanofiber production through electrospinning is relatively simple [1,2], the mats generated by the nanofibers can closely resemble the three-dimensional structure of the extracellular matrix of certain tissues [3–5], and parameters of the electrospinning technique can be altered to yield a final product of specific structure and function [4,5]. The latter is a unique quality of electrospinning and a principal factor in utilizing the method for tissue engineering and many other applications.

Typically, a synthetic, biodegradable polymer, such as poly-L-lactic acid (PLLA) [6], polyglycolic acid (PGA) [7], or polycaprolactone (PCL) [6–8], is dissolved in an appropriate solvent to generate a moderately viscous solution which is suitable for electrospinning. The design of the collector for the electrospinning device can have a marked effect on the morphological characteristics of the nanofibrous mat produced. For instance, rotating collectors may be employed to obtain aligned nanofibers suitable for engineering nerve tissue [9]. Magnetic fields have been incorporated into other designs in order to orient the nanofibers in uniaxial arrangements as well [10]. In most instances, however, the nanofibrous mat is collected onto a smooth surface, resulting in a thin, two-dimensional mat or "sheet" of nanofibers.

Two-dimensional mats of nanofibers have demonstrated notable benefits in repairing damage to tissues such as skin [11], which lack the need for a more complex, macroscopic three-dimensional structure. In fact, much of the current research in tissue engineering with nanofibers is focused on modification and manipulation of the fibers at the microscopic level. A number of studies have addressed the generation of nanofibers containing functional groups which facilitate cell attachment [12–14]. Others are concentrating on development of sophisticated techniques to produce multi-layered fibers that differ in composition between the inside and outside of an individual fiber [6–9,15]. Most of the approaches mentioned, then, identify various areas for potential improvement in tissue engineering, but they remain limited in specific applications where macroscopic three-dimensional structures are required to regenerate large tissues and organs.

While numerous investigations remain concerned with alterations to nanofiber structure and composition at the microscopic level, a few have attempted to fabricate larger, three-dimensional structures with varying degrees of success. For example, Chang et al. have developed methods of producing three-dimensional tubes of nanofibers which could be appropriate for tissue engineering of blood vessels, although this method introduces a third

---

* Corresponding author. Department of Polymer Science, Goodyear Polymer Center, Room 1201C, The University of Akron, 171 University Avenue, Akron, OH 44325-3909, USA. Tel.: +1 330 972 8483.

E-mail address: wlandis@uakron.edu (W.J. Landis).

0032-3861/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2013.10.014

dimension to the fibrous mat only by rolling two-dimensional nanofiber sheets into simple, hollow tubes [16]. Such an approach is not an effective method for producing tissue scaffolds for entire organs in which greater three-dimensional complexity is a necessity. In separate studies, Grigoni et al. have fabricated a heart valve prosthesis from PCL using electrospinning [17]. The trileaflet shape of the valve demonstrated a significant increase in overall three-dimensional complexity, but the prosthesis remained relatively thin and unsuited for applications requiring increased thickness and strength of the scaffold.

Blakeney et al. have developed and patented a method to produce nanofiber structures having overall three-dimensional structure resembling a cotton ball [18]. They refer to their material as Focused, Low density, and Uncompressed nanoFibrous (FLUF) mesh. The FLUF meshes produced have a lower density of fibers when compared to flat nanofiber mats and provide a suitable three-dimensional environment for the infiltration and growth of INS-1 cells [18]. In this regard, experimental data illustrated that lower density and greater spacing between individual fibers contributed to more rapid infiltration and proliferation of cells [18]. However, the “cotton ball” scaffolds do not exhibit the necessary strength or structure for the engineering of other cells and tissues such as those from bone and other mineralized connective tissues. In addition, there are reports of electrospun scaffolds fabricated using solutions of alginate and polyethylene oxide (PEO) which produce three-dimensional structures because of charge repulsion between individual fibers as a result of the negatively charged alginate [19]. As with FLUF scaffolds, the alginate-PEO scaffolds lack sufficient mechanical strength to be effective for engineering of bone tissue.

With regard to tissue engineering of bone, independent studies have reported the design and development of tissue-engineered constructs in the shape of human digits (phalanges) [20,21]. These engineered models consisted in part of the thin tissue (periosteum) covering the long bones obtained from young calves. Periosteum was dissected and then wrapped and sutured about biodegradable, solvent-cast polymer scaffolds composed of PCL/PLLA (75:25) and resembling the size and form of human distal middle phalanges. The periosteum/scaffold constructs were then implanted in athymic, immunodeficient mice (lacking the means for rejecting foreign tissue such as that from calves and other species) for 20 and 40 weeks. Constructs retrieved from the mice at various time intervals of implantation and development demonstrated that bone tissue could be reproducibly regenerated in three dimensions by utilizing the periosteum as a viable source of bone progenitor cells [20,21]. Further, the addition to these constructs of certain growth factors, such as osteogenic protein-1 (OP-1) and basic fibroblast growth factor (bFGF), expedited cell proliferation and differentiation. These molecules, applied directly to cells or provided to them through release and delivery vesicles or other means, have been shown to lead to more rapid formation of bone and other tissues [22–24].

Growth factor addition is not the only method to promote bone formation for tissue engineering. Indeed, electrospun nanofibers have resulted in increased cell attachment and proliferation when compared to cells cultured in a monolayer environment [25]. In this context, the authors have attempted previously to incorporate nanofibers into experimental digit designs by suturing thin, pre-formed sheets of PGA nanofibers around the PCL/PLLA scaffolds prior to application of periosteum (unpublished results). These experiments, however, were unsuccessful largely because of the difficulty in maintaining direct contact at the interface between the nanofibers and the underlying PCL/PLLA scaffold. In the absence of direct contact between the tissue scaffold and periosteum, osteoprogenitor cells were unable to infiltrate the scaffold and grow.

Suturing mats of nanofibers to, rather than wrapping them around, the scaffolds is an alternate approach to direct contact. The application, however, is time-consuming and requires expertise to produce a suitable nanofiber-covered scaffold and subsequent construct. Additionally, as the complexity of the underlying construct increases, so does the number of sutures needed to ensure the nanofiber sheet remains in close contact with it.

A simple and novel method to circumvent the difficulties in designing an intimate contact between a nanofiber mat and pre-existing polymer scaffold is to apply nanofibers directly to the surface of the scaffold utilizing the electrospinning process. The surface to be coated must be made electrically conductive, and thereby maintained at an electrical potential that attracts the charged nanofibers, and placed in the path of the electrospun nanofibers as they are produced. Naturally conductive materials are easily coated, but poorly conductive materials, such as PCL/PLLA or most other polymers, are more difficult to coat. In initial studies by this laboratory, solvent-cast PCL/PLLA (75:25) scaffolds were placed onto a flat, grounded electrical collector, directly in the path of the nanofiber jet, but the design resulted in only a few nanofibers being deposited onto the surface of the scaffolds.

In order to facilitate and possibly improve efficient collection of nanofibers on the surface of other pre-formed PCL/PLLA polymer scaffolds, a very fine stainless steel needle was inserted through the scaffold. The needle presence resulted in a much more effective electrical connection between the scaffold and the flat plate collector. The needle provides an attractive electrical potential near the surface of the scaffold and also can provide an electrical connection to ions migrating on the surface of the polymer scaffold and collected nanofibers, thereby maintaining a potential that collects more fibers. Further, the addition of the needle allowed the scaffold to be supported above the collector so as to promote nanofiber deposition over the top, sides, and bottom of the PCL/PLLA scaffold. The design offers a new means of producing a three-dimensional nanofiber closely covering the scaffold surfaces. The deposition of electrospun nanofibers in this manner expands electrospinning technology to greater numbers of applications in which three-dimensional coatings of a wide nature are advantageous.

2. Methods and materials

2.1. Preparation of nanofiber-coated scaffolds

The basic electrospinning apparatus developed and utilized by Reneker and Yarin [2] was experimentally adapted with the addition of a single, fine-point needle to the flat plate collector. A 1.0% (w/v) solution of PLLA (700 kDa, Polysciences, Warrington, PA) in chloroform (ACS reagent grade $\geq$99.8%, Sigma–Aldrich, St. Louis, MO) was prepared and stirred continuously over a 12 h period. The solution was then loaded into a 5 mL syringe (Luer-Lok tip, BD, Franklin Lakes, NJ) having a blunt-tip needle (25 gauge, ½ inch in length, BD) attached to it. The syringe was placed into the syringe pump (NE-300, New Era Pump Systems, Farmingdale, NY), and the positive electrode from a high-voltage power supply (ES60-10W, Gamma High Voltage Research, Ormond Beach, FL) was connected. A fine (0.5 mm diameter, 35 mm length) stainless steel needle was attached to a flat, grounded collector, and the end of the needle was inserted by hand through each of a number of poorly conductive, pre-formed rectangular-shaped tissue scaffolds (0.6 cm × 0.5 cm × 0.5 cm in dimensions) composed of PCL/PLLA (75:25 ratio of PCL:PLLA; Gunze Co., Japan). Nanofibers of PLLA were generated and deposited onto the surface of the scaffolds using an applied voltage of 13.1 kV and working distance of 6 cm between the electrospinning tip and the tip of the grounded needle.