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Magnesium oxide-poly(ε-caprolactone)-chitosan-based composite nanofiber for tissue engineering applications

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ABSTRACT

The ability to produce composite nanofibers of inorganic particles and synthetic polymers represents a significant advancement in the development of composite materials for potential biomedical applications. In this study, composite nanofibers of magnesium oxide (MgO), poly(ε-caprolactone) (PCL) and chitosan (CS) with diameters in the range of 0.7–1.3 μm were fabricated by electrospinning their blend solutions in trifluroethanol and water. To support the potential use of these nanofibrous membranes for biomedical applications their physicochemical properties such as morphology, mechanical strength, and integrity in aqueous medium, were studied. Cellular compatibility was determined using cell viability assays and microscopy imaging, with the results showing that the nanofibrous membranes support 3T3 cell viability and attachments. The new composite nanofibrous membranes developed in this study have the ability to mimic the physical structure and function of tissue extracellular matrix (ECM) and thus have potential for many tissue engineering applications.

1. Introduction

Engineered composite nanofibers have shown great potential in different biomedical applications including but not limited to drug delivery, wound healing, tissue engineering, implant coatings etc. [1–4]. Nanofibers having high surface-area-to-volume ratio produce scaffolds with unique physicochemical properties that can closely resemble the properties of extracellular matrix (ECM) components found in natural tissues [5]. For the last few years, electrospinning has been recognized as an efficient and reliable technique to create nanofibers [6–9]. Electrospinning is a simple and versatile technique that allows the engineering of scaffolds with micro-to-nanoscale topography and with porosity that can be tuned to match the tissue ECM [10].

Composite nanofibers derived from natural and/or synthetic biopolymers and ceramic particulates are gaining popularity in biomedical applications because they capitalize on the favorable biological properties of the natural polymer and the ceramic, and superior mechanical properties of the synthetic polymer [11]. However, effective synthesis of well-blended composite fibers remains a great challenge due to the poor miscibility between polymers and ceramic particles at the molecular level. As a result of phase separation, poorly blended composite nanofibers exhibit weak mechanical strength and uncontrollable material properties [11,12].

A large body of published work in the area of chitosan (CS) and polycaprolactone (PCL) blends demonstrates the growing interest in CS-PCL composite fibers for biomedical applications where mechanical strength, biocompatibility and stability of nanofibers *in vitro* and *in vivo* are important [13,14]. CS, a polysaccharide derived from the exoskeletons of crustaceans shares structural similarity with glycoasamine glycan (GAG), a major component of tissue ECM. Therefore, the nanostructured morphology of the CS-PCL composite fibers better represents the ECM of tissues and serve as an excellent framework for cell adhesion, proliferation, and differentiation. Due to chitosan's versatile characteristics, it makes an excellent choice for controlled release formulations, including non-viral vectors for DNA-gene and drug delivery, imaging, wound healing, and smart implant coating applications [15–17]. The complementary polymer, PCL, an aliphatic synthetic polymer, is a widely-used material in tissue engineering. PCL is biocompatible and has mechanical properties superior to natural polymers [18]. On the other hand, PCL lacks desirable cell affinity, primarily due

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to its hydrophobicity and lack of cell recognition sites. This results in decreased cell proliferation and differentiation [18]. Most natural and synthetic polyblends such as gelatin/PCL and collagen/PCL require chemical crosslinking agents to retain their structure and maintain mechanical properties. Unfortunately, the crosslinking agents simultaneously yield toxic residues [18]. However, appropriately constructed PCL-CS/MgO composites offer the benefit of integrating the favorable biological properties of CS with the favorable mechanical properties of PCL without requiring chemical crosslinking to retain their structure and desirable mechanical properties. MgO, a ceramic, is an inorganic salt of magnesium that releases Mg^{++} ions. Mg^{++} is the second most abundant intracellular cation, and is important to human metabolism. Recent studies have shown that the divalent cations such as Mg^{++} play a crucial role in providing effective function of nerve tissue, and in repairing nerve damage as well as in bone remodeling and skeletal development [19–21]. The choice of Mg was further motivated by its excellent biocompatibility, biodegradation into non-toxic products and its proven role in different processes such as cellular respiration, protein synthesis, membrane integrity, ATPase function and oxidative phosphorylation [22–24].

Among a large number of inorganic particulates used to design composite nanofibers, there has been a growing interest in magnesium oxide (MgO)-based composite materials because of its decontamination potential for catalytic detoxification of toxic chemicals as well as protection from UV light [25–28]. These composites have great potential to protect against chemical warfare stimulants [25,27,28]. Although there has been some success in producing MgO-based composite nanofibers, very limited studies have been done on MgO-incorporated nanofiber membrane as potential scaffold material for tissue engineering applications [26,29]. In this research, therefore, we seek to improve the miscibility of MgO in poly (ε-caprolactone) (PCL) as well as poly (ε-caprolactone)-chitosan (PCL-CS) solution systems, and to study the mechanical and biological properties of the composite nanofiber electrospun from the solutions.

We fabricated nanofiber membranes of PCL-CS/MgO of different compositions by electrospinning the blend solutions of PCL and MgO in trifluoroethanol, and CS in water. Physicochemical properties, such as morphology, mechanical strength, and integrity in aqueous medium as well as cellular compatibility of the nanofibrous membrane were determined.

2. Materials and methods

2.1. Materials

Chitosan (MW 2.5 k; Lot No. HL130109G) was purchased from Creative PEGWorks Inc. (Chapel Hill, NC). 2,2,2-Trifluoroethanol (TFE) was obtained from Alfa Aesar (Ward Hill, MA). PCL (Mn 70–90 kDa), and MgO (nanopowder, < 50 nm particle size) were purchased from Sigma Aldrich. Stainless steel dispensing needle (21 gauge and 3.81 cm long, product number 75165A757), fluorinated ethylene propylene tubing (0.32 cm inner diameter) and Luer lock syringe needle fittings were obtained from McMaster-Carr (Atlanta, GA). Luer-lock syringes (catalog number: 14-829-45) was obtained from Fisher Scientific (Pittsburgh, PA).

2.2. PCL/MgO and PCL-CS/MgO solution preparation

PCL and CS were dissolved in separate beakers at a concentration of 10% (w/w) in TFE and DI water respectively. PCL/MgO solutions were created by mixing PCL and MgO in different ratios (Table 1). Subsequently, a PCL-CS/MgO solution was created by mixing CS solution drop-wise to the solution of PCL and MgO. The solution mixtures were vortexed manually until each solution reached a homogeneous blend ready for electrospinning. Weight ratio of PCL/CS was maintained at 80/20 for all the CS based blend solutions.

Table 1
Fiber sample compositions.

Set A

Sample	Concentration of PCL (wt%)	Relative amount of PCL	Relative amount of MgO
PCL	10	100	0
PCL/MgO	10	90	10
PCL/MgO	10	75	25
PCL/MgO	10	50	50

Set B

Sample	Concentration of CS (wt%)	Concentration of PCL (wt%)	Relative amount of PCL-CS	Relative amount of MgO
PCL-CS	10	10	100	0
PCL-CS/MgO	10	10	90	10
PCL-CS/MgO	10	10	75	25
PCL-CS/MgO	10	10	50	50

2.3. Electrospinning of PCL/MgO and PCL-CS/MgO nanofibers

A previously prepared polymer solution of PCL/MgO and PCL-CS/MgO was individually fed into the syringe of 10 mL and then placed into a syringe pump (Model 78-01001, Fisher Scientific, Pittsburgh, PA, USA). The syringe pump was set to a flow rate of 2.5 mL/h. The syringe tip was positioned ~7 cm from a fiber collecting drum at an angle of ~30° to the horizontal. A 25–27 kV high voltage power supply (Model CZE100PN30, Spellman High Voltage Electronics Corporation, Hauppauge, NY, USA) was used to charge the solution. The positive lead from the high voltage power supply was fixed to a 21-gauge hypodermic needle. The fibers formed were deposited onto an aluminum sheet wrapped over a rotating grounded collector.

2.4. Nanofiber morphology study

The surface morphology of nanofiber membranes was analyzed by SEM (Hitachi SU8000, Tokyo, Japan). Prior to imaging, a small section of the fibers was sputter coated with gold in a Polaron SEM coating system for 90 s at 15 mA. Images of the samples were taken at an accelerating voltage of 10 kV and 5 μA current. The diameter of these electrospun fibers was determined through SEM images with the use of ImageJ Pro Plus 6.0 software (NIH, USA). Three SEM images from different location of each composition were utilized. Twenty different nanofibers were randomly chosen in each SEM image to measure the diameter in pixels. The number of pixels was converted into μm using the scale factor. Finally, the average diameter of the nanofibers was calculated based on the converted ImageJ data.

The core–shell structure of the electrospun PCL/MgO composite nanofibers was examined in a Tecnai G2 Twin transmission electron microscope (TEM) at 200 kV. The samples for TEM were prepared by directly depositing the as-spun nanofibers onto a copper grid.

Stability and degradation of nanofiber membranes were also studied through SEM images. Sterilized nanofiber membrane of PCL, PCL/CS and PCL-CS/MgO (30 × 30 mm) immersed in 40 mL of 1X Phosphate Buffer Saline (PBS) solution were incubated for 3 weeks in a Shaking Incubator (Dubnoff Shakebath-2876, Thermo Fisher Scientific, Fair Lawn, NJ, USA) at 37 °C and 50 rpm. Nanofibers after incubation were removed from the PBS solution, rinsed with DI water and lyophilized.