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Controlled protein release from electrospun biodegradable fiber mesh composed of poly(ε-caprolactone) and poly(ethylene oxide)

Taek Gyoung Kim [a], Doo Sung Lee [b], Tae Gwan Park [a,*]

[a] *Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea*
[b] *Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon, Kyoungki-do 440-746, South Korea*

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Abstract

A blend mixture of poly(ε-caprolactone) (PCL) and poly(ethylene oxide) (PEO) was electrospun to produce fibrous meshes that could release a protein drug in a controlled manner. Various biodegradable polymers, such as poly(L-lactic acid) (PLLA), poly(ε-caprolactone) (PCL), and poly(D,L-lactic-*co*-glycolic acid) (PLGA) were dissolved, along with PEO and lysozyme, in a mixture of chloroform and dimethylsulfoxide (DMSO). The mixture was electrospun to produce lysozyme loaded fibrous meshes. Among the polymers, the PCL/PEO blend meshes showed good morphological stability upon incubation in the buffer solution, resulting in controlled release of lysozyme over an extended period with reduced initial bursts. With varying the PCL/PEO blending ratio, the release rate of lysozyme from the corresponding meshes could be readily modulated. The lysozyme release was facilitated by increasing the amount of PEO, indicating that entrapped lysozyme was mainly released out by controlled dissolution of PEO from the blend meshes. Lysozyme released from the electrospun fibers retained sufficient catalytic activity.

Keywords: Electro-spinning; Biodegradable; Nanofibers; Protein release; Lysozyme

1. Introduction

Electrospun ultrafine nanofibers have been explored during the past several years as potential biomedical device including tissue engineering scaffolds, wound dressing materials, and drug delivery carriers. Recently, nanofibrous polymeric meshes prepared by an electrospinning technique have gained much interest for delivering various bioactive agents in a sustained manner, such as antibiotics, anti-tumor agents, proteins, and plasmid DNA (Luu et al., 2003; Kim et al., 2004; Casper et al., 2005; Chew et al., 2005; Zeng et al., 2005a). The electrospinning process enables a diverse range of drugs to be directly encapsulated within the bulk phase of nanoscale fibers by dissolving or dispersing them in the organic solvent used for electrospinning. The resultant fibrous mesh possesses a three-dimensional open porous structure with a high specific surface area, providing an ideal condition for controlled drug delivery. It has been shown that drug release patterns from nanofibrous meshes can be tailored by various formulation conditions such as polymer type, polymer concentration, blending of different polymers, surface coating, and the state of drug molecules in an electrospinning medium (e.g. emulsion or suspension, direct dissolution, and coaxial electrospinning) (Zeng et al., 2003; Jiang et al., 2005; Xu et al., 2005; Zeng et al., 2005a). In addition, drug solubility and compatibility with the polymer solution have decisively influenced drug release profiles by altering the drug distribution inside the electrospun nanofibers (Zeng et al., 2005b; Jiang et al., 2006). More recently, electrospun core/shell nanofibrous polymer meshes prepared by coaxial electrospinning were also utilized for delivering protein drugs in a sustained manner. The core-shell structured fibers showed a wide range of protein release profiles by varying the electrospinning parameters. However, most biodegradable nanofibers directly entrapping water soluble drugs exhibited high burst effects with poor controlled release patterns, probably due to incompatibility of drug/polymer/solvent system and slow degradation of biodegradable polymer (Zeng et al., 2003, 2005b).

Biodegradable nanofibrous polymer scaffolds with an open pore structure have been extensively investigated for tissue

* Corresponding author. Tel.: +82 42 869 2621; fax: +82 42 869 2610.
E-mail address: tgpark@kaist.ac.kr (T.G. Park).

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engineering applications, since they have a nanofibrous skeletal structure similar to that of the extracellular matrix (ECM) present in the living tissue. We previously reported a study on the surface immobilization of a cell adhesive Gly-Arg-Gly-Asp-Tyr (GRGDY) peptide on the electrospun PLGA nanofibrous mesh (Kim and Park, 2006). Amine functionalized nanofibers were produced by electrospinning a blend mixture of PLGA and PLGA-b-PEG-NH_2 di-block copolymer, followed by covalent conjugation with the peptide. The cell adhesive peptide functionalized meshes exhibited enhanced cell adhesion, spreading, and proliferation. The ECM mimicking electrospun nanofibrous meshes can be used as attractive scaffold materials for tissue regeneration (Mo et al., 2004; Khil et al., 2005). To further mimic the function of the ECM, growth factor releasing nanofibrous and biodegradable meshes are highly desirable, because they could modulate diverse cellular functions such as differentiation. In particular, nanofibrous meshes releasing growth factors can be potentially used as wound dressing material that provides sustained release of growth factors at the wound site. Many growth factors have been used for wound healing and tissue regeneration due to their potent mitogenic effects (Pandit et al., 2000; Breitbart et al., 2001).

In this study, biodegradable fibrous meshes that can release a protein drug in a sustained manner were produced by directly dissolving protein molecules in an electrospinning solvent medium. Lysozyme was used as a model protein. Various biodegradable polymers such as PLLA, PCL, and PLGA were blended with PEO in varying ratios, co-dissolved with lysozyme in a mixed solvent of chloroform/DMSO, and electrospun to produce lysozyme loaded fibrous meshes. Hydrophilic PEO was incorporated into the hydrophobic bulk phase of biodegradable fibers in order to facilitate lysozyme release by forming extractable pore channels upon incubation in the buffer medium. Lysozyme release patterns were examined by varying the formulation parameters such as polymer type and blend ratio. Enzyme activity of the released fractions was analyzed.

2. Materials and methods

2.1. Materials

PEO (Polyox WSRN-80, Mw: 200,000) was obtained from Union Carbide Corp. (Danbury, CT). Poly(D,L-lactic-*co*-glycolic acid) (PLGA RG756 LA/GA = 75/25, Mw: 100,000) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Poly(ε-caprolactone) (PCL, Mw: 65,000) and poly(L-lactic acid) (PLLA, Mw: 50,000) were supplied by Aldrich (Milwaukee, WI) and Polysciences Inc. (Warington, PA), respectively. Lysozyme (from chicken egg white, 50,000 units/mg protein) (E.C. 3.2.1.17, mucopeptide *N*-acetylmuramylhydrolase), Micrococcus lysodeikticus, and fluorescamine were obtained from Sigma (St. Louis, USA). Micro-bicinchoninic acid (BCA) assay kit was from Pierce (Rockford, IL). All other chemicals were of analytical grade.

2.2. Electrospinning

A blend mixture of PEO and PLLA, PCL, or PLGA (7/3 weight ratio) was dissolved in chloroform at a concentration of 15% (w/v). PEO/PCL blends with varying ratios (9/1, 7/3, and 5/5) were dissolved in chloroform at a concentration range of 10–15% (w/v). The final volume of each polymer blend solution was 3 ml. Lysozyme was first dialyzed against distilled deionized water to remove residual salts. After adjusting pH to 3.0 with 1.0 M HCl, the solution was lyophilized. Salt-free dry lysozyme was dissolved in DMSO at a concentration of 30 mg/ml. The lysozyme solution (0.2 ml) was mixed with the polymer blend solution, followed by gentle stirring. The electrospinning apparatus used in the present study was constructed based on our previous study (Kim and Park, 2006). Each polymer/lysozyme solution was added into a 5 ml syringe with a metal blunt needle (22G) and then mounted in a programmable syringe pump (model 210, KD Scientific Inc., USA) operated at 20 μl/min. The positive lead from high voltage power generator (CPS-40 K03VIT, Chungpa EMT Co., Korea) was connected to the needle tip and a DC voltage of 15 kV was applied. Stretched and solidified polymeric fibers were deposited on a rotating mandrel-type collector placed 12 cm away from the needle. All electrospinning processes were carried out under ambient conditions.

2.3. Characterization of electrospun fiber meshes

The morphological observation of each electrospun fiber was carried out with a scanning electron microscope (SEM, Philips 535M, Netherlands) after sputter coating with Au particles. From the SEM images, each fiber diameter was determined by using an image analyzer (Image J, developed by the National Institute of Health, USA). For visual observation of encapsulated lysozyme in the fiber, lysozyme was pre-conjugated with fluorescamine in DMSO, and then the fluorescent lysozyme/polymer composite solution was electrospun onto a slide glass. Distribution of fluorescent lysozyme in the fiber was examined by a laser scanning confocal microscope (LSCM, Carl Zeiss LSM5100, Germany).

2.4. In vitro lysozyme release

The circular pieces of lysozyme loaded fibrous mesh (ca. 20 mg) were placed, in triplicate, in a 12 well tissue culture plate and immersed in 2 ml of 33 mM phosphate buffer saline (PBS, pH 7.4, 0.02% NaN_3) solution at 37 °C in a humidified 5% CO_2 environmental incubator. At pre-determined time intervals, 1 ml of release medium was collected and replaced with an equal volume of fresh buffer medium. The amount of lysozyme in the collected solution was measured by using a micro-BCA protein assay kit.

2.5. Polymer erosion

Blend PEO/PCL electrospun meshes were incubated in 5 ml of PBS solution at 37 °C under static condition. The incubation medium was changed daily. The samples were retrieved after