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Assembly of particle-fiber composites by electrohydrodynamic jetting using counter-charged nozzles: Independent release control

Harim Bae, Jonghwi Lee *

Department of Chemical Engineering and Materials Science, Chung-Ang University, Seoul 156-756, South Korea

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ABSTRACT

The optimal design of an electrospun scaffold for tissue engineering is contingent on its functionality to mimic the native extracellular matrix (ECM) as much as possible. Using core-sheath particles for encapsulation, the scaffolds of particle-fiber hierarchical structures could play the dual roles of structural matrix and controlled delivery system of active ingredients. Herein, electrohydrodynamic jetting of countercharged nozzles was investigated to combine fibers and core-sheath particles based on the neutralization phenomenon between electrospun poly(L-lactide-co-D,L-lactide) fibers and electrosprayed poly(lactide-co-glycolide) particles. It was revealed that the key features of particle-fiber composites could be conveniently designed by controlling the composition of the binary mixture of ethyl acetate and benzaldehyde (BA) for electrospraying. With an increase in the BA concentration, the areal density of the particles on the fibers (degree of combination) increased threefold, and the total loading amount of lysozyme (model active ingredient) also increased threefold, while particle size and fiber diameter did not change significantly. The composites prepared from a high BA content case provided sustained release profiles, which are independent from the degradation kinetics of fibers, such that lysozyme could be released for more than a month. The use of a binary solvent mixture for this process seems to be an effective strategy for developing future functional scaffolds for tissue engineering.

Introduction

In recent tissue engineering applications, biodegradable and biocompatible scaffolds have been specifically designed to support the proliferation and regeneration of various tissues [1–3]. The rational design of a scaffold depends on its ability to support cells and release functional factors, such as angiogenic factors or anti-inflammatory drugs, that can provide cells with consistent signals [4–8]. Electrospinning technology is suited for the development of functionalized scaffolds for biomedical applications, owing to the close resemblance of the resulting three-dimensional architectures to the native extracellular matrix (ECM) in terms of physical, mechanical, and chemical features [9–14]. However, the various limitations of electrospun nanofiber mats with respect to functional modification have been recognized.

Direct incorporation of functional factors into electrospun fibers has been investigated using coaxial electrospraying [9]. By using proper solvents, stable coaxial electrospraying successfully encapsulated biomolecules into the cores of the fibers. However, the release kinetics of these biomolecules has been difficult to engineer, and initial burst release has been reported in many papers, although stable coaxial electrospinning was employed [5,9,11,15]. Furthermore, the release kinetics of encapsulated biomolecules is always related to the degradation kinetics of the fibers, which prevent the preparation of ideal scaffolds that mimic the ECM.

In our previous study, we developed a novel *in situ* technique combining electrosprayed particles and electrospun fibers to enhance the functionality of fiber mats, by the electrohydrodynamic jetting of countercharged nozzles (EJC), which is reproducible and scalable [16–18]. It used two jetting methods simultaneously, electrospinning and coaxial electrospraying, which were countercharged. Electrosprayed particles and electrospun fibers combine and are neutralize owing to the electrostatic attraction between their surfaces. There is strong electric repulsion among the electrosprayed particles, which intrinsically prevents aggregation of particles and promotes uniform attachment onto fiber surfaces. *In situ* attachment during particle forming improves the attachment strength of particles [19,20]. Functional factors can be stably encapsulated in biodegradable particles by using coaxial electrospraying on one side of the EJC, being immobilized *in situ* onto electrospun fibers. Unlike conventional surface-functionalized

* Corresponding author. Tel.: +82 1092701349.
E-mail address: jong@cau.ac.kr (J. Lee).

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fibers, this method does not require additional chemical or mechanical treatments, which use severe environments to encapsulate biomolecules [16–18]. The resulting particle-fiber composites in that study demonstrated that the mechanical strength of the fibers was not affected by the surface attachment of particles, and that the functional factors that were encapsulated in the particles could have their own release mechanism, independent of fiber degradation [16]. Furthermore, local release at a target site was achieved without spreading out by *in vivo* cell mobility, and the presence of fiber mats provided mechanical cues and guides to cell growth, in addition to the sustained release of biomolecules from the particles [16].

Many particle-fiber composite systems have since been investigated [21]. Jet-spraying has been utilized to attach particles to the surfaces of fibers, but the controllability of the composite morphology and release kinetics has not improved [22]. In all previous papers, the release control of the biomolecules largely depended on the material properties of the particles and the fibers, and the release kinetics was not easy to control, since it was significantly dependent on interwoven processing parameters [21–23]. Therefore, efficient variables that can control the release kinetics should be developed to effectively engineer particle-fiber composite scaffolds.

Based on our previous successful development of particle-fiber composite scaffolds, the aim of this study is to develop an effective strategy to control the key properties, such as release kinetics. Herein, the effects of the composition of a binary solvent mixture was systematically investigated using a combination of poly(D,L-lactide-co-glycolide) (PLGA) and poly(L-lactide-co-D,L-lactide) (PLA), and it was found that the use of a binary solvent mixture was a simple and effective method of controlling the release kinetics. The solvent properties were indeed pivotal in determining the attraction between particles and fibers, as well as the release kinetics of the composite scaffolds.

Experimental

Materials

PLA 70:30 (Resomer LR708, i.v. 5.5–6.5) and PLGA 50:50 (Resomer 503H, i.v. 0.32–0.44) were purchased from Boehringer Ingelheim (Ingelheim, Germany). Polyvinylpyrrolidone (PVP, M_w = 29,000 g/mol), lysozyme (from chicken egg white), benzaldehyde (BA, ReagentPlus, ≥99%), rhodamine B, potassium buffer solution, and a BCA assay kit (QuantiPro BCA Assay Kit) were supplied by Sigma–Aldrich (MO, USA). N,N-dimethylformamide (DMF) and ethyl acetate (EA) were obtained from Duksan (Gyeonggi, Republic of Korea). Tetrahydrofuran (THF) was purchased from Samchun (Seoul, Republic of Korea). All materials were used as-received, without any purification.

Electrohydrodynamic jetting

A schematic diagram of the EJC process is shown in Fig. 1. It consisted of two nozzles, a negatively charged electrospinning one and a positively charged electrospraying one [17]. The electrospinning solution consisted of 4 wt% PLA in a mixed solvent of DMF and THF (1:1 v/v). During electrospinning, the flow rate was 0.7 mL/h, which was fixed by a syringe pump (KDS 100, KD Scientific, MA, USA), and a 19-gauge needle was used. An optimized voltage of −6.3 kV was supplied by a DC negative voltage supply (H.V. model, NanoNC, Seoul, Republic of Korea). Coaxial electrospraying was used to prepare protein-encapsulated particles for the electrospraying portion of Fig. 1. The core solution was prepared by dissolving 0.3 g PVP and 20 mg lysozyme (model active ingredient) in 1 mL H_2O. The sheath solution was comprised of 0.1 g PLGA and

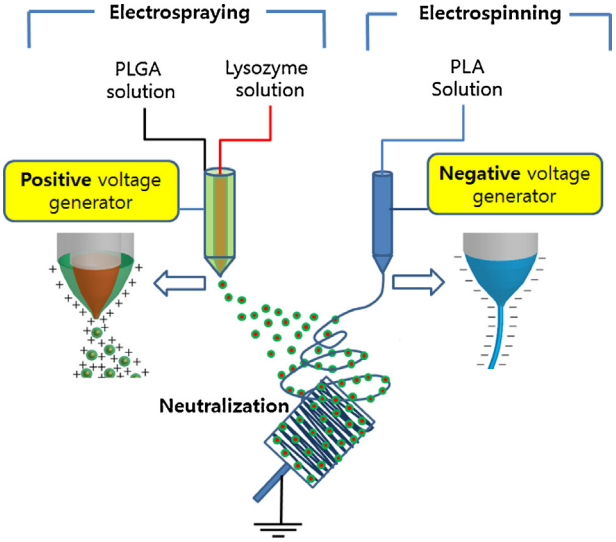

Fig. 1. *In situ* combination of encapsulated particles and fibers *via* electrohydrodynamic jetting of countercharged nozzles (EJC). The countercharged particles and fibers combine and neutralize in the middle.

1 mL mixed solvents of EA and BA (7:3, 5:5, 3:7, or 1:9 v/v). A homemade concentric spinneret, whose outside and inner needles are 18 and 26 gauge, respectively, was used with flow rates of 0.6 (sheath) and 0.01 (core) mL/h. A DC positive high-voltage power supply (Convertech SHV model, Seoul, Republic of Korea) provided +7.0 kV for the coaxial electrospraying. Both the electrospraying and electrospinning nozzles were placed 200 mm above the ground, and the distance between the nozzles was 165 mm. The resulting particle-fiber composites were collected on a square frame of steel wire (6 × 6 cm^2) (Fig. 1). The same conditions were used for single jetting of the coaxial electrosprayer with an aluminum foil plate. All of the processes were carried out at 24 ± 2 °C and 40 ± 10 RH%, and the collected samples were placed in a vacuum at room temperature (RT) overnight. Since they have been successfully employed for PLGA [24], BA and EA were chosen, and since PLGA completely dissolves only in a solvent system with a BA content greater than 30 v/v%, EA:BA = 9:1 (v/v) was not investigated.

Characterization

To observe the jetting mode of the nozzle tips, a charge-coupled device (CCD) camera (Luminera LW575C, 800 × 600 pixels, Ottawa, Canada) was used at 1/5 s. The morphology of the fiber-particle composites was characterized using a scanning electron microscope (SEM, S-3400N, Hitachi, Japan) (15 kV). The samples were mounted on carbon tape and sputter-coated with platinum using an ion sputter (E-1010, Hitachi, Japan) at 15 mA for 100 s. The average fiber diameter and particle size were quantified by analyzing SEM micrographs using an image program, Image J (National Institutes of Health, USA). To quantify average areal density (number of particles per unit fiber surface area), at least thirty SEM micrographs (100 × 100 μm) were analyzed. The number of particles was counted, and fiber surface area was calculated from the length and average diameter of the fibers in the images. Confocal laser scanning microscopy (CLSM, Carl-Zeiss, Oberkochen, Germany) was employed to observe the distribution of lysozyme encapsulated in the microparticles. For fluorescence contrast in confocal microscopy, 0.01 wt% of rhodamine B was added to the core solution.

Solution conductivities were determined at RT using a Meterlab CDM210 conductivity meter (conductivity accuracy ±0.2%, Radiometer Analytical, Lyon, France) with a conductivity probe (2-pole