Antibacterial Nanofibrous Mats Composed of Eudragit for pH-Dependent Dissolution

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ABSTRACT: A pH-responsive nanofibrous mesh was prepared for the controlled release of antibiotics in response to pH changes. Eudragit EPO (EPO) and Eudragit L100 (L100) were injected through inner and outer needle and simultaneously electrospun through coaxial nozzles composed of inner and outer needles. Various amounts of EPO and L100 were coejected with tetracycline through the needle and simultaneously electrospun to the fibrous meshes. The mass erosion rates of the meshes at pH 6.0 gradually decreased as the amounts of EPO increased, whereas those at pH 2.0 showed negligible differences; these differences were confirmed by scanning electron microscopy and monitoring the dry weight changes. At pH 6.0, the fibrous structures of the meshes rapidly disappeared compared to those under acidic conditions because Eudragit L100 is localized to the shell of the nanofiber during the electrospinning process. Both the pH changes and the blend ratio of the two polymers significantly affected the tetracycline release; tetracycline was rapidly released from the meshes at pH 6.0, whereas the release rates were attenuated at pH 2.0. Tetracycline was released faster from the mesh at higher blend ratios of EPO for both pH values. The electrostatic interaction between EPO and L100 is expected to yield different release profiles of tetracycline. Consequently, higher amounts of encapsulated drugs were released from the mesh at neutral pH and successfully inhibited bacterial growth. J Pharm Sci 104:2611–2618, 2015
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INTRODUCTION

Drug delivery to the desired site has been considered an inevitable challenge for decades. For this reason, various types of spherical particles have been developed, such as nanoparticles and microspheres.[1,2] However, these particles are systemically transported and do not remain at specific positions, resulting in an insufficient absorption time. Furthermore, the long-term use of antibiotics at high doses can cause resistance to these drugs.[3] Therefore, the time a drug spends in a diseased region needs to be prolonged with sustained release approaches to ensure high drug concentrations at targeted sites and avoid excess drug use.[4] Another problem of these drug delivery systems is that the drug is easily damaged. Acidic conditions and stomach enzymes may denature the drug before it reaches the desired site, such as the small intestine or colon. Therefore, many studies have been conducted to overcome this harsh environment. The pH values of organs vary by site, and various carriers have been developed with pH-dependent dissolution properties for local drug delivery.[5] Alonso and coworkers[6] developed Eudragit L100 coated chitosan microspheres for the drug delivery to colon. Fast dissolution of chitosan microcores was efficiently attenuated by Eudragit L100 layers in stomach, which lead to successful delivery of the encapsulated drug to the colon.[6]

Electrospun nanofibers are one of the promising biomedical devices for drug delivery that have shown long retention times at the wound site because of the porous, thin, and flexible structure.[7,8] Furthermore, these materials can be fabricated via electrospinning, which utilizes simple and inexpensive equipment.[9] Because of the highly porous and nano-scaled structures, electrospun nanofibers have high surface-to-volume ratios, which permits the easy transports of nutrients, water, and oxygen. In addition, many bioactive molecules, such as drugs, proteins, and genes, can be incorporated on the surface of the nanofibers via physical or chemical methods. In a previous study by our group, nanofibers composed of PCL and PCL–poly (ethylene glycol) (PCL–PEG) mixtures were used to deliver various growth factors for facilitating wound healing.[10] Blagbrough's group used electrospun nanofiber to antibiotics delivery system by staking of two and three different nanofiber layer.[11,12] Therefore, nanofibers are a promising candidate for drug delivery in mucous tissues because of their sustained release and high incorporation of the drug. Eudragit, which was approved by the Food and Drug Administration for oral drug delivery systems, is a biocompatible polymer based on methacrylate and methyl methacrylate.[13] The dissolution characteristics of Eudragit depend on its substituents.[14] Eudragit EPO (EPO) is a cationic polymer that contains dimethylamino groups and dissolves at values below pH 5.0.[15] Eudragit L100 (L100) contains carboxyl groups, is anionic, and soluble above pH 6.0.[16] Because of its unique dissolution characteristics, Eudragit is applied as coating materials. For example, drugs were coated with Eudragit L100–55 and Eudragit S100 to be delivered to the colon.[17] Ji-Shan's group investigated L100-coated chitosan microspheres that encapsulated bovine serum albumin (BSA)

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and demonstrated the pH-dependent sustained release of BSA at different pH conditions.[18] In another study, Eudragit L100–55 nanofiber was fabricated, and diclofenac sodium was loaded as a model drug.[7] The drug showed sustained release with a pH-dependent profile. Eudragit is advantageous for site-specific drug delivery because of its pH-dependent solubility, which favors alternative drug release. The unique dissolution properties for specific Eudragit varieties allow for site-specific drug release from Eudragit nanofibers.

In this study, we fabricated nanofibers using coaxial electrospinning for pH-dependent release of tetracycline. EPO and L100 were simultaneously injected from an inner and outer nozzle. Tetracycline was used as a model drug, which was co-electrospun with EPO solution. The complex ratio of EPO and L100 in nanofibers was controlled with the flow rate. The resultant materials were characterized based on their morphology, pH-dependent dissolution rate, drug release profile, and a bacteria inhibition test.

MATERIALS AND METHOD

Materials

Eudragit L100 [poly(methacylic acid-co-methyl methacrylate)] [molecular weight, (MW) 125,000 g/mol] and Eudragit EPO [poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate)] (MW 47,000 g/mol) were kindly donated by EVONIK (Incheon, South Korea). Tetracycline hydrochloride was purchased from Sigma–Aldrich (St. Louis, Missouri). HITTM-DH5α was purchased from RBC bioscience (Taipei, Taiwan). Difco™ LB Agar was purchased from BD (Mississauga, Ontario, Canada). Agar powder and hydrochloric acid solution were obtained from Junsei (Tokyo, Japan). All other chemicals were of analytical grade.

Preparation of Coaxial Eudragit Nanofibrous Mat

Eudragit L100 was dissolved in a mixture of methanol and N,N-dimethylacetamide (DMAc) (5:1, v/v) at a concentration of 11.0% (w/v). Eudragit EPO was dissolved at a concentration of 26.0% (w/v) in the same solvent mixture used for Eudragit L100. A dual nozzle composed of an inner (25 G) and an outer (18 G) nozzle was employed for coaxial electrospinning (NanoNC, Seoul, South Korea). Eudragit EPO solution containing tetracycline and Eudragit L100 solution was simultaneously injected via the inner and outer portions of the dual nozzle, respectively, and electrospun to ground by high voltage, 15 kV, at 23°C, in 33% humidity. The flow rate was adjusted to obtain various weight ratios of Eudragit EPO to Eudragit L100. The amount of tetracycline codissolved in the inner polymer solution was simultaneously varied to ensure that the mass of the drug relative to the mass of the Eudragit nanofibrous mat remained constant after electrospinning. The solutions were electrospun onto large pieces of aluminum foil at 18 kV, and the distance between the ground and the nozzle was 12 cm. The coaxially electrospun Eudragit nanofibrous mats were morphologically analyzed using a field emission–scanning electron microscope at the Central Laboratory Kangwon National University (FE-SEM, S-4300, Hitachi, Japan), and the diameters of the nanofibrous mat were image-analyzed with the Image Pro plus 6.0 software.

Remaining Mass Profile of Eudragit Nanofibrous Mat

The dissolution properties of the Eudragit nanofibrous mat were assessed using various combinations of Eudragit L100 and Eudragit EPO. The Eudragit nanofibrous mats were soaked in 1 mL of 0.2 M potassium chloride buffer (pH 2.0) and 0.1 M citric acid buffer (pH 6.0). The nanofibrous mats were incubated with gentle shaking at room temperature. At pre-determined time intervals, the nanofibrous mat was washed five times with distilled water and freeze-dried. The mass erosion rates were determined by quantifying the amount of the remaining nanofibrous mats after incubation. The percentages of remaining mass were calculated using the following equation:

$$Remaining\ mass = \left(\frac{weight\ of\ dissolved\ nanofiber}{initial\ weight\ of\ Eudragit\ nanofiber}\right) * 100 \quad (1)$$

Release Profile of Tetracycline

The release profile of tetracycline from the Eudragit nanofibrous mat was monitored at pH 2.0 or pH 6.0. The Eudragit nanofibrous mats were soaked in 0.2 M potassium chloride solution (pH 2.0) or 0.1 M citric acid solution (pH 6.0) in a 12-well plate. The plate was incubated with vigorous shaking at 200 rpm at room temperature. The dissolved samples were transferred to 1-mL micro-tubes and centrifuged at 9,950 g for 2 min. The amount of released tetracycline in the supernatant was measured at 274 nm, and tetracycline dissolved in potassium chloride solution or citric acid solution was used as a standard for the respective release fractions. The remaining tetracycline in the nanofibrous mat was also fluorescently visualized with an *in vivo* imaging system (IVIS) at Korea Basic Science Institute. Sections were removed from the Eudragit nanofibrous mat with a biopsy punch ($\phi = 5$ mm) and incubated in 0.2 M potassium chloride buffer (pH 2.0) and 0.1 M citric acid buffer (pH 6.0). After a 10-min incubation period, the supernatant was discarded and the remaining nanofibrous mast was washed with distilled water. The fluorescence of tetracycline in the nanofibrous mat was then examined by IVIS at $\lambda_{ex} = 410$–440 nm and $\lambda_{ex} = 445$–490 nm (IVIS-200; PerkinElmer, Waltham, MA) at Korea Basic Science Institute.

Bacterial Inhibition Test

To confirm the ability of the tetracycline-laden mats to inhibit bacteria, the growth inhibition of bacteria was monitored as a function of the fraction of released drug at different pH values. *E. coli* (DH5α; RBC Bioscience, Taipei, Taiwan) was cultured in LB medium with vigorous shaking at 37°C for 12 h. After re-suspending *E. coli* at 10^4 CFU/mL in 1 mL of LB medium, 20 μL of the supernatant, which contained the fraction of tetracycline released after 12 h, was added to the bacteria-containing LB medium. After orbital shaking at 200 rpm and 37°C for 12 h, the absorbance of the culture broth was measured at 600 nm to quantify the inhibition of *E. coli* growth. Untreated bacteria were used as a control to determine the relative antibacterial efficacy of the mats.

RESULTS AND DISCUSSION

Figure 1 describes the preparation of Eudragit nanofibrous mats by coaxial electrospinning for anti-bacterial treatments.

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