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Electrospinning of PLGA/gum tragacanth nanofibers containing tetracycline hydrochloride for periodontal regeneration

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ABSTRACT

Controlled drug release is a process in which a predetermined amount of drug is released for longer period of time, ranging from days to months, in a controlled manner. In this study, novel drug delivery devices were fabricated via blend electrospinning and coaxial electrospinning using poly lactic glycolic acid (PLGA), gum tragacanth (GT) and tetracycline hydrochloride (TCH) as a hydrophilic model drug in different compositions and their performance as a drug carrier scaffold was evaluated. Scanning electron microscopy (SEM) results showed that fabricated PLGA, blend PLGA/GT and core shell PLGA/GT nanofibers had a smooth and bead-less morphology with the diameter ranging from 180 to 460 nm. Drug release studies showed that both the fraction of GT within blend nanofibers and the core–shell structure can effectively control TCH release rate from the nanofibrous membranes. By incorporation of TCH into core–shell nanofibers, drug release was sustained for 75 days with only 19% of burst release within the first 2 h. The prolonged drug release, together with proven biocompatibility, antibacterial and mechanical properties of drug loaded core shell nanofibers make them a promising candidate to be used as drug delivery system for periodontal diseases.

1. Introduction

Drug delivery systems are engineered technologies for the controlled release of therapeutic agents to achieve therapeutic purposes in humans or animals. In recent years, the market for drug delivery technology (DDT) has increased tremendously [1], and it is forecasted to reach $136 billion by 2021 [2]. Controlled drug release systems have shown benefits over conventional drugs [3], such as improved adequacy, reduced side effects, improved patient compliance, and reduced toxicity [4,5]. Electrospinning is one of the developed techniques, which enables the design and production of nanostructured drug carriers with high loading capacity, encapsulation efficiency, multi-drug delivery with ease of operation, and cost-effectiveness [6,7]. In most cases, drugs are blended with the polymeric solution to produce drug incorporated nanofibers, which might result in low delivery efficiency and burst release [8], while other electrospinning techniques such as emulsion or coaxial electrospinning showed capabilities to overcome some of these problems [9,10]. Use of nanofibrous scaffolds loaded with antibiotic drugs for biomedical applications especially for treatment of infections after tissue damages such as burn, ulcers, surgery or periodontal disease has evoked considerable interest [11]. Mefoxin incorporated poly(lactide-co-glycolide) membrane displayed a controlled release of the drug for over 6 days [12]. Kenawy et al. [13], fabricated tetracycline hydrochloride (TCH) loaded poly(ethylene-co-vinyl acetate), poly(lactic acid) and their blends, then they reported controlled release of drug over 5 days. However, controlled release of antibiotics is required for a longer period of time for treatment of some of the chronic infections such as periodontal diseases. Nevertheless, long term release of hydrophilic drugs (such as TCH) from nanofibrous scaffolds is still challenging due to high solubility of the drug molecule in aqueous mediums. It was previously demonstrated that the compatibility between hygroscopic properties of drug and polymer is essential to obtain a sustained drug release from nanofibrous delivery system [14,15].

Poly lactic-*co*-glycolic acid (PLGA) is a Food and Drug Administration (FDA) approved synthetic polymer, and is one of the most attractive polymeric candidates used for the fabrication of drug delivery devices [16]. Gum tragacanth (GT), on the other hand, is a branched, heterogeneous and anionic polysaccharide with properties such as moisture absorption, hydrocolloid formation, water solubility, drug holding and releasing abilities and it is categorized as generally recognized as safe

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(GRAS) material at a level of 0.20–1.30% in food stuffs. This natural biopolymer is a mixture of two soluble and insoluble polysaccharides. Tragacanthin, a galacturonic acid part of tragacanth which is water soluble and branched with high molecular weight which gives highly viscous solutions and bassorin, the other part of tragacanth, is a complex of methoxylated acids that is insoluble in water and swells to form a gel or viscous solution [17–19]. It is approved as a food additive in European Union and has the number E 413 in the list of additives confirmed by the Scientific Committee for Food of the European Community [20]. GT also exhibited significant potency for wound healing in the form of mucilage or blended nanofibers with PCL or PVA because of an acceleration in collagenation and proliferation phases of the wound repair [21–23]. Thus, we hypothesized that incorporation of hydrophilic drugs into composite nanofibers of PLGA and GT could provide a more sustained and prolonged release of the drug, due to better hygroscopic compatibility of the drug and polymeric matrix.

Here, for the first time, we aim towards the fabrication of composite scaffolds of PLGA and GT at various ratios via blending and coaxial electrospinning. Further we investigated the controlled release of TCH incorporated within these nanofibers, along with the physical characteristics (i.e. wettability, porosity), mechanical properties and cytocompatibility of the composite nanofibers, which are critically important for a nanofibrous mat to be employed as scaffolds for periodontal disease treatment.

2. Experimental procedure

2.1. Materials

PLGA with lactic acid:glycolic acid (LA:GA) ratio of 75:25 with an intrinsic viscosity of 0.72 dl g^{-1} was purchased from Boehringer Ingelheim Pharma GmbH & Co. (Ingelheim, Germany). Gum tragacanth used in this study was a high quality ribbon type, collected from the stems of Fluccosus species of *Astragalus* bushes, grown in the central areas of Iran. *TCH* (purity >95%) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was purchased from Sigma Aldrich. Human dermal fibroblasts (HDFs) were obtained from American Type Culture Collection. Dulbecco modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin streptomycin solution and trypsin-ethylene diamine tetra acetic acid were purchased from Gibco, Invitrogen Corp., USA.

2.2. Blend and core–shell electrospinning

Electrospinning was performed to prepare blend nanofibers of PLGA-GT (PG) in three different weight ratios, including 100:0, 75:25, 50:50 (wt.%). Polymers were dissolved in HFP for making a total concentration of 16% (*w*/*v*). For fabrication of drug containing nanofibers, 5% (*w*/*w*) TCH (based on the total weight of PLGA and GT) was added and stirred for 30 min. Prepared solution was loaded into individual 3-mL syringe attached to a 25G blunted stainless steel needle and a high voltage of 15 kV was applied to the tip of the needle. The flow rate of the solutions was maintained at 1.0 mL/h using a syringe pump (KDS 100, KD Scientific, Holliston, MA). For fabrication of core shell nanofibers, PLGA was dissolved in HFP to obtain 16% (*w*/*v*) solution which was used as the shell. GT was dissolved in water to obtain 2% (*w*/*v*) solution and stirred overnight to be used as the core solution. The polymer solutions were separately fed into 3 mL standard syringes attached to a coaxial nuzzle. The inner diameter of shell capillary was 0.84 mm, while the smaller capillary had outer and inner diameters of 0.56 mm and 0.30 mm, respectively. A high voltage of 15 kV (Gamma High Voltage Research, Ormond Beach, FL) was applied, while the flow rate of the shell and core was maintained at 1.0 mL/h and 0.2 mL/h, respectively, and the polymer solution was drawn into fibers. To make TCH incorporated core shell fibers TCH was added to the core solution (5% *w*/*w*) based on the total amount of PLGA and GT, considering the concentration and flaw rate of core and shell solutions. Nanofibers were deposited on aluminum wrapped collector at a distance of 15 cm from the needle tip, dried overnight under vacuum and used for characterization, drug release, and cell proliferation experiments.

2.3. Characterization of nanofibers

The morphology of the electrospun nanofibers was studied under a Field Emission Scanning Electron Microscope (FESEM; JEOL JSM-6701-F, Japan) after sputter coating with gold (JEOL JFC-1200 fine coater, Japan) at an accelerating voltage of 15 kV. Diameters of the electrospun fibers were analyzed from the SEM images using image analysis software (Image J, National Institutes of Health, USA). Core–shell structure of drug loaded PLGA-GT (PG(cs)-TCH) nanofibers was examined using transmission electron microscopy (TEM) (JEOL JFM-3010, Japan). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic analysis of the electrospun scaffolds was fulfilled using a Nicolet Avatar 380 spectrometer (Thermo Nicolet, Waltham, MA) over the range of 600–3800 cm^{-1} at a resolution of 4 cm.

The pore size of the nanofibrous scaffolds was studied using a CFP-1200-A capillary flow porometer (PMI, New York, NY). Three samples of each type of nanofibers with the same thickness and a dimension of 2×2 cm^2 were used for measuring the pore size. Galwick with a surface tension of 15.9 dynes/cm (PMI, New York, NY) was used as the wetting liquid. Wettability of the nanofibers was determined via contact angle measurement, and a sessile drop method-based video contact angle system (VCA Optima, AST Products, Billerica, MA) was used for this purpose. The size of the distilled water droplet was set at 1.0 μL.

The mechanical properties of the electrospun membranes were determined by a uniaxial tensile machine (Instron5943, Canton, MA) with a load cell capacity of 10 N and cross head speed of 5 mm min^{-1}. All nanofiber tape samples were cut in the form of rectangular shape with dimensions of 30×10 mm^2. At first, a white window paper template was cut and nanofibrous tapes were glued onto the top and bottom areas of the window. It was placed between the grips of the tensile testing machine and after closing the grips, the other sides of the window papers were cut by a scissor. Tensile test was carried out for the as obtained dry electrospun mats, and the 48 h phosphate-buffered saline (PBS) hydrated scaffolds. A minimum of six specimens of individual scaffolds were tested.

2.4. Nanofiber degradation studies

To perform the biodegradability test, the fibers on cover slips were immersed in PBS (pH of 7.4) and incubated for a period of 40 days at 37 °C. At each specific time point, the scaffolds were washed and subsequently dried in a vacuum oven for 48 h. The morphology changes were studied by FESEM.

2.5. TCH release from electrospun nanofibers

Release of TCH from electrospun nanofibers was measured using a UV/VIS instrument (Shimadzu 3600, UV–VIS-NIR Spectrophotometer). For this, the drug containing nanofibrous mats were accurately weighed, placed in tightly capped glass bottle, soaked in 1 mL of PBS (pH 7.4) and kept in shaking incubator at 37 °C and 150 rpm. The UV absorbance of TCH released in buffer solution was determined at $\lambda max = 362$ nm and converted to the TCH concentration, according to the calibration curve of TCH in the same media [24]. The cumulative release of TCH against release time was further plotted. Fig. 1 gives the graphical representation of the preparation of nanofibers and studying release properties of them.

2.6. Cell culture and proliferation studies

In vitro biocompatibility of electrospun mats was evaluated using human dermal fibroblast (HDF) cells. The proliferation test