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Material performance

PHB-PEO electrospun fiber membranes containing chlorhexidine for drug delivery applications

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ABSTRACT

Fiber meshes of poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate)/poly(ethylene oxide) (PHB/PEO) with different concentrations of chlorhexidine (CHX) were prepared by electrospinning for assessment as a polymer based drug delivery system. *The electrospun fibers were characterized at morphological, molecular and mechanical levels.* The bactericidal potential of PHB and PHB/PEO electrospun fibers, with and without CHX, was investigated against *Escherichia coli (E. coli)* and *Staphylococcus aureus* (*S. aureus*) by disk diffusion susceptibility tests. Electrospun fibers containing CHX exhibited bactericidal activity. PHB/PEO-1%CHX displayed higher CHX release levels and equivalent antibacterial activity when compared to PHB/PEO with 5 and 10 wt% CHX. Bactericidal performance of samples with 1 wt% CHX was assessed by Colony Forming Units (CFU), where reductions of 100% and 99.69% against *E. coli* and *S. aureus* were achieved, respectively.

1. Introduction

Material engineering approaches allow the design of materials with increasing complexity and functionality for application in the development of drug delivery systems [1]. Both natural and synthetic polymers are being used in controlled drug release to maximize system efficiency [1,2]. "Drug release" refers to the process in which drug solutes migrate from the initial position in the polymer system to the polymer outer surface and then to the medium [1]. This process is affected by multiple complex factors such as the structural characteristics of the material system, release environment and possible interactions between them [1].

According to Zeng et al. [3], polymer based drug delivery systems show advantages when compared to the conventional dosage forms as they allow, for example, the therapeutic effect with reduced toxicity and enhance compliance of the patients by delivering drugs at a controlled rate over a period of time to the site of action [3]. However, some problems such as the low efficiency of the drug delivery systems and drug disintegration at the beginning of the process are still unresolved [3]. Drugs can be encapsulated directly into fibers processed by electrospinning, thus conferring on the electrospun fibers the function of drug carriers [3]. The study of electrospun fiber carriers for drug delivery is very limited [3]. Reported electrospun drug delivery systems include transdermal, oral sustained, targeted, implantable, tissue engineering and transmembrane delivery [4]. Drug delivery with polymer fibers is based on the principle that the delivery rate of the drug can be controlled by tailoring surface area of both drug and

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carrier [5]. Several factors that can affect the drug release from electrospun fibers are fiber geometry and thickness, membrane porosity, composition, crystallinity and swelling, to name a few [6].

Synthetic and natural biodegradable polymers have received special attention in pharmaceutical research [2]. Delivery systems based on biodegradable polymers are used because these polymers degrade into compounds that can be readily excreted from the body, thus obviating the need for later removal at the end of the treatment period [7].

Biodegradable polymers are used to control the drug release rate by diffusion through the polymer matrix or the pores within the matrix, and/or by degradation of the polymer chain and erosion of the matrix [2]. However, the control of the drug release rate from a delivery system that is susceptible to a degradation process is difficult, as the release rate may change over the degradation process [2].

PHB is a biocompatible polymer obtained from natural sources with a high degree of crystallinity. It is also characterized by high brittleness, poor processability and poor thermal stability [8]. Due to its natural origin, PHB has potential for biomedical applications, including drug delivery systems [9]. It has been evaluated for controlled drug release systems, surgical structures, wound dressings, orthopedic devices, tissue engineering and skin substitute materials [10].

Chlorhexidine (CHX) is one of the most efficient antimicrobial agents [11]. This drug has been widely used in a largely range of applications due to its antimicrobial activities against Gram-positive and Gram-negative bacteria and fungi, and non-toxicity toward mammalian cells [11,12]. It is used in several products for oral protection and, in general, for dentistry applications due to its antiseptic and disinfectant action on wounds [13]. Various studies [11,12,14–16] have been conducted on CHX release, however, only a few works are devoted to CHX release with electrospun fibers. Chen et al. [12] studied electrospun cellulose acetate fibers containing CHX as a bactericide for Gram-negative *Escherichia coli* and the Gram-positive *Staphylococcus epidermidis*. It was concluded that CHX bound on cellulose acetate fibers is still capable of killing the bacteria with a reduction of 99.9% of the viable bacteria in 1 hour [12].

In an electrospinning process, a strong electrostatic field is applied to a polymer solution held in a syringe and feed through a needle [17]. The fiber jet travels through the atmosphere allowing the solvent to evaporate, thus leading to the deposition of solid polymer fibers on the collector [17].

In the present work PHB/PEO fiber membranes containing different amounts of CHX were produced by electrospinning. The influence of the presence of the drug on fiber diameter and average size distribution, as well as the evolution of the mechanical properties of the membranes was characterized. Further, drug immobilization on the fibers was confirmed and the CHX release kinetics evaluated. Bactericidal performance of PHB/PEO samples 1 wt% CHX was assessed by Colony Forming Units (CFU), against both the *gram-negative* strain *E. coli* and *gram-positive* strain *S. aureus*.

2. Experimental

2.1. Materials

Poly(hydroxybutyrate), (PHB, molecular weight of ~531112 Da) from sugar cane was supplied by PHB Industrial. Poly(ethylene oxide), (PEO, molecular weight of ~100 000 Da) was supplied by Polysciences and Chlorhexidine, (CHX, molecular weight of ~505.45 Da) was purchased from Sigma Aldrich. The polymer solutions of PHB and PHB/PEO (90/10, wt%) with different final CHX concentrations (1, 5 and 10 wt%) were dissolved in a blend of N,N dimethylformamide (DMF, from Merck) and chloroform (CF, from M*erck)* (80/20 v/v) to achieve a final polymer concentration of 10% (w/w). The polymer solutions with CHX were dissolved at 70 °C under stirring until complete dissolution.

2.2. Electrospinning

Electrospinning was performed by the method described previously [18]. Briefly, the polymer solution was placed in a glass syringe (10 mL) fitted with a steel needle with a diameter of 0.5 mm. Electrospinning was conducted in a home-made controlled temperature chamber at 40 °C, a relative humidity between 45–55% and applying a voltage of 20 kV with a power supply from *Glassman* (model PS/FC30P04). A syringe pump (from *Syringepump*) was used to feed the polymer solutions into the needle tip at a rate of 10 $mL.h^{-1}$. The as-spun randomly oriented fibers were collected on a grounded collecting plate and stored at room temperature.

2.3. Electrospun fiber membrane characterization

Electrospun fiber membranes were coated with a thin gold layer using a sputter coater (*Polaron,* model SC502), and their morphology was analyzed using scanning electron microscopy (SEM) (Quanta 650 from FEI) with an accelerating voltage of 10 kV. The fiber average diameter and their size distribution was calculated over approximately 40 fibers using SEM images at 5000X magnification and Image J software. Contact angle measurements (sessile drop in dynamic mode) were performed at room temperature in a Data Physics OCA20 device using ultrapure water as test liquid. The contact angles were measured by depositing water drops (3 μL) on the sample surface and analyzed with SCA20 software. At least 6 measurements on each sample were performed in different membrane locations, and the average contact angle was taken as the result for each sample.

Infrared measurements (FTIR) were performed at room temperature with an ABB FTLA 2000 apparatus in transmission mode from 4000 to 500 cm^{-1}. FTIR spectra were collected after 10 scans with a resolution of 4 cm^{-1}. The measurements were performed with dry potassium bromide pellets (KBr).

The mechanical properties of the electrospun fiber membranes (dimensions of 40 mm × 5 mm × 40 μm) were characterized by stress-strain experiments in tensile mode with a Linkam TST 350 universal testing machine. The