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Biocompatible electrospun nanofibers containing cloxacillin: Antibacterial activity and effect of pH on the release profile

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ABSTRACT

The effectiveness of antibiotics toward resistant strains of bacteria has brought serious concerns related to human and animal health. Controlled drug release systems, especially those based on polymer and polymer-based nanostructures appears as a remarkable approach, once they can potentially improve the therapeutic outcomes toward bacterial infections, while requiring lower amounts of drugs. The current study was designed to investigate the incorporation and release profile of a drug loaded in biodegradable electrospun nanofibrous membranes, based on the drug-polymer interactions, as well as its ability to inhibit bacterial growth. For that purpose, nanofibrous membranes of Ecovio® (EC), a polymer blend composed by poly (lactic acid) (PLA)/poly (butylene adipate-co-terephthalate) (PBAT), loaded with different cloxacillin (CLOX) contents were successfully produced *via* electrospinning technique. Electrospun nanofibers of EC unloaded and loaded with drug presented smooth surface with a mean diameter close to 600 nm. The physical-chemical characterizations by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) confirmed the successful drug encapsulation achieved by electrospinning technique. *In vitro* studies revealed that the developed drug-loaded nanofibrous membrane was successful in inhibiting *S. aureus* growth. The cumulative release of drug from EC nanofibrous membranes containing 20% of CLOX was demonstrated to be pH dependent, where the antibiotic release rate was much faster for pH 7.3 than that for pH 5.5. In this way, the mechanism involved in the release could be either Fickian or non-Fickian depending on the pH environment. The simple and efficient strategy presented here to develop antimicrobial nanofibrous membrane make them promising for drug delivery carrier and wound dressing applications.

1. Introduction

The decrease of effectiveness of antibiotics in treating infectious diseases has accelerated in recent years, bringing serious concerns to human and animal health professionals [1,2]. The extensive and inadequate usage of antibiotic for treating human and agricultural livestock have contributed to develop resistant strains of bacteria, forcing a shift to more expensive and more broad-spectrum antibiotics [3]. However, the development of new antibiotics have not been enough to deal with this issue, and consequently, nowadays much of the research efforts are focusing on the strategies to improve the therapeutic outcomes using the available antimicrobials [4,5]. In addition, the precise delivery of antimicrobials can contribute to enhance local drug concentrations, which is capable of decreasing the emergence of bacterial resistance, reducing dose-related systemic toxicity and side effects [6,7]. In this sense, the modulation of antibiotic release, *e.g.* an initial fast release of an antibiotic and subsequently a more sustained and prolonged release of other antibiotics, would be highly desired [8].

Owing to the impressive progress in materials science, a broad range of nanostructured drug carriers with diverse sizes, architectures and surface properties have been explored for delivery applications of multiple drugs, including micelles, liposomes, nanoparticles, and nanofibers [4,5,9–12]. Among these nanocarriers, electrospun nanofibers have been applied for drug release purposes due to their advantages, including: (i) cost-effectiveness and ease of processing, (ii) high drug loading and encapsulation efficiency, and (iii) ability to modulate the

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drug release [6,13–15]. The resulting nonwoven fibrous structure along with the versatility of polymer selections and polymer blend combinations make electrospun fibers an ideal platform for the release of different drugs [16,17]. This approach also enables the fabrication of nanofibers loaded with drugs simply by adding those agents directly into the polymer solutions prior to electrospinning [18–20].

Herein, we report the design and fabrication of electrospun nanofibrous membranes based on a biodegradable polymer for the efficient release of cloxacillin benzathine (CLOX). CLOX, a semi-synthetic penicillin and β-lactam antibiotic, was chosen as a model drug because of its activity against most common infections (*e.g.* mastistis) caused by Gram-positive bacteria such as *S. aureus* [21]. We investigated the feasibility of encapsulating CLOX into Ecovio® nanofibers (ECNF) *via* electrospinning, once studies on the drug release behavior using nanofibers of EC, a biocompatible and biodegradable polymer with good mechanical properties [22,23], are rarely available in the literature. The formed EC nanofibers loaded with different concentrations of CLOX were characterized using different physical-chemical techniques. Afterward, we studied the *in vitro* release profile of CLOX from the nanofibrous membranes and examined the antibacterial effects of these membranes against *S. aureus* by using growth inhibition zone test, as depicted in Scheme 1. We demonstrate that it is possible to obtain versatile drug release profiles, which can be tailored for specific applications by choosing, for example, the pH environment.

2. Experimental

2.1. Materials

Ecovio® (F2224), a commercial blend composed by 55 wt% Poly (butylene adipate-co-terephtalate (PBAT, containing 55% of aromatic segments) and 45 wt% Poly(acid lactic) (PLA), was obtained from BASF.

Chloroform (≥99.8%), *N*,*N*-dimethylformamide (≥ 99.8%) (DMF) and NaH_2PO_4 anhydrous salt were purchased from Synth (Brazil). Na_2HPO_4 anhydrous salt was provided from Cromoline (Brazil). Methanol solvent (≥ 99.0%) was purchased from Vetec (Brazil). Cloxacillin benzhatine (CLOX) antibiotic were obtained from Hebei Huari Pharmaceuticals Corporation as a solid white powder (HPLC grade, 97,6%). Acetonitrile (HPLC grade, 99.9%) and formic acid (HPLC grade, 50% in water) were purchased from Sigma-Aldrich. For microbiological essays, Agar and Mueller-Hinton Broth were obtained from Kasvi and Himedia, respectively.

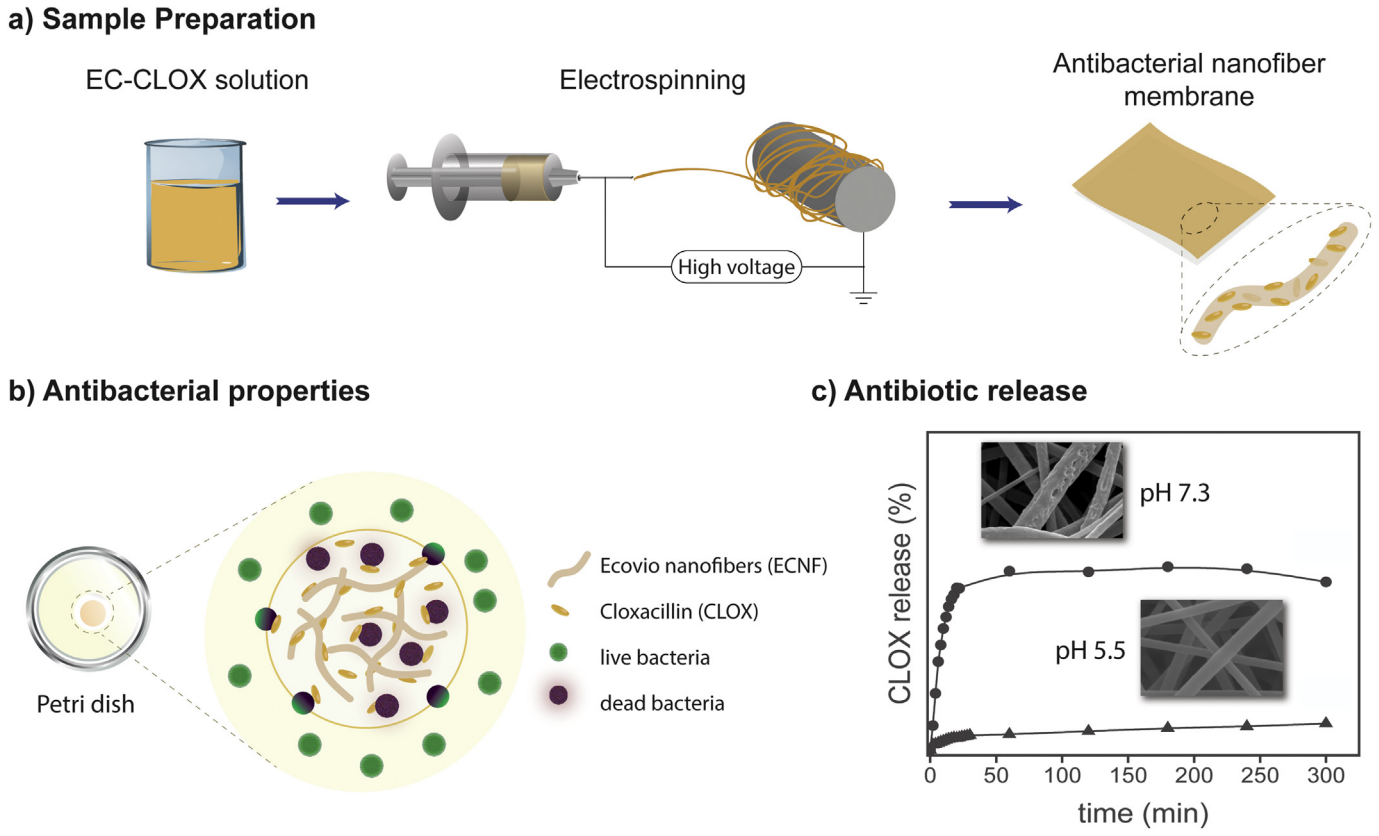
Table 1
Sample composition for electrospinning nanofibers of EC containing CLOX.

Sample	EC concentration (% *w/v* in respect to the solvent)	CLOX concentration (% *w/w* in respect to the polymer)
ECNF	10	0
ECNF-CLOX10	10	10
ECNF-CLOX20	10	20

EC = Ecovio® F2224 = blend of Poly(acid lactic)/Poly(butylene adipate-co-terephtalate (PLA/PBAT); CLOX = Cloxacillin benzhatine.

2.2. Preparation of polymeric/drug solution and electrospinning parameters

Polymer solution was prepared by dissolving EC pellets (10% *w/v*) in a methanol/DMF/chloroform solvent mixture (5/10/85, % *v/v/v*). For fabricating the antibiotic-loaded nanofibrous membranes, initially CLOX (10% and 20% *w/w*, in respect to the mass of polymer) was dissolved in methanol/DMF and sonicated for 20s. Then, chloroform and EC (10% *w/v*) were added to CLOX solution and stirred for 2 h,



Scheme 1. Schematic representations of a) preparation of CLOX encapsulated EC nanofibrous membranes, b) antibacterial properties of ECNF-CLOX nanofibers and c) CLOX release profiles at different pHs.