European Journal of Pharmaceutical Sciences 122 (2018) 347–358

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Impact of PCL nanofiber mat structural properties on hydrophilic drug release and antibacterial activity on periodontal pathogens

Špela Zupančič[a], Liis Preem[b], Julijana Kristl[a], Marta Putriņš[c], Tanel Tenson[c], Petra Kocbek[a,*], Karin Kogermann[b,*]

[a] Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta. 7, 1000 Ljubljana, Slovenia
[b] Institute of Pharmacy, Faculty of Medicine, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[c] Institute of Technology, Faculty of Science and Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

ARTICLE INFO

Keywords:
Nanofibers
Hydrophilic drug
Drug release mechanism
Antibacterial activity
Periodontopathogenic bacteria
Wettability

ABSTRACT

Electrospinning enables to design and manufacture novel drug delivery systems capable of advancing the local antibacterial therapy. In this study, two hydrophilic drugs – metronidazole and ciprofloxacin hydrochloride – were loaded both individually and in combination into hydrophobic poly(ε-caprolactone) (PCL) matrix using electrospinning. We aimed to develop prolonged release drug delivery systems suitable for the treatment of periodontal diseases and understand how different rarely studied structural features, such as nanofiber mat thickness, surface area, wettability, together with intrinsic properties, like solid state and localization of incorporated drugs in nanofibers, affect the drug release. Furthermore, the safety of nanofiber mats was assessed *in vitro* on fibroblasts, and their antibacterial activity was tested on selected strains of periodontopathogenic bacteria. The results showed that the structural properties of nanofiber mat are crucial in particular drug-polymer combinations, affecting the drug release and consequently the antibacterial activity. The hydrophobicity of a PCL nanofiber mat and its thickness are the key characteristics in prolonged hydrophilic drug release, but only when wetting is the rate-limiting step for the drug release. Combination of drugs showed beneficial effects by inhibiting the growth of all tested pathogenic bacterial strains important in periodontal diseases.

1. Introduction

Periodontal diseases are classified as inflammatory diseases that cause several abnormalities affecting the periodontium, including bleeding, pain, formation of periodontal pockets, detachment and bone loss, tooth mobility and loss due to the increased number of periodontopathogenic bacteria in the biofilm along with an unbalanced immune response (Hajishengallis, 2014). Periodontal pockets provide an excellent location for drug delivery, and thus antibiotics can be precisely delivered to the infected site. Local antibacterial treatment has several advantages over systemic administration, such as allowing higher antibiotic levels while avoiding systemic toxicity, prolonged retention of antibiotic at the target site with the consequent decreased possibility of emergence of bacterial resistance, and enhanced patient compliance (Palmeira-de-Oliveira et al., 2015; Ulubayram et al., 2015; Zupančič et al., 2015b). Thus, patients with periodontal diseases should benefit from local antibacterial therapy.

Electrospun antibiotic-loaded nanofibrous mats are promising, widely applicable, novel delivery systems in the local treatment of infectious diseases, including periodontal diseases (Pelipenko et al., 2015). They need certain specific characteristics to ensure efficient and prolonged drug delivery. Drug release should be controlled so that the local concentration of antibiotic can be kept above the minimum inhibitory concentration (MIC) for at least one week to achieve good antibacterial efficacy and prevent the development of antibiotic resistance (Zupančič et al., 2015b). The delivery system needs to be biocompatible, with biodegradability and bioadhesiveness also being desired. Since drug release from electrospun hydrophilic polymer mats is usually immediate, hydrophobic polymer mats are preferred. One of the most extensively studied synthetic hydrophobic biodegradable polymers for nanofiber preparation is poly(ε-caprolactone) (PCL). It is a polymer approved by the Food and Drug Administration (FDA), is chemically stable, biocompatible, biodegradable, affordable, and can be easily electrospun (Dash and Konkimalla, 2012; Pelipenko et al., 2015).

* Corresponding authors.
E-mail addresses: Spela.Zupancic@ffa.uni-lj.si (Š. Zupančič), liis.preem@ut.ee (L. Preem), Julijana.Kristl@ffa.uni-lj.si (J. Kristl), marta.putrins@ut.ee (M. Putriņš), tanel.tenson@ut.ee (T. Tenson), petra.kocbek@ffa.uni-lj.si (P. Kocbek), karin.kogermann@ut.ee (K. Kogermann).

https://doi.org/10.1016/j.ejps.2018.07.024
Received 5 February 2018; Received in revised form 9 July 2018; Accepted 10 July 2018
Available online 12 July 2018
0928-0987/

Although PCL has many characteristics favoring the preparation of dosage forms of controlled drug release, incorporation of hydrophilic drugs into its matrix can be challenging (Dash and Konkimalla, 2012). Even though some studies have reported prolonged drug release over 24 h (Potrč et al., 2015; Sultanova et al., 2016), a few days (Amna et al., 2013; Srikar et al., 2008), one week (Karuppuswamy et al., 2015; Xue et al., 2014; Zupančič et al., 2016), two weeks or even longer (Zamani et al., 2010), some reports are about fast drug release lasting from only few minutes (Siafaka et al., 2016) to few hours (Potrč et al., 2015; Zupančič et al., 2015a). Several explanations for such drug release behavior have been proposed. Release can depend on the nature of the incorporated drug (Potrč et al., 2015), the polymer concentration in the electrospinning solution, polymer molecular weight (Srikar et al., 2008), drug-polymer interactions (e.g. hydrogen bonding), intermolecular forces binding drug to the polymer surface in the presence of water (hydrophobic interactions) (Preem et al., 2017; Srikar et al., 2008), drug loading (Karuppuswamy et al., 2015; Potrč et al., 2015; Xue et al., 2014; Zamani et al., 2010), nanofiber morphology (Zupančič et al., 2015a), and medium used for the preparation of polymer solution (Zamani et al., 2010). Not to mention, that the drug release experimental conditions (e.g. temperature, agitation etc.) may affect the drug release from electrospun nanofibers. Different mechanisms of drug release from electrospun nanofiber mats have been suggested, such as drug diffusion (Karuppuswamy et al., 2015; Sultanova et al., 2016), erosion (Karuppuswamy et al., 2015), and drug desorption from polymer matrix (Srikar et al., 2008). These mechanisms depend mostly on the intrinsic material and nanofiber properties, whereas the effect of the structural properties of nanofiber mat on drug release has scarcely been explored. One such extrinsic structural feature is nanofiber mat porosity, which due to air capture in pores between the nanofibers decreases nanofiber mat wettability compared to polymer films. As both intrinsic and extrinsic properties of the nanofiber mats govern their biopharmaceutical behavior, understanding the link between these properties, drug release and antibacterial activity is crucial to achieve successful therapeutic outcomes. For example, the importance of PCL nanofiber hydrophobicity has already been shown in the case of tissue engineering in which high hydrophobicity decreases cell adhesion and proliferation (Ghasemi-Mobarakeh et al., 2008; Ku and Park, 2010) and degradation of polymer matrix in aqueous media (Cui et al., 2008).

Our aim was first to develop PCL nanofiber mats loaded with two different hydrophilic antibacterial drugs, metronidazole (MTZ), which is well known in the current protocols for the treatment of periodontal disease, and ciprofloxacin (CIP), with proven antibacterial activity against periodontopathogenic bacteria. Second, the effects of the rarely studied structural features, such as nanofiber mat thickness, surface area, and wettability, as well as intrinsic properties, including solid state and localization of incorporated drugs in the nanofibers on drug release, were systematically examined to find critical parameters that can distinguish and explain the drug release from nanofiber mats. Finally, safety of the nanofiber mats was assessed *in vitro* on fibroblasts, and their antibacterial activity was tested on selected strains of periodontopathogenic bacteria *in vitro*.

2. Experimental section

2.1. Materials

Acetic acid (100%) and formic acid (98–100%) were from J. T. Baker, Germany. PCL (M_w = 80 kDa) and MTZ were purchased from Sigma Aldrich, USA. CIP HCl was obtained from Alfa Aesar, Germany. Potassium dihydrogen phosphate, sodium hydroxide, methanol, and acetonitrile were purchased from Merck, USA. Triton X-100 was purchased from BioTop, Austria. All chemicals were used as received without any further purification or modification.

2.2. Nanofiber mat preparation

A solvent mixture of acetic and formic acid in a ratio 3:1 (w/w) was used for preparing all 15% (w/w) PCL solutions, since this mixture enabled dissolution of hydrophobic polymer and hydrophilic drugs used in this study. An hour before electrospinning the drugs or a combination of both, in a weight ratio of 1:1 was added to achieve the theoretical 5% (w/w) drug loading in the electrospun mat. The polymer solutions had been stirred at room temperature and electrospun right after all the components had been fully dissolved to minimize undesirable PCL hydrolysis (Gil-Castell et al., 2017). Nanofibers were produced using vertical electrospinning technology with a Fluidnatek LE-100 apparatus (Bioinicia, Spain) with a monoaxial nozzle at 21 ± 2 °C and the relative humidity of 35 ± 2%. Positive high voltage (16–17 kV) was applied to the nozzle and the collector was negatively charged with −5 kV. Nanofibers were electrospun from a polymer solution at a flow rate of 1 mL/h. To obtain a nanofiber mat of homogenous thickness, a rotating drum of 9.55 cm diameter was used as a collector, with a rotation speed of 150 rpm. The distance between the nozzle and the collector was 15 cm. The nozzle was simultaneously moving perpendicular to the direction of the drum rotation, at a speed of 6.0 mm/s and in the range of 3 cm. The process was run for different times to produce nanofiber mats of different thicknesses.

2.3. Preparation of physical mixtures

To understand the solid state of a drug within nanofibers, physical mixtures of PCL and drug powder were prepared as reference materials. PCL powder was made by mechanical crushing of PCL filaments (prepared using melt electrospinning set-up in a Nano NC electrospinning robot, South Korea, at 200 °C, a relative humidity (RH) of 16%, a voltage of 4 kV, a roller speed of 30 rpm, a flow rate of 2 mL/h) together with liquid nitrogen in a mortar and pestle. We fixed the powder particle size with a sieve (mesh size 500 μm). The amount of MTZ, CIP HCl or a combination of them was the same as described in Section 2.2.

2.4. Physical characterization

2.4.1. Conductivity of the drug solutions

The conductivity of drug solutions, with the same solvent composition and drug content as used for electrospinning, was measured by a MC226 Conductivity Meter and electrode Inlab 741 (Mettler Toledo, Switzerland).

2.4.2. Nanofiber and nanofiber mat morphology and topography

As described elsewhere (Pelipenko et al., 2013), the nanofiber mats were fixed onto the metallic studs with double-sided conductive tape and examined with a field emission scanning electron microscope (SEM, Supra 35 VP, Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 1 kV, with a secondary detector. SEM equipment allowed a high-resolution imaging of the non-coated samples at low accelerating voltage with negligible electrical surface charging. Therefore, the samples examined were used in their native state without any possible artefacts due to coating.

2.4.3. Determination of thickness, density, and porosity of nanofiber mats

Nanofiber mats were cut at 3 random locations and pasted to the studs at an angle of 90°. The thickness of the cross-sections was determined by stereomicroscopy (Olympus SZX12, Japan). A nanofiber mat of 2.6 cm^2 surface area was weighed and its density, $\rho_{NF\ mat}$, was calculated by dividing the weight by the volume. Porosity, ε, was calculated by using Eq. (1), where PCL bulk density, (ρ_{PCL} = 1.145 mg/mm^3) was given by the supplier.

$$\varepsilon(\%) = 100 - \frac{\rho_{NF\ mat}}{\rho_{PCL}} \times 100 \quad (1)$$