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Protein release from electrospun nonwovens: Improving the release characteristics through rational combination of polyester blend matrices with polidocanol

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ABSTRACT

Nonwoven scaffolds consisting of poly-ε-caprolactone (PCL), poly(lactic-*co*-glycolic acid) (PLGA) and polidocanol (PD), and loaded with lysozyme crystals were prepared by electrospinning. The composition of the matrix was varied and the effect of PD content in binary mixtures, and of PD and PLGA content in ternary mixtures regarding processability, fiber morphology, water sorption, swelling and drug release was investigated. Binary PCL/PD blend nonwovens showed a PD-dependent increase in swelling of up to 30% and of lysozyme burst release of up to 45% associated with changes of the fiber morphology. Furthermore, addition of free PD to the release medium resulted in a significant increase of lysozyme burst release from pure PCL nonwovens from approximately 2–35%. Using ternary PCL/PD/PLGA blends, matrix degradation could be significantly improved over PCL/PD blends, resulting in a biphasic release of lysozyme with constant release over 9 weeks, followed by constant release with a reduced rate over additional 4 weeks. Based on these results, protein release from PCL scaffolds is improved by blending with PD due to improved lysozyme desorption from the polymer surface and PD-dependent matrix swelling.

1. Introduction

Nonwovens prepared by electrospinning are extremely versatile scaffolds allowing the controlled and localized delivery of various drugs in a broad variety of applications ranging from tissue engineering to topical gene delivery. The large surface area of electrospun nonwovens, their versatility with regards to attainable structures as well as the large number of biocompatible polymers suitable for elecrospinning such as poly-ε-caprolactone (PCL), poly (lactic-*co*-glycolic acid) (PLGA), poly(ethylene glycol) (PEG) or poly (L-lysine) (PLL) constitute the exceptional suitability of nonwoven scaffolds for drug delivery (Meinel et al., 2012; Wendorff et al., 2012). PCL is frequently used as a polymer matrix for various drug delivery applications, including the production of electrospun nonwovens, because of its biodegradability, biocompatibility, advantageous material characteristics such as low glass transition- and melting temperature, and broad solvent compatibility as well as its excellent mechanical properties (Cipitria et al., 2011; Dash and Konkimalla, 2012; Qin and Wu, 2012). Moreover, PCL has been used in several FDA approved drug delivery systems and implants, as suture material and adhesion barrier (Gunatillake and Adhikari, 2003). Despite these advantages, release of hydrophilic, high molecular weight drugs was frequently found to be challenging because of PCLs semicrystalline nature, slow degradation and high hydrophobicity (Chen et al., 2000; Wang et al., 2009), requiring copolymerization or blending with hydrophilic polymers or polymers possessing a higher degradation rate such as PLGA (Anderson and Shive, 1997; Briggs and Arinzeh, 2014; Liu et al., 2008; Puhl et al., 2014).

In previous studies we observed that the combination of PCL nonwovens and protein crystals allows control of both, amount of burst release as well as long-term release rate through variation of protein crystal–fiber size ratio, degree of loading and polymer matrix composition (Puhl et al., 2014). However, despite the

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significant variability achieved through the novel combination of electrospun fibers and protein crystals, the low amount of total released protein and limited control over release rate required further optimization. Furthermore, blending of PCL with PEG and PLGA alone, while in part successful, still resulted in slow and incomplete protein release.

Within this study we explore the mode of protein release from pure and blended PCL matrices containing protein crystals. We hypothesize that besides polymer degradation and drug diffusion, wettability of the nonwoven, swelling of the polymer matrix and desorption play an important role in protein release from nonwoven scaffolds. Therefore, we studied the effect of matrix composition on protein release using electrospun nonwovens consisting of a novel blend of PCL, polidocanol (PD) and PLGA. PD is used in pharmaceutical and cosmetic formulations as an O/W emulsifier and as an active pharmaceutical ingredient for topical therapy, as local anesthetic and antipruritic drug at concentrations between 3 and 8% m/m, and as sclerosant after injection into varicose veins at concentrations between 0.25 and 3% m/m. Because of its surface activity and well-established topical application, PD may represent a valuable excipient to improve the material characteristics of PCL nonwovens.

2. Materials and methods

PCL (Mw 70,000–90,000), chicken egg white lysozyme and trifluoroacetic acid (HPLC grade) were purchased from Sigma–Aldrich (Munich, Germany). Polidocanol (Macrogoli aether lauri-ilicum 9, Ph.Eur. 6.0) was bought from Fagron (Barsbüttel, Germany). Poly(D,L-lactide-*co*-glycolide) 50:50 (Resomer RG 502H, Mw 7000–17,000) was a kind gift from Evonik Industries (Essen, Germany). Acetonitrile (gradient grade) was purchased from VWR Prolabo (Fontenay-sous-Bois, France). Poly(ethylene glycol) 6000 was obtained from MERCK Schuchardt (Hohenbrunn, Germany). Chloroform, ethanol, sodium phosphate, sodium hydroxide, sodium chloride, sodium azide and acetic acid were of analytical grade.

2.1. Lysozyme crystallization and crystal size determination

Lysozyme crystallization was performed according to the method of Falkner et al. (2005) with modifications as described before (Puhl et al., 2014). Lysozyme was dissolved in 100 mM sodium acetate buffer pH 3.5 at a concentration of 8.0 mg/ml. Precipitation buffer consisting of 20% sodium chloride, 10% PEG 6000 and 500 mM sodium acetate at pH 3.5 at 8 °C was poured quickly into the lysozyme solution and stirred at 500 rpm. Mixing ratio of precipitation buffer to lysozyme solution was 2:1. After centrifugation at 3500 × *g* for 5 min to separate crystals, they were washed twice with ethanol/chloroform (volume ratio 3:10). Finally, crystals were dried over night under mild vacuum. Average particle size of crystals was measured by laser diffraction analysis using ethanol as dispersion medium (LS 230, Beckman Coulter, Brea, CA).

2.2. PCL electrospinning

Electrospinning was performed using a custom-built apparatus consisting of a DC power supply HCP 140-350000 (FuG Elektronik, Rosenheim, Germany) and a syringe pump (Type 540200, TSE Systems, Bad Homburg, Germany). Nonwovens were produced by electrospinning onto a stationary copper plate using a similar setup as described before (Pham et al., 2006; Puhl et al., 2014). Protein crystals were dispersed in ethanol at a concentration of 3.0 mg/ml and homogenized for 20 s using an ultrasonic bath (Branson 3200, Bransonic, Danbury, CT). Subsequently, chloroform and the particular polymers were added. Ethanol and chloroform were mixed in a volume ratio of 1:6. Ratios of polidocanol and PLGA were calculated as dry mass in relationship to PCL (m/m%) and compositions are summarized in Table 1. No sedimentation, dissolution or disintegration of the protein crystals was observed during processing in polymer solution. The resulting suspensions were filled into a syringe with an attached gauge 22 metal needle. The needle was centered within a copper ring of 20 cm diameter consisting of copper wire of 2 mm diameter placed in a vertical plane around it and both were connected to the DC power supply and charge was adjusted to 27 kV. The flow rate was set to 10 ml/h and the stationary copper plate was placed at a distance of 40 cm from the needle. The lysozyme crystal loading of the nonwovens was calculated individually. Average loading was 0.15 ± 0.04% m/m. All data is depicted as results from triplicated experiments and represents the average ± standard deviation (SD).

2.3. Morphology and fiber diameter

SEM images were recorded using a JSM-7500F field emission scanning electron microscope (Jeol, Tokyo, Japan) with an acceleration voltage of 2 kV. ImageJ (NIH, Bethesda, MD) was used to determine the fiber diameter. Sixty individual fiber diameters were manually measured vertically to the fiber surface for every preparation. Samples were cut out of the incubated nonwovens and washed with purified water, dried under mild vacuum overnight and studied by SEM using fresh nonwovens as reference.

2.4. Wettability and sorption rate of the nonwovens

Contact angle measurements were performed using casted films as substitutes for electrospun nonwovens using a drop shape analysis (DSA 10, Krüss GmbH, Hamburg, Germany). Films were used instead of nonwovens to avoid bias by variable capillarity and uneven surfaces, respectively. Polymer blends were coated on a glass microscope slide and allowed to dry under ambient conditions. A drop of release medium was placed on the polymer films and contact angle was measured over time.

Sorption of PBS release medium into capillaries of the nonwovens was determined similar as described before (Puhl et al., 2014). According to the Washburn theory the sorption rate is correlated to contact angle and wettability of a nonwoven (Washburn, 1921).

$$t = A \times m^2 \tag{1}$$

$$A = \frac{\eta}{c \times \rho^2 \times \sigma \times \cos\theta} \tag{2}$$

$$\frac{m^2}{t} = \frac{c \times \rho^2 \times \sigma \times \cos\theta}{\eta} \tag{3}$$

where m is the sorbed mass of water, η is the viscosity of the immersion liquid, c is the capillarity of the sample, ρ is the density of the immersion liquid, σ is the surface tension of the immersion

Table 1
Compositions of the electrospun nonwovens. All ratios are expressed as % m/m.

Name	PCL (%)	PD (%)	PLGA (%)
PCL	100	–	–
PCL/PD2	98	2	–
PCL/PD10	90	10	–
PCL/PD20	80	20	–
PCL/PD50	50	50	–
PCL/PD20/PLGA2	78	20	2
PCL/PD20/PLGA10	70	20	10