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Antibiotic incorporation in jet-sprayed nanofibrillar biodegradable scaffolds for wound healing

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ABSTRACT

In view of preparing antibiotic-loaded structures that can be used as dressing to prevent or contain wound infections, this study evaluates biodegradable nanofibrillar matrices obtained by jet-spraying and containing ciprofloxacin (CIF). The matrices were prepared from different blends of poly-(ε-caprolactone) (PCL) and poly-D,L-(lactic acid) (PDLLA) in view of controlling mechanical properties, biodegradation and antibiotic release rate. The effect of CIF incorporation was assessed in regard of matrices fiber diameter, mechanical properties and degradation while antibiotic release from the polymer blends of different PCL/PDLLA ratios was measured in buffers of different pH to better mimic the wound context. Finally, antibiotic activity of the nanofibrillar matrices and their ability to be colonized by skin cells were evaluated.

Non-woven nanofibrillar matrices could be obtained from various polymer blends by jet-spraying and CIF crystals incorporation was easily obtained. The crystals were dispersed in the fibers, without complete embedding. Antibiotic incorporation resulted in a slight increase of fiber diameter and did not modified the mechanical properties of the various matrices composed of different polymer blends. Unlike fiber diameter, degradation and mechanical properties of the fibrillar matrices, CIF release profiles were not controlled by the polymer blend ratios. However, sustained release was observed over more than 23 days. Due to the antibiotic pH-dependent solubility, burst release was more prominent in acidic conditions, which mimic the pH of undamaged skin. Finally the incorporated antibiotic was efficient in inhibiting bacterial growth of *E. coli* and *B. subtilis* whereas human fibroblasts were able to colonize the CIF-loaded matrices.

1. Introduction

Wound healing and tissue regeneration is an interplaying multifactorial mechanism spanning over different phases (Stroncek and Reichert, 2008). After skin injury, haemostasis preventing blood loss is swiftly followed by the inflammation phase that allows tissue repair by cleansing of the lesion, fibroblasts proliferation and angiogenesis. The ensuing skin repair phase is characterized by the formation of a granulation tissue supporting the skin barrier restoration, the narrowing of wound margins and the synthesis of unorganised extracellular matrix (ECM). Finally, unnecessary cells are eliminated, leaving an unorganised dermal ECM, to be remodelled over months.

Bacterial infection after skin injury is the main failure cause of wound repair (D'Avignon et al., 2011; Murray et al., 2008). Indeed, the damaged tissue is very rapidly colonized by the resident microbial flora or by exogenous agents carrying bacteria. On a wider perspective, wound infection is considered responsible for most of post-surgery complications. Many products have been developed to counteract or prevent bacterial infections, such as Cutimed® Sorbact® that provides an antimicrobial treatment to colonized and infected wounds or Urgosorb® silver and Acticoat 7® that release silver ions to create a barrier effect against infection. Nevertheless, those dressing are designed to be renewed regularly and cannot be used as scaffolds supporting tissue formation. Moreover the use of silver in dressing have been reported to stain the healing area and the surrounding skin (Walker et al., 2006; Zweiker et al., 2014).

In consequences, researchers have tried to get antibiotic-releasing matrices to prevent or counteract bacterial infections in wounds. A wide range of antibiotics were used, such as aminoglycosides (Zilberman et al., 2015; Egozi et al., 2015), penicillins (Yao and Webster, 2009), fluoroquiniolones (Toncheva et al., 2012) or tetracyclines (Hong et al., 2008). The considered antibiotic should have a wide spectrum and

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target skin infection bacteria. Complying with these requirements, fluoroquinolones such as ciprofloxacin (CIF) have demonstrated a strong efficiency on a wide range of bacteria. In addition, CIF solubility is pH dependent with slow dissolution at neutral pH and high dissolution at acidic pH. This specificity strengthens its potential for application in skin wound and on adjacent intact skin, as skin environment pH is close to 5.5 (Ali et al., 2016).

As a support for antibiotic delivery, nanofibers were widely considered over the last decade to prevent and decrease bacterial infection risks after skin injury and wound (Egozi et al., 2015; Toncheva et al., 2012; Alhusein et al., 2016). In this context, electrospinning was increasingly evaluated to produce antibiotic-loaded nanofibers, owing to easily controlled production parameters (temperature, voltage …) (Hu et al., 2014; Cho et al., 2015; Tang et al., 2016) and resulting adjustable fibers diameter, narrow dispersion and good reproducibility. Moreover, electrospun matrices have demonstrated a strong potential in drug incorporation, ranging from antibiotics and anticancer to nucleic acids (DNA, RNA) and growth factors (Alhusein et al., 2016; Hu et al., 2014). However, major drawbacks of this method remain, such as low nanofiber production rate (1 mL/h) and low porosity altering the final functionality of the electrospun matrices (Eichhorn and Sampson, 2005), the latter being directly linked with limited cell adhesion and weak cell colonization (Hamrang, 2014). To overcome these shortcomings, an alternative approach has recently emerged based on the spraying of a polymer solution by an air flow, leading to the formation of polymer nanofibers while solvent is evaporating (Keloglu et al., 2016; Sohier et al., 2014a; Sohier and Biomimetic, 2010). Jet-spraying presents various advantages such as high productivity rate (100 mL/h), production parameters adjustments and high porosity (up to 96%) (Sohier et al., 2014a) of the resulting nanofibrillar matrices. As a result, jet-sprayed matrices can be readily colonized by cells (Sohier et al., 2014a, 2014b; Brennan et al., 2015) and could therefore potentially be used not only as dressing but as well as a scaffold to enhance wound healing. Indeed, such 3D and highly porous structures (> 90% porosity) supports cells colonization, tissue ingrowth by production of extracellular matrix proteins, diffusion of nutrients, oxygen and wastes and consequently provides a dermal equivalent for an efficient epithelial barrier reconstruction (Garric et al., 2012; Lawrence and Madihally, 2008). However, contrarily to electrospinning that has exhibited a wide versatility using numerous polymers, nanofibrillar matrices obtained by jet-spraying have so far only been reported with a limited number of polymers (Keloglu et al., 2016; Brennan et al., 2015), and never with incorporated compounds.

A known solution to obtain drug release from nanofibers consist in blending different types of polymers to modify drug diffusion or polymer degradation (Santoro et al., 2016; Pawar et al., 2015). Those blends consist in non-biodegradable (polyolefins, polyacrylate) or biodegradable polymers (natural or synthetic) with a strong predominance for synthetic biodegradable polyesters, which present the advantages of ease of production, purity, and adjustable biodegradation. The most studied are poly-(lactic-co-glycolic acid) (PLGA), poly-(lactic acid) (PLA) and poly-(ε-caprolactone) (PCL) due to their biocompatibility and relative biosafety during degradation (Jeong et al., 2004). Moreover, polyesters can be solubilized into chloroform contrarily to natural polymer such as collagen or chitosan and are consequently suitable for jet-spraying.

Lactic acid based polymers, with a relatively fast degradation rate, seem to be beneficial in the wound healing context through delivery of lactic acid during degradation (Porporato et al., 2012). A recent study highlighted this benefit through an increasing neovascularization and wound healing linked to lactate released during polymer degradation (Chereddy et al., 2013; Trabold et al., 2016). Furthermore, the presence of lactic acid in the wound allows a quick restoration of the normal acidic skin environment which enhances fibroblast proliferation, inhibits microbial proliferation and increases cells oxygenation (Schneider et al., 2007). However, PLGA does not appear suitable to produce scaffolds that allow cell support and could be integrated into the wound, due to its fast degradation rate swiftly impairing the polymer structure, as described by Lu et al. (2000). Indeed, *in vitro* and *in vivo* degradation of PLGA foams demonstrated a half-life of 3 months for PLGA of 85/15 (%lactic group/%glycolic group) and less than one month for PLGA 50/50. In case of submicronic PLGA 50/50 fibers *in vitro* degradation, You et al. (2005) demonstrated fibers merging from 8 days followed by a total merging in 20 days whereas PLA kept a fibrillar structure up to 45 days. PCL, on the contrary, present a slow biodegradability of about 3 years (Abedalwafa et al., 2013), but is associated with good mechanical strength (if chosen with a higher molecular weight than PLA) and elasticity of higher value than PLA, that could be of value for handling of the resulting matrices.

Therefore, we proposed to blend low molecular weight poly-D,L-(lactic acid) (PDLLA) to PCL in view of controlling biodegradation during tissue regeneration while insuring (i) nanofibrillar morphology which can be used as guide for cell colonization and (ii) proper mechanical properties and sufficient mechanical strength to withstand stresses for its manipulation.

The objective of this study was therefore to design and evaluate jet-sprayed biodegradable matrices associated with an antibiotic, with a view to improve the treatment and prevention of wound infections. Unlike common wound dressing, the produced matrices could remain in the wound to support cell colonization and tissue formation.

First, CIF, PDLLA and PCL were blended and the production of antibiotic-loaded nanofibrillar matrices by jet-spraying was evaluated. An experimental design was performed to associate production parameters to nanofibers morphology and resulting matrices of different blend ratios were evaluated structurally and mechanically. Similarly, matrices ageing was evaluated in aqueous conditions, to determine the effect of PDLLA content on matrices degradation and fragmentation. Antibiotic incorporation and effect on resulting nanofibrillar structure were assessed while *in vitro* release was monitored in pH 7.4 and pH 5.5 buffers to mimic the different environments in and around skin wounds. Antibiotic efficacy of the CIF-loaded matrices was evaluated through antibiograms of both *Bacillus subtilis* (gram positive) and *Escherichia coli* (gram negative). Finally, the ability of the antibiotic-loaded matrices to be colonized by human fibroblasts was evaluated *in vitro*.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic acid) (PDLLA) of Mw = 50,000 g mol^{-1}; PDI = 2.08 was produced by zinc catalyzed polymerization while poly-(ε-caprolactone) (PCL) Capa6800 of Mw = 80,000 g mol^{-1}; PDI = 1.7 was acquired from Perstorp (Malmö, Sweden). Both polymers contained carboxylic end-groups. Phosphate Buffer Saline (PBS) and Lysogeny broth (LB) Lennox agar were purchased from Euromedex (Strasbourg, france), sodium phosphate dibasic anhydrous was ordered from Biobasic inc. (Markham, Canada), and citric acid (99%), CIF (> 98%), 4′,6-diamidino-2-phenylindole (DAPI), paraformaldehyde (PFA), chloroform and Penicillin-streptomycin were purchased from Sigma-Aldrich (Darmstadt, Allemagne). Cellcrown scaffolds (1 cm) were purchased from Scaffdex (Tampere, Finland). DMEM/F-12 GlutaMAX and trypsine-EDTA (0.25%) were bought from Gibco. Tissue-tek OCT was purchased from Sakura Finetek (Torrance, USA). All reagents and solvents were of analytical grade and used as received. *Escherichia coli* W3110 and *Bacillus subtilis* were kindly offered by the 'Bacterial pathogens and protein phosphorylation' research group of the Molecular Microbiology and Structural Biochemistry research unit (MMSB, UMR 5086, Institute of biology and chemistry of proteins, Lyon, France). Normal human dermal fibroblasts (NHDF) were acquired from PromoCell (Heidelberg, Germany).