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In vitro and in silico characterization of fibrous scaffolds comprising alternate colistin sulfate-loaded and heat-treated polyvinyl alcohol nanofibrous sheets

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ABSTRACT

A multilayer mat for dispensing colistin sulfate through a body surface was prepared by electrospinning. The fabricated system comprised various polyvinyl alcohol fibrous layers prepared with or without the active ingredient. One of the electrospun layers contained water-soluble colistin sulfate and the other was prepared from the same polymer type and composition without the active drug and was finally heat-treated. The heat treatment modified the supramolecular structure and conferred the polymer nanofibre with the rate-controlling function. The microstructure of different layers was tracked by positron annihilation lifetime spectroscopy, and detailed morphological analysis of the fibre mats was performed using a scanning electron microscope. The drug-release profiles of various layer arrangements were studied in relation to their antimicrobial activity. The finite element method was applied to overcome the challenge of diffusion-controlled drug release from multilayer polymer scaffolds. The finite element method was first verified using analytical solutions for a simple arrangement (one drug-loaded swellable fibre and one rate-controlling nonswellable fibre) under perfect sink conditions and in a well-stirred finite volume. The effect of alternate layer arrangements on the drug-release profiles was also investigated to plan for controlled topical drug release from fibrous scaffolds. This design is expected to aid in increasing local effectiveness, thus reducing the systemic loading and the consequent side effects of colistin.

1. Introduction

Life-threatening bloodstream infections caused by multiresistant bacteria are increasing worldwide (Peleg and Hooper, 2010). Carbapenems are considered the last-resort agents for the treatment of infections caused by highly antimicrobial-resistant organisms such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii*; and species of Enterobacteriaceae that produce extended-spectrum β-lactamase or plasmid-mediated AmpC β-lactamase. However, the prevalence of carbapenem-resistant gram-negative pathogens has dramatically increased in the last decade (Akova et al., 2012; Dortet et al., 2014). The increasing rates of infections caused by these bacteria have stimulated the clinical usage of an old but reintroduced antibacterial agent, colistin. Colistin is often the only agent against carbapenemase-producing Enterobacteriaceae, MBL- producing *Pseudomonas aeruginosa* or *Acinetobacter baumannii* and multidrug resistant *Stenotrophomonas maltophilia.* Colistin is the only agent that achieves adequate serum levels exceeding the MICs (Minimum Inhibitory Concentrations) of these bacteria (Kwa et al., 2005; Arnold et al., 2011). However, the use of colistin had almost ceased (after the 1970s) owing to a high incidence of nephrotoxicity and neurotoxicity. Newer studies have demonstrated less toxicity and good efficacy (Dhariwal and Tullu, 2013). The disruption of the epidermal barrier in any type of wound provides a fertile environment for microbial growth and becomes the portal of entry for life-threatening infections (Church et al., 2006). Burn injuries, diabetic feet, and

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decubitus wounds are difficult to treat with systemically administered antibiotics because of difficulty in reaching the damaged tissue with compromised blood circulation. This has encouraged the use of topically applied antibiotics.

Research on fibrous drug delivery systems has been attracting increasing attention in the last decade. Electrospinning (Electrospun®) is most commonly used to fabricate different polymer-based fibrous structures. High-speed rotary spinning, which uses centrifugal force for fibre formation, is another well-known technique. The average diameters of the produced fibres are in the range of micrometres. The nano- and microfibres have significant pharmaceutical importance in both drug delivery systems and tissue engineering. Electrospun fibres with a high surface area to volume ratio and structures mimicking the extracellular matrix have shown great potential in tissue engineering and local and systemic drug delivery (Cui et al., 2010). The high surface to volume ratio of electrospun scaffolds can enhance cell attachment, drug loading, and mass transfer properties (Sill and von Recum, 2008). The electrospun nanofibrous scaffolds can also be used as carriers of both hydrophilic and hydrophobic drugs, where the drug release profile can be finely controlled by modulation of the scaffold's morphology, porosity, and composition (Kim et al., 2004).

Drug release from electrospun fibres is a mainly diffusive mechanism. In diffusion-driven systems, the release kinetics is determined by the concentration gradient, diffusivity of the substance inside the polymer matrix, and the mean diffusion distance. In these systems, two cases can be considered: one in which diffusion occurs through the bulk of the polymer and the other in which diffusion occurs through a membrane/layer. The latter represents the process of barrier diffusion, which is similar to reservoir devices used in core-shell fibres, composite fibres, or multilayered constructs. On the other hand, in diffusion through the bulk of the polymer, the driving force of diffusion is the concentration gradient inside the polymer matrix. This leads to an initially increased release, i.e. burst release. In a diffusion-across-a-barrier system, the driving concentration gradient occurs between the reservoir and release environment, which are separated by a barrier. In such a system, a near-constant concentration gradient can be created if the rate of transport across the diffusion barrier is sufficiently low compared with the reservoir size and the rate of substance clearance outside the barrier; thus, a near-constant rate of release can be achieved (Natu et al., 2011; Szentivanyi et al., 2011). The linear release profile is eventually affected by erosion of the barrier, depletion of the reservoir, or substance build-up in the surrounding tissue. The diffusion distance may be safely assumed to be constant for electrospun fibres. Various post-treatment modalities have been developed to effectively control the drug release from the fibre. These can be grouped in two main categories: physical and chemical. The first category includes functionalization of the electrospun fibres with biomolecules using coating. The second involves chemical vapour deposition or fluorination (Im et al., 2010), which enables the shift from a diffusion-from-a-polymer matrix system to a diffusion-across-a-barrier system (Szentivanyi et al., 2011).

Nanofibrous systems typically consist of various biocompatible and/or biodegradable polymer excipients. Almost all of them are official in various pharmacopoeias. Polyvinyl alcohol (PVA), which is water soluble and often used in various pharmaceutical applications, is one such polymer base material. The produced fibrous web is a potential material for drug delivery and wound cover.

Analytical approaches have obvious limitations in the design of combined controlled-release multilayer scaffolds. Since experimentation could be time-consuming and costly, numerical methods such as the finite element method (FEM) show good potential because they enable evaluation and comparison of various design options prior to device fabrication, and thus, can significantly reduce the number of experiments required for the development and optimization of controlled-release devices (Zhou and Wu, 1997; Wu and Zhou, 1998). Liu et al. (2009) investigated the application of an inverse finite element analysis technique to identify material parameters of polymer gels via nanoindentation creep testing, optimization, and finite element simulation. The effects of the initial drug concentration with nonuniform distributions along the radial direction of hydrogel carriers on drug release were examined by finite element simulation of two-dimensional hydrogel swelling processes (Xu et al., 2013). The results showed that the drug release rate changes along with hydrogel swelling, and the major influencing factors of the drug release rate are water convection and the drug diffusion coefficient, which are affected by water volume fraction, drug concentration distribution in the matrix, and carrier radius. FEM was also applied to gain further insight into the release and distribution behaviour of inhomogeneous coating layer thicknesses around the strut, which may result from the coating process and thus influence drug release and distribution (Seidlitz et al., 2011).

In the present study, microfibrous PVA-based covers with different functions were produced by electrospinning technique. One type of the prepared electrospun layers contained the water-soluble antibiotic colistin sulfate, while the other layer was heat-treated in order to function as a rate-controlling membrane. The primary purpose was to ensure controlled topical drug release on the infected wound by alternating the drug-loaded and rate-controlling fibrous layers in order to increase the effectiveness and decrease the dose and consequent side effects of colistin.

In addition, a numerical evaluation method based on the FEM was used to investigate the effect of different arrangements of fibrous drug-containing layers and membranes on the extent and profile of drug release in order to achieve the optimal design of controlled topical drug-release fibrous scaffolds.

2. Materials and methods

2.1. Fibre formation

2.1.1. Drug-loaded fibre formation with electrostatic spinning

The nanofibrous drug delivery base was prepared from PVA by electrospinning (18–88, Ph. Eur., Merck, Darmstadt, Germany). The preparation of electrospun samples was done under a laminar chamber (FASTER Laminar BH-EN 2004 Fume/Bio Safety Hood), which was located in a 22 °C (±2 °C) temperature, air-conditioned room. The electrostatic spinner used for the experiments was equipped with an NT-35 high-voltage DC supply (MA2000). The utilized electrical potential, attached to the spinneret, was set at 25.1 kV. A grounded aluminium plate of area $250 \times 250\,mm^2$ covered with aluminium foil was used as the collector. The distance between the spinneret and collector was 15 cm and the experiments were performed at room temperature (25 °C ± 2 °C). The solution was dosed by the Alaris GH® syringe pump with a 1.4 ml/h flow rate and 1 h spinning duration. The uniform circularly symmetric samples were removed from the aluminium foil half an hour after the spinning procedure. The selected active ingredient, colistin sulfate (CAS: 1264-72-8, Sigma-Aldrich Chemie, USA), was dissolved in aqueous PVA polymeric gel of 15% w/w, which was prepared at 80 °C with 4 h stirring to obtain 2% w/w dry weight of active ingredient and then it was further stirred for 3 h. The spinning process was carried out under aseptic conditions in FASTER Laminar chamber BH-EN 2004 Fume/Bio Safety Hood (Ferrara, Italy).