European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 108–118

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Antimicrobial PLGA ultrafine fibers: Interaction with wound bacteria

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ARTICLE INFO

Article history:
Received 22 January 2011
Accepted in revised form 3 March 2011
Available online 9 March 2011

Keywords:
Ultrafine fibers
Fusidic acid
Electrospinning
Drug release
Wound bacteria
Interaction

ABSTRACT

The structure and functions of polymer nanofibers as wound dressing materials have been well investigated over the last few years. However, during the healing process, nanofibrous mats are inevitably involved in dynamic interactions with the wound environment, an aspect not explored yet. Potential active participation of ultrafine fibers as wound dressing material in a dynamic interaction with wound bacteria has been examined using three wound bacterial strains and antimicrobial fusidic acid (FA)-loaded electrospun PLGA ultrafine fibers (UFs). These were developed and characterized for morphology and in-use pharmaceutical attributes. In vitro microbiological studies showed fast bacterial colonization of UFs and formation of a dense biofilm. Interestingly, bacterial stacks on UFs resulted in a remarkable enhancement of drug release, which was associated with detrimental changes in morphology of UFs in addition to a decrease in pH of their aqueous incubation medium. In turn, UFs by allowing progressively faster release of bioactive FA eradicated planktonic bacteria and considerably suppressed biofilm. Findings point out the risk of wound reinfection and microbial resistance upon using non-medicated or inadequately medicated bioresorbable fibrous wound dressings. Equally important, data strongly draw attention to the importance of characterizing drug delivery systems and establishing material–function relationships for biomedical applications under biorelevant conditions.

1. Introduction

Cutaneous wound healing is a dynamic process involving overlapping phases of hemostasis, inflammation, tissue regeneration, and remodeling with scar formation [1,2]. The healing process can be delayed by diverse factors, mainly bacterial colonization and infection of the wound [3]. Healing acceleration with functional and esthetic results remains the main goal of efficient wound care. Wound dressings play a key role in this respect by providing a mechanical protective effect, an optimum microenvironment for tissue regeneration and by controlling bacterial infection [4]. A wide range of passive, interactive, and bioactive wound dressing materials with different clinical merits have been developed [4,5]. Since the turn of the millennium, ultrafine fibers and nanofibers with a diameter ranging from several micrometers down to tens of nanometers have evolved as a soft porous scaffold for tissue regeneration and wound healing applications [5,6]. Their unique structural and functional properties have demonstrated the potential to revolutionize wound management. Nanofibers are fabricated by different methods, most commonly electrospinning using well-documented processing and characterization methodologies [7,8]. A wide variety of natural and synthetic polymers, polymer blends, copolymers as well as hybrid and composite polymer systems are used for their production [5,7].

As wound dressing biomaterials, nanofibrous mats perform two important functions, temporary substitute for the native ECM and potential carrier system for the controlled delivery of antibacterial agents and other wound healing enhancers. Because of their resemblance to the fibrillar highly porous structure and size scale of the native ECM, plain nanofibers inherently promote the hemostasis phase of wound healing and initiate tissue repair by facilitating cell attachment and proliferation [5,9]. They reduce wound scarring by giving cells a better roadmap for self-repair [5]. Moreover, nanofibrous mats promote wound cleanliness by restricting bacterial invasion via the sieve effect. The role of nanofibrous wound dressings can be further enhanced by functionalization with antimicrobial drugs and other wound healing promoters such as silver nanoparticles [10] and bioactive agents [11]. The large surface area of the nanofibers results in efficient drug release by mass transfer [7], a process that can be modulated by controlling characteristics of the nanofibrous membrane [12,13] and by functionalization with drug-loaded nanoparticles incorporated or adsorbed on nanofibers [14,15] as well as surface graft polymerization [16].

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doi:10.1016/j.ejpb.2011.03.002

Studies dealing with polymer nanofibrous wound dressing materials usually focused on the implication of their structure and antimicrobial function in effective wound healing [17,18]. However, eventual infection control and tissue repair involve an inevitable dynamic interaction of the fibrous mat with the wound environment including bacteria. Irreversible attachment of bacterial cells to a substratum or interface resulting in colonization and biofilm formation has been recently reviewed [19]. Colonization of nanofibers with wound bacteria might considerably affect their characteristics as wound dressing materials. Surprisingly, there have been virtually no literature reports documenting the interaction of nanofibers with wound bacteria, particularly the potential effect of wound bacteria on the structural integrity and functions of nanofibrous mats.

The objective of the study was to gain more insight into potential nanofibers-wound bacteria interactions. To this end, antimicrobial biodegradable electrospun fusidic acid (FA)-loaded PLGA ultrafine fibers as wound dressing material were developed and characterized. PLGA has been selected as an FDA-approved biodegradable and biocompatible copolymer. PLGA with different glycolic acid to lactic acid ratio produces fibers with suitable mechanical properties and a wide range of diameters and degradation rates [5]. Because of PLGA hydrophobicity, it is electrospun from organic solvents which allows faster electrospinning at a lower voltage compared to electrospinning settings required for water soluble polymers such as PVA [5,7]. The developed electrospun fibrous mats were used to examine in vitro interaction with three wound bacterial strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus* standard strain, and methicillin-resistant ($MRSA_1$) clinical isolate. The study addressed the effect of wound bacteria on the structural integrity and function of the ultrafine fibrous mat, mainly in terms of matrix degradation and drug release properties and the effect of the FA-loaded mat characteristics on the in vitro antimicrobial activity.

2. Materials and methods

2.1. Materials

The following materials were used: Fusidic acid (gift of Pharaonia Pharm. Co., Alexandria, Egypt), Medisorb® Poly (lactide-co-glycolide) (PLGA) 50:50 DL 3A, MW 50 kD and inherent viscosity 0.36 dl g^{-1} (Alkermes, Inc., Cincinnati, Ohio, USA), dichloromethane (DCM), Biotech. grade 99.9% (Sigma. Aldrich, USA), absolute ethanol, potassium dihydrogen orthophosphate, sodium hydrogen phosphate dibasic, sodium chloride, and sodium hydroxide, analytical grade (Adwic, El-Nasr Pharmaceutical Co., Egypt), and nutrient agar (Oxoid Ltd., Basingostok, Hampshire, England). Bacterial strains used were standard *S. aureus* ATCC 6538P (Sa_{st}) and *Ps. aeruginosa* ATCC 9027 (Ps_{st}) strains and a methicillin-resistant *S. aureus* clinical isolate ($MRSA_1$) isolated from an infected wound (Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt). Bacteria were maintained at 4 °C as slant cultures of sterile nutrient agar for a maximum of 1 month. Long-term preservation was performed by freezing in 15% glycerol broth.

2.2. Development and characterization of plain and FA-loaded PLGA ultrafine fibers (UFs)

PLGA ultrafine fibers (UFs) were prepared using the electrospinning technique [17,18] in an air-conditioned laboratory at an ambient temperature of ≈25 °C and relative humidity of <65%. The electrospinning apparatus was equipped with a high-voltage DC power supply (ALE 402, TDK-Lambda Americas, Inc., USA) set to 25 kV and a syringe with a blunt-tip stainless steel spinneret (0.9 mm diameter). The distance between the spinneret and the fiber collector was kept constant at 10 cm. PLGA solution (5 ml) in DCM was gravity-fed to the spinneret. A copper collector covered with aluminum foil and a nonwoven synthetic porous mat for the ease of peeling of the electrospun mat was used. For the preparation of FA-loaded UFs, the drug was dissolved in the DCM polymer solution. Preliminary trials were made to adjust the processing parameters. The effect of two formulations variables, polymer content and initial drug loading, was examined.

2.3. Characterization of PLGA ultrafine fibers (UFs)

2.3.1. Scanning Electron Microscopy (SEM)

Samples of UFs were mounted on metal stubs using double-sided adhesive tape onto which the UF fibrous meshes were fixed. The samples were then coated with gold using an ion sputtering coater (JFC-1100E, JEOL, Japan) and the gold coated samples scanned using SEM (JEOL, model JFC-1100E, Japan). The mean fiber diameter was determined using image analysis software and at least 10 randomly selected fiber segments.

2.3.2. Determination of drug content and % entrapment efficiency

FA was extracted from UFs by shaking an accurately weighed amount (20 mg) of drug-loaded UFs in 10 ml absolute ethanol in well-closed screw-capped 20 ml vials at ambient temperature (≈25 °C). Vials were shaken intermittently for 24 h, time proven sufficient for complete drug extraction. A 1-ml sample was diluted threefold with absolute ethanol, and FA concentration was determined spectrophotometrically in absolute ethanol at λmax, 220 nm using UV–Visible spectrophotometer (Thermospectronic, Helios alpha, NC 9423 UV A 1002E, England). The % entrapment efficiency was calculated using the following equation:

$$\text{Entrapment efficiency } \% = \frac{\text{Weight of drug in UFs}}{\text{Theoretical drug loading}} \times 100$$

Results are the average of three determinations.

2.3.3. Differential scanning calorimetry (DSC)

The thermal behavior of the electrospun fibers was investigated by DSC (DSC-6, CT, Perkin Elmer instruments, USA) under a nitrogen atmosphere. DCS traces were recorded between 25 and 400 °C at a constant rate of 10 °C/min. Indium standard was used to calibrate the DSC temperature and enthalpy scale. An empty pan was used as reference.

2.3.4. Degradation of PLGA UFs

Degradation of plain and FA-loaded PLGA UFs was assessed at 37° in PBS pH 7.4 as degradation medium by monitoring the change in pH [20] using a digital pH-meter (Schott Geräte CG-820, Germany). Samples of PLGA UFs mats were immersed in 10 ml of the degradation medium in 20-ml screw-capped glass vials for 60 days without agitation. Prior to each measurement set, the pH-meter was calibrated and data reproducibility checked by replicate pH measurement of selected samples. Results are the average of two measurements.

2.3.5. In vitro drug release

Samples of FA-loaded UFs mats, 2×2 cm^2, were immersed in PBS pH 7.4/1% ethanol as release medium in capped Erlenmeyer flasks containing 30 ml of the medium. The medium was selected based on a preliminary solubility study. Flasks were shaken at 50 rpm in a thermostatically controlled shaking water bath (GFL,