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Development of Suberin Fatty Acids and Chloramphenicol-Loaded Antimicrobial Electrospun Nanofibrous Mats Intended for Wound Therapy

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ABSTRACT

Suberin fatty acids (SFAs) isolated from outer birch bark were investigated as an antimicrobial agent and biomaterial in nanofibrous mats intended for wound treatment. Electrospinning (ES) was used in preparing the composite nonwoven nanomats containing chloramphenicol (CAM; as a primary antimicrobial drug), SFAs, and polyvinylpyrrolidone (as a carrier polymer for ES). The X-ray powder diffraction, differential scanning calorimetry, scanning electron microscopy, atomic force microscopy, and texture analysis were used for the physicochemical and mechanical characterization of the nanomats. ES produced nanofibrous mats with uniform structure and with an average fiber diameter ranging from 370 to 425 nm. Microcrystalline SFAs and crystalline CAM were found to undergo a solid-state transformation during ES processing. The ES process caused also the loss of CAM in the final nanofibers. In the texture analysis, the SFAs containing nanofibers exhibited significantly higher maximum detachment force to an isolated pig skin ($p < 0.05$) than that obtained with the reference nanofibers. CAM exists in an amorphous form in the nanofibers which needs to be taken into account in controlling the physical storage stability. In conclusion, homogeneous composite nanofibrous mats for wound healing can be electrospun from the ternary mixture(s) of CAM, SFAs, and polyvinylpyrrolidone.

Introduction

In the recent years, polymeric nonwoven nanofibrous networks have gained an increasing attention as potential drug delivery systems. One promising application area for nanofibers is to use them as medicated wound dressings.[1-7] Some of the most favorable properties of nanofibers in such applications include their ability to mimic the fibrillar structure of natural extracellular matrix, the high surface area to volume ratio, interconnecting porous structure with high permeability, and the ability to incorporate a wide range of drugs in an amorphous form within the fibers.[8] Furthermore, the polymeric nanofibers have also been reported to hold a promise of healing wounds without leaving scars.[9] Therefore, using appropriate nonwoven nanofiber mats as localized drug delivery vehicles could remarkably improve the wound-healing process and also reduce the systemic absorption of the drug.[2,10]

Nanofibers can be prepared by a number of different methods including the electrospinning (ES), self-assembly, and phase separation.[11] ES is the most widely used technique for preparing

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polymeric nanofibers due to the fact that it is the only method that can be further developed for mass production of one-by-one continuous nanofibers from various polymers.[12] Thus, electrospun nanofibers exhibit excellent performance when applied as wound dressings[11] or for localized antibiotic drug delivery.[13] ES is an effective method, which uses electrostatic forces to produce fibers from polymer solutions or melts. The main principle is that a polymer solution is held at the end of a capillary tube by surface tension forces. The capillary tube is introduced to an electric field, and consequently, an electric charge is induced on the surface of polymer solution. When the applied electric charge reaches to a critical value, the electrical repulsive forces overcome the surface tension forces, and a charged jet of polymer solution is ejected from the tip of the capillary tube. An unstable and rapid whipping of the jet that occurs between the capillary tip and collector induces the solvent to evaporate.[14]

Today, there is an increasing interest to find suitable polymers and/or excipients that have adequate properties for ES, and consequently, polymeric nanofiber applications. Suberin is a composite biopolyester found in the suberized cell walls of higher plants, where it plays the fundamental role of a protective barrier between the organism and its environment.[15] In plants, suberin forms important barriers, which limit water and nutritient transport and protect plants from the invasion of pathogens. For example, outer birch bark (OBB, *Betula* spp.), contains up to 35 % of suberin and 30 % of betulin.[16] Chemically, suberin is a natural surface wax-like material consisting of a polyaliphatic domain in association with a polyaromatic domain.[17] The polyaromatic domain is mainly derived from ferulic acid. The polyaliphatic domain is a biopolyester primarily composed of oxygenated fatty acid derivatives. The polyaliphatic domain is mainly responsible for the barrier function of suberin for water and solutes.[18] Suberin that is formed in outer tissues also acts as an antimicrobial barrier against pathogens.[19] In the present study, suberin fatty acids (SFAs) were isolated from OBB by depolymerizing of suberin polymer using alkali solvent extraction,[16] and SFAs were used as a synergistic antimicrobial biomaterial in electrospun nanofibers intended for wound healing.

The purpose of applying antibiotics and other antibacterials in the medicated wound dressings is mainly to prevent or combat infections and thus accelerate the wound-healing process.[20,21] Chloramphenicol (CAM) was chosen as a model antibacterial agent because of its wide antibacterial spectrum with a bactericidal activity on gram-negative and gram-positive bacteria.[22] CAM has also bacteriostatic effects against most of the microorganisms.[23] Because of its broad antibacterial activity and high antimicrobial effectiveness, CAM is often used as a standard agent in topical wound-healing preparations.[24,25]

Polyvinylpyrrolidone (PVP) is a synthetic polymer, which consists of *N*-vinyl-pyrrolidone groups.[26] The different degree of polymerization results in PVP grades of various molecular weights. PVP is mainly used in peroral solid dosage forms, but it can also be used as a suspending, stabilizing, or viscosity-increasing agent in topical and oral solutions and suspensions.[27] Recently, its suitability has been demonstrated also in transdermal dressings and drug delivery systems,[28] as well as in the electrospun nanomats for wound healing[29] as a filament-forming matrix (carrier polymer).

The aim of the present study was to develop the multifunctional medicated nanofibrous wound dressings containing SFAs (of OBB origin) as a synergistic antimicrobial biomaterial and synthetic PVP as a carrier polymer for ES. Special attention was paid to the effects of SFAs on the formation and physical solid-state stability of nanofibers. CAM was used as a model antibiotic in the polymeric nanofibrous systems.

Materials and Methods

Materials

The SFAs biocomposite material (Batch Pilot 1/14.01.2013, VTT Technical Research Centre of Finland Ltd., Espoo, Finland) was used as received. SFAs were isolated from OBB (*Betula* spp.) by a novel extractive hydrolysis method, in which betulin and SFAs are directly isolated from bark using alkaline 2-propanol as a solvent, followed by extraction of SFAs as sodium salts with hot water leaving water insoluble betulin residue. Finally, SFAs-sodium salts were acidified to obtain SFAs.[16] The chemical composition of SFAs mixture was similar to previously published results in the literature.[30] PVP (Kollidon K90) was obtained from BASF Aktiengesellschaft (Germany). CAM was purchased from Sigma-Aldrich. The primary solvent used in the ES studies was ethanol (96%). Pig ears were purchased from Nurtura AS (Bardufoss, Norway).

Electrospinning of Nanofibers

The solutions for ES were prepared by dissolving the solids (CAM, SFAs, and PVP) in ethanol (96% m/V). The nanofibers were composed of (I) PVP; (II) PVP + CAM (2.1%); (III) SFAs + PVP (1:4); (IV) SFAs + PVP (1:4) + CAM (2.1%); (V) SFAs + PVP (1:7); and (VI) SFAs + PVP (1:7) + CAM (2.1%). Table 1 summarizes the theoretical compositions of electrospun nanofibrous systems (in milligrams). The components were dissolved in 5 mL of ethanol, to prepare ES solutions. For comparison, the respective physical mixtures (PMs) were also prepared by gently mixing the materials in a mortar using geometric dilution. An automated robotized ES system ESR200RD Series, NanoNC (Korea) was used for fabricating nanofibrous mats at controlled conditions. A needle distance from the collector was 6 cm. The voltage applied between a needle tip and collector was 9.0 kV, and injection rate was 1.5 mL/h. The electrospun nanofibers were collected onto an aluminum foil and put into ziploc bags. All samples were kept in refrigerator (8°C) and 0% relative humidity (RH) above silica gel in desiccator for 12 h before analysis to reduce the amount of residual ethanol in the nanofibrous mats.

Surface Topography and Morphology of Nanofibers

Scanning Electron Microscopy

The surface morphology and diameter of nanofibers (n = 100) were investigated with a high-resolution scanning electron microscope (SEM; Zeiss EVO® 15 MA, Germany) and Image J.[31,32] The nanofibrous samples were mounted on aluminum stubs with a conductive carbon film and were magnetron-sputter coated with a 3-nm gold layer in an argon atmosphere before microscopy.

Atomic Force Microscopy

The surface topography and morphology of nanofibers were investigated at nanoscale with atomic force microscopy AFM; (Autoprobe CP, Thermomocroscopes). The surface topography images were obtained from a 20 μm × 20 μm area. The AFM mapping was performed in a contact mode with a cantilever of 0.12 N/m

Table 1
The Theoretical Solid-State Composition of Experimental Electrospun Nanofibers (in Milligrams, Solids Were Dissolved in 5 mL of Ethanol)

Material	Formulation/Theoretical Composition (mg)					
	I	II	III	IV	V	VI
SFAs	0	0	48	48	48	48
PVP	240	240	192	192	336	336
CAM	0	5	0	5	0	5