ELSEVIER

BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
12 (2016) 1375 – 1385

nanomedicine Nanotechnology, Biology, and Medicine

nanomedjournal.com

Original Article

Drug functionalized microbial polysaccharide based nanofibers as transdermal substitute

Priya Vashisth, PhD[a], Amit Kumar Srivastava, MPharm[b], Hemant Nagar, MPharm[b], Navdeep Raghuwanshi, MTech[a,b], Shruti Sharan, MSc[a], Kumar Nikhil, PhD[a], Parul A. Pruthi, PhD[a], Rajesh P. Singh, PhD[a], Partha Roy, PhD[a], Vikas Pruthi, PhD[a,*]

[a]*Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India*
[b]*Sapience Bio-Analytical Research Lab Bhopal, Madhya Pradesh, India*

Received 22 April 2015; accepted 31 January 2016

Abstract

In order to promote the natural healing process, drug-functionalized nanofibrous transdermal substitute was fabricated using gellan as chief polymer and polyvinyl alcohol (PVA) as supporting polymer via electrospinning technique. These fabricated nanofibers physiochemically mimic the extracellular matrix (ECM) which supports the cell growth. For neo-tissue regeneration in a sterilized environment, amoxicillin (Amx) was entrapped within these nanofibers. Entrapment of Amx in the nanofibers was confirmed by FESEM, FTIR, XRD and TG analysis. *In vitro* cell culture studies revealed that the fabricated non-cytotoxic nanofibers promoted enhance cell adherence and proliferation of human keratinocytes. A preliminary *in vivo* study performed on rat model for full thickness skin excision wound demonstrated the prompt re-epithelialization in early phase and quicker collagen deposition in later phases of wound healing in case of Amx-functionalized gellan/PVA nanofibers. Data collectively confirmed the potential usage of gellan based electrospun nanofibers as transdermal substitute for faster skin restoration.
© 2016 Elsevier Inc. All rights reserved.

Key words: Gellan; Transdermal substitute; Nanofibers; Electrospinning; Wound healing

Wound healing is a complex multi-step process which involves the reestablishment of dermal and epidermal tissues by the means of various cellular and biochemical process. This process includes successive cascade of events involving inflammation, migration, proliferation, and maturation phases.[1] Though skin itself has the natural ability of wound healing, open wounds are soft target for microorganisms which cause infection at wound site as well as affect the nearby healthy tissues which consequently delays the wound healing process. Therefore, an antibiotic treatment along with an appropriate wound dressing is often helpful to eliminate infection.[2,3]

Apart from protecting microbial invasion, the ideal wound dressings should have the swelling capability for absorbing excess wound exudates and high porosity for gas permeability.[4,5] Recently, electrospun biomaterials have gained increasing attention as wound dressing material as they fulfill the above described criteria of an ideal wound dressing and also possess nonwoven structural resemblance to skin.[6,7] The high porosity and variable pore-size distribution of electrospun nanofibers effectively provide the required air for cell respiration whereas its high surface area to volume ratio could quickly activate the cell signaling pathway.[8] Most significantly the morphological resemblance of nanofibers to the natural extracellular matrix (ECM) could support fibroblast's growth in order to repair the damaged tissue.[9]

At present, nanofibers synthesized from natural biodegradable polymers have gained a special attention for wound healing as they are histocompatible, non-antigenic and can easily be washed off from the wound surface.[10] Gellan is a natural anionic exocellular polymer secreted from *Pseudomonas elodea* which mainly consists of repeated tetrasaccharide units of glucose, glucuronic acid and rhamnose residues in 2:1:1 ratio joined in a linear

We wish to confirm that there are no known conflicts of interest associated with this publication.

This study is supported by Council of Scientific and Industrial Research (CSIR), Government of India.

*Corresponding author. Tel.: +91 1332 285530; fax: +91 1332 286151x273560.
E-mail addresses: vikasfbs@iitr.ernet.in, vikasfbs@gmail.com (V. Pruthi).

http://dx.doi.org/10.1016/j.nano.2016.01.019
1549-9634/© 2016 Elsevier Inc. All rights reserved.

Please cite this article as: Vashisth P, et al, Drug functionalized microbial polysaccharide based nanofibers as transdermal substitute. *Nanomedicine: NBM* 2016;12:1375-1385, http://dx.doi.org/10.1016/j.nano.2016.01.019

fashion.[11,12] It is an economically abundant biopolymer with remarkable properties such as biodegradability, biocompatibility and swelling capabilities which make it an appropriate candidate for effective wound healing.[13–15]

This investigation is the first attempt to develop gellan based nanofibrous transdermal substitutes using a versatile technique electrospinning. The electrospinning of gellan solution alone was challenging as it shows a complex gelling behavior even at low concentration (1 wt% in deionized water). Therefore, to overcome this limitation, PVA was used as a copolymer of gellan. PVA was found to lower the repulsive forces of highly negatively charged gellan solution and subsequently allowed its electrospinning. PVA is a biodegradable, nontoxic, non-carcinogenic, biocompatible polymer with appropriate mechanical and swelling properties which make it a suitable candidate for wound dressing.[16,17] This study reports on the fabrication of cytocompatible drug functionalized gellan/PVA nanofibrous transdermal substitutes and their preclinical trial on rat excision model to prove its efficacy for wound healing.

Methods

Materials

Gellan (Gelrite; average M_w 1000 kg/mol) was purchased from Sigma Aldrich. PVA (M_w = 140,000), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cell culture-grade dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), and all analytical grade chemicals were obtained from Himedia (India). Human keratinocytes (HaCaT) cell line was procured from National Center for Cell Science (NCCS), Pune, India.

Fabrication of nanofibrous transdermal substitute via electrospinning

Aqueous gellan solution (1 wt%) was prepared in deionized water at 90 °C. Aqueous PVA (10 wt%) solution was prepared separately and mixed with gellan solution in equal ratio. For the preparation of Amx-functionalized gellan/PVA nanofibers, Amx drug (5 mg/ml) was mixed with the prepared gellan/PVA solution. Electrospinning process was carried out in a high electric voltage (21 kV) environment and at a fixed solution flow rate of 0.1 ml/h under ambient temperature (25 ± 2 °C) and humidity (30% ± 1%) conditions as reported earlier by our group.[18] The random uniform gellan/PVA nanofibers were collected on aluminum collector placed at a horizontal distance of 18 cm from the needle tip. The electrospun nanofibers were kept overnight in desiccator for complete removal of moisture.

Physiochemical characterization of nanofibrous transdermal substitute

Field emission scanning electron microscopy (FESEM)

The morphological features of the fabricated nanofibers were examined using FESEM (Quanta 200F, FEI). For analysis, the nanofibrous samples (10 × 10 mm) were sputter coated with gold using a sputter coater (Bal-tech SC005, Balzers, Switzerland) for 60 s and observed at an accelerating voltage of 10 kV. The average diameter of the nanofibers was calculated over 100 different points from the FESEM images using image analysis software (ImageJ, NIH, USA).

Fourier transformed infrared spectroscopy (FTIR)

The presence of secondary interactions between drug and polymeric nanofibrous matrix was determined by FTIR analysis (Thermo Nicolet Nexus 6700, US). For the same, the dried test samples (Amx, PVA nanofibers, PVA-Amx nanofibers, gellan/PVA nanofibers and gellan/PVA-Amx nanofibers) were first finely crushed, mixed with potassium bromide (KBr) and pressed to form discs. Subsequently, the infrared absorptions of test samples were recorded in the mid-infrared scanning range of 4000-400 cm^{-1} with the resolution of 4 cm^{-1}.

X-ray diffraction (XRD) analysis

Physical state of test samples was further examined by using powdered X-Ray diffractometer (Bruker, AXS D8 Advance). For analysis, the test samples were first mounted on the metal sample holder. Subsequently, the XRD patterns were recorded by using a slit detector with Cu Kα radiation over the 2θ range from 5° to 100° with the scanning rate of 2° min^{-1}.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out using a TGA instrument (EXSTAR, TG/DTA 6300, Hitachi, Tokyo, Japan) under nitrogen atmosphere. A sealed empty pan was used as the reference. Firstly, the samples of about 8-10 mg were kept under vacuum for 24 h prior to testing and then the precisely weighed samples were sealed in aluminum pans. The samples were heated from 25 °C to 500 °C at a scanning rate of 10 °C/min.

In vitro cell culture of human keratinocytes on nanofibrous transdermal substitute

The cytocompatibility of the nanofibrous transdermal substitute was evaluated by keeping the UV sterilized test formulations (PVA nanofibers, Amx-functionalized PVA nanofibers, gellan/PVA nanofibers and Amx-functionalized gellan/PVA nanofibers) in 24-well microtiter plates individually. Subsequently, the HaCaT cells (1 × 10^5 cells/ml) were added to each well along with DMEM medium supplemented with 10% fetal bovine serum (FBS), 50 IU/ml penicillin and 50 μg/ml streptomycin.[19] The culture plates were incubated at 37 °C in a CO_2 incubator maintaining 5% CO_2. Cellular harvests were collected after fixed incubation period and washed thrice with PBS (pH 7.4) to remove the unattached cells.

In vitro cell proliferation assay

Cell proliferation on different nanofibrous samples was quantified using colorimetric MTT assay. In this assay, the yellow tetrazolium salt (MTT) is reduced to form intracellular purple formazan crystals by the activity of dehydrogenase enzymes secreted by mitochondria of metabolically active cells. The purple formazan crystals can be solubilized to mark the absorbance at 490 nm. The amount of formazan crystals formed is directly proportional to the number of viable cells. Briefly, after culturing the keratinocytes on different nanofibrous samples for a period of 1, 3, 5 and 7 days, they were incubated

Download English Version:

https://daneshyari.com/en/article/877357

Download Persian Version:

https://daneshyari.com/article/877357

Daneshyari.com