Available online at www.sciencedirect.com

ScienceDirect

St. Petersburg Polytechnical University Journal: Physics and Mathematics 2 (2016) 322–328

www.elsevier.com/locate/spjpm

Tissue reconstruction of skin failures and soft-tissue injuries using regenerative medicine methods

Natalia V. Smirnova[a,*], Natalia O. Petrova[b], Inna O. Lebedeva[a], Pavel V. Popryadukhin[a,c], Irina P. Dobrovalskaya[a,c], Vladimir E. Yudin[a,c]

[a] *Peter the Great St. Petersburg Polytechnic University, 29 Politekhnicheskaya St., St. Petersburg 195251, Russian Federation*
[b] *Biotechnological Company "BIOCAD", 34a Svyazi St., Strelna, St. Petersburg 198515, Russian Federation*
[c] *Institute of Macromolecular Compounds RAS, 31 Bolshoy Pr. V.O., St. Petersburg 199004, Russian Federation*

Available online 15 December 2016

Abstract

The electrospinning technique has been used to manufacture a composite material based on nanofibers made of aliphatic copolyamide and composite nanofibers made of chitosan and chitin nanofibrils. Experimental in vivo studies of the designed material as wound covering were carried out to treat a vast and multilayered wound on a rat's back. After 28 days and nights of observation, complete epithelialization of the wound surface was established to occur in the experimental rat group. Histological analysis of scar tissue showed the presence of a small minority of capillaries and a low amount of infiltrating cells. The survival of animals was 100%. At the same time, in the control group of animals, lethality was observed in 11% of cases, and suppurative complications were observed in 100% of cases. Thrombocyte gel prepared from the peripheral blood of the patients was used to increase the rate of tissue regeneration, and to reduce the infection probability. The curative effect was proved to increase when wound dressings and autologous blood product separation, i.e., thrombocyte gel and platelet-rich plasma, were applied simultaneously.

Keywords: Aliphatic copolyamide; Chitosan; Chitin nanofibrilla; Electrospinning; Wound covering; Composite nanofiber.

Introduction

The problem of healing damaged skin and soft tissues using surgical and conservative methods has not yet been completely resolved. The major factors hindering the granulation and epithelialization processes are tissue degeneration, oxidative damage, moisture imbalance in the wound, infections and other complications in the area of the surgical wound, trauma or burn. Scar tissue or other structural changes to the surgical site reduce the patient's quality of life. Currently, a number of techniques aimed at boosting wound healing and improving the structural and functional properties of the newly formed tissue have been developed. Most of these techniques use wound dressings varying by their compositions and functional characteristics [1–3].

* Corresponding author.
E-mail addresses: nvsmirnoff@yandex.ru (N.V. Smirnova), nataly_petrova@mail.ru (N.O. Petrova), innale@list.ru (I.O. Lebedeva), pavel-pn@mail.ru (P.V. Popryadukhin), zair2@mail.ru (I.P. Dobrovalskaya), yudin@hq.macro.ru (V.E. Yudin).

http://dx.doi.org/10.1016/j.spjpm.2016.11.008

An optimal wound dressing should provide gas and moisture exchange necessary for full cell integration and activity. Wound surface should also be reproduced, i.e., the material must be flexible and easy to handle. Modern wound dressings are minimally invasive, as the newly formed epithelial layer must not be damaged or destroyed. Finally, optimal wound dressing should prevent the wound from becoming infected through penetration of foreign pathogens. Incubation of local pathogens should also be averted; for this purpose the dressing should ensure effective exudate removal.

Porous film materials based on polymeric nanofibers obtained by electrospinning possess all of the above-listed properties. This method allows to produce fibers from 50 nm to 4500 nm in diameter from a variety of polymers. Film materials based on nanofibers typically have low density, high porosity, water and gas permeability [4–7], and pore sizes ranging from tens to hundreds of micrometers.

Recently, porous film materials have been used as matrices for cell technologies. The chemical composition and the porous structure of the materials contribute to adhesion of stem or somatic cells on the surface of the fibers and ensure the metabolic processes necessary for effective cell proliferation, movement and differentiation.

Our earlier study [5] described the preparation of nanofibers from an alcohol-soluble aliphatic copolyamide (CoPA), a copolymer of poly(ε-caprolactam)

$$[-NH-(CH_2)_5-CO-]_n$$

and poly(hexamethylendiaminadipate)

$$[-NH(CH_2)_6NHCO(CH_2)_4CO-]_n.$$

The second polymer, widely used for biomedical materials, is chitosan. It is a biocompatible and biodegradable polymer, a polysaccharide derivative whose macromolecules consist of β-(1–4) D-glucosamine and *N*-acetyl-D-glucosamine monomers. The biodegradation products of chitosan are nontoxic and become part of the natural metabolic reactions of the body as chitosan decomposes.

However, electrospinning nanofibers from a chitosan solution are known [8–10] to be complicated by its polyelectrolyte properties. To stabilize the electrospinning of chitosan-based nanofibers, water-soluble polymers such as polyethylene oxide (PEO), polyvinyl alcohol (PVA), methylcellulose (MC), and polyvinyl pyrrolidone (PVP) [8–10] are introduced into the solution. Adding these polymers in a concentration of up to 50 wt.% of the amount of chitosan (which is necessary for nanofibers to stably form) adversely affects the finished material, increasing its hygroscopicity and reducing its mechanical properties.

Ref. [11] established that chitosan composite fibers containing chitin nanofibrils 20 nm in diameter and 600–800 nm in lengths typically exhibit increased strength and elasticity. Additionally, administering chitin nanofibrils into the spinning solution of chitosan stabilizes the formation process. A similar beneficial effect was described for electrospinning nanofibers containing chitin nanofibrils [12].

In view of the above, this paper proposes a method for fabricating a two-layered wound dressing that consists of an outer layer of CoPA-based nanofibers and an inner layer of composite nanofibers based on chitosan and chitin nanofibrils. Nanofibers of non-resorbable CoPA provide the necessary mechanical properties for the dressing, as well as metabolic processes with the external environment. The layer of chitosan and chitin nanofibrils directly contacting the wound surface simultaneously provides hemostatic and bactericidal effects and non-invasiveness of the dressing; additionally, the polymer is gradually resorbed during integration with an active biological environment. Once the dressing is removed, a non-resorbable CoPA layer separates from the wound surface, while the chitin–chitosan layer remains and promotes epithelialization.

Dealing with deeper wounds where soft tissues are damaged in addition to skin requires applying spatial reconstruction techniques and stimulating regeneration throughout the defect. In this case, it is effective to use tissue-engineered preparations consisting of a polymer matrix, cellular components and cellular secretion products [13]. We propose combining an experimental wound dressing with the separation products of the patient's blood for increasing the efficiency of tissue regeneration and achieving greater hemostatic, antimicrobial and analgesic effects.

Platelet-rich plasma and other blood separation products have been used in human and veterinary medicine for over 20 years. Numerous studies have proved the therapeutic effect of these products resulting from biological activity of the growth factors contained in platelets.

Platelets are known to contain both specific and non-specific growth factors initiating mitogenesis and connective tissue regeneration. The most important of them are the platelet-derived growth factor (PDGF), the transforming growth factor (TGF-β), the vascular endothelial growth factor (VEGF), the