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Use of ginsenoside Rg3-loaded electrospun PLGA fibrous membranes as wound cover induces healing and inhibits hypertrophic scar formation of the skin

Xiaoming Sun[a,1], Liying Cheng[a,1], Wankun Zhu[d], Changmin Hu[b,d], Rong Jin[a], Baoshan Sun[a], Yaoming Shi[a], Yuguang Zhang[a,*], Wenguo Cui[b,c,*]

[a] Department of Plastic and Reconstructive Surgery, Ninth People's Hospital Affiliated to Medical School of Shanghai Jiao Tong University, 639 Zhi Zao Ju Road, Shanghai 200011, PR China
[b] Orthopedic Institute, Soochow University, 708 Renmin Road, Suzhou, Jiangsu 215006, PR China
[c] Department of Orthopedics, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou, Jiangsu 215006, PR China
[d] School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai, PR China

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ABSTRACT

Prevention of hypertrophic scar formation of the skin requires a complex treatment process, which mainly includes promoting skin regeneration in an early stage while inhibiting hypertrophic formation in a later stage. Electrospinning PLGA with the three-dimensional micro/nano-fibrous structure and as drugs carrier, could be used as an excellent skin repair scaffold. However, it is difficult to combine the advantage of nanofibrous membranes and drug carriers to achieve early and late treatment. In this study, Ginsenoside-Rg3 (Rg3) loaded hydrophilic poly(D,L-lactide-co-glycolide) (PLGA) electrospun fibrous membranes coated with chitosan (CS) were fabricated by combining electrospinning and pressure-driven permeation (PDP) technology. The PDP method was able to significantly improve the hydrophilicity of electrospun fibrous membranes through surface coating of the hydrophilic fibers with CS, while maintaining the Rg3 releasing rate of PLGA electrospun fibrous membranes. Experimental wounds of animal covered with PDP treated fibrous membranes completely re-epithelialized and healed 3–4 days earlier than the wounds in control groups. Scar elevation index (SEI) measurements and histologic characteristics revealed that Rg3 significantly inhibited scar formation 28 days post-surgery. Moreover, RT-PCR assays and western blot analysis revealed that at day 28 after wound induction the expression of VEGF, mRNA and Collagen Type I in the scars treated with Rg3 was decreased compared to control groups. Taken together PLGA-Rg3/CS electrospun fibrous membranes induced repair of tissue damage in the early stage and inhibited scar formation in the late stage of wound healing. These dual-functional membranes present a combined therapeutic approach for inhibiting hypertrophic scars of the skin.

1. Introduction

Hypertrophic scarring (HS) is a pathological wound healing process, which can lead to joint contractures, joint stiffness, chronic pain, chronic itching, and inability to sweat [1]. Patients with hypertrophic scars suffer from a reduced quality of life [2]. Many patients with hypertrophic scars suffer from serious chin-chest adhesions or mouth deformities. Therefore, it is important to inhibit hypertrophic scar formation. Currently, the main methods for both treating and preventing scars include: injection preparations, surface coating, radiotherapy, surgery, laser treatment, photodynamic therapy and cryotherapy [3,4]. However, these conventional methods of inhibiting hypertrophic scar formation directly intervene in the process of excessive fibroblast proliferation or promote fibroblast apoptosis. Moreover, these methods fail to take into account the importance of promoting skin regeneration in the early stage, while inhibiting scar formation in the late stage. Ghahary et al. reported that the incidence of hypertrophic scars increased to 78% when a wound was epithelialized later than 21 days [5]. Therefore, optimal prevention of hypertrophic scars should allow efficient tissue repair while taking the necessary steps to inhibit the scar formation at a later stage. 20(R)-ginsenoside Rg3 (Rg3), a ginsenoside from Panax ginseng, has been shown to have many biological activities, including anti-inflammatory and anti-tumor effects [6].

* Corresponding author. Tel.: +86 21 63089562, fax: +86 512 67781420.
E-mail addresses: zhangyg18@126.com (Y. Zhang), wgcui80@hotmail.com (W. Cui).
[1] These authors contributed equally.

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Many studies indicated that Rg3 interfered with various steps of angiogenesis [7]. For instance, Rg3 was able to down-regulate VEGF expression [8,9]. Liu et al. reported that Rg3 also inhibited fibroblast growth, a property that could be useful for prevention of hypertrophic scars [10]. Nevertheless, Rg3 is a poorly soluble crystalline drug, and is mainly used as orally administered capsules for tumor treatments [11]. It is difficult to prepare a formulation of Rg3 in the form of a membrane suitable for tissue repair. Because of agglomeration and poor solubility of Rg3, the efficiencies of Rg3 by traditional means such as spray-drying particles and microcapsules were very low [12]. To increase Rg3 solubility and improve absorption, the preparation of an effective drug delivery for Rg3 is critical.

Electrospinning has become an increasingly popular method for fabricating fibrous membranes as drug delivery devices and for various tissue engineering materials [13,14]. Electrospinning has been recognized as a versatile polymer processing technique. The setup for electrospinning could be simply summarized as a stream of a polymer solution being subjected to a high electric field and formation of nano/micro fibers. In drug delivery applications, drugs could be incorporated into the fibers to achieve drug controlled releasing. Such controlled drug delivery systems could improve therapeutic efficacy, reduced toxicity and offer site-specific delivery [15,16]. Therefore, electrospun fibers carrying Rg3 could serve as an efficient drug delivery device to achieve sustained Rg3 release and could form an ideal membrane to promote cell growth and tissue repair.

Poly(D,L-lactide-co-glycolide) (PLGA), a biomedical polymer approved by the U.S. Food and Drug Administration [17], has been widely used as tissue repair and reconstruction material, as well as a drug delivery device [18–20]. PLGA as an easily processable synthetic polymer has a slower rate of biodegradation and a more reliable mechanical property compared to the natural polymers. On the other hand, lactic acid and glycolic acid, the degradation products of PLGA can be excreted by metabolic pathways [21]. Therefore, PLGA has been extensively used in tissue engineering scaffolds. However, due to the hydrophobicity of the polyester type (PLGA) electrospun fibers and the poor solubility of the Rg3, PLGA-Rg3 mixed fiber tends to be relatively hydrophobic which is not conducive for cell growth on the surface of the fibers. Ultimately, this results in the fibrous membranes being incompatible with the surrounding tissue, which often results in inflammation of the tissue at the incipient stage. In addition, this hydrophobic three-dimensional electrospun fibrous membrane only functions as a drug delivery device, but cannot be membrane substrate for the induction of tissue repair. Thus, promoting cytocompatibility of the fibrous membranes to allow cell growth on the membrane, while simultaneously inhibiting fibroblast hyperplasia in the late stage, remains a challenge.

Chitosan (CS) derived from the crab and shrimp shell chitin by deacetylation, is a natural alkaline polysaccharide that is not only non-toxic, but also does not cause a rejection reaction and is biodegradable [22,23]. Additionally, CS has been shown to provide hemostasis, odynolysis, bacteriostasis, and promotes cell growth and accelerated wound healing [24]. Therefore, coating the surface of the hydrophobic fibers with CS can improve the surface hydrophilicity of the electrospun fibers. Currently, there are many techniques to introduce chitosan into electrospun fibers, such as sodium hydroxide hydrolysis and grafting-coating [25], photoinitiated surface-grafting polymerization [26] and aminolysis method [27,28]. In recent reports, PLGA-chitosan hybrid electrospun fibrous membranes were obtained by co-electrospinning [29] or coaxial electrospinning [30], which is better suited for fibrous membranes and drug delivery systems. However, CS easily dissolved into cell culture medium during the culturing accelerating the rate of drug release. Therefore, we considered the above methods as not suitable for a combination device that provides a substrate for cell growth and acts as drug vehicle, because excessive latter processes would (1) change the fibrous structure; (2) cause the dissolution of the drug in the fiber; (3) change the quality of the drug; and (4) introduce excessive other substances.

According to solution-diffusion and pore-flow transport models, pressure-driven permeation (PDP) is a one-component solution through a membrane, which has been widely used in the coating technology [31,32]. This technology is very fast, easy, and suitable for coating a polymer onto drug particles or membranes [33]. Therefore, PDP is more suitable for coating chitosan onto the surface of the drug containing fibers used in this report. Chitosan can be uniformly coated onto the fiber surface using PDP. Accordingly,

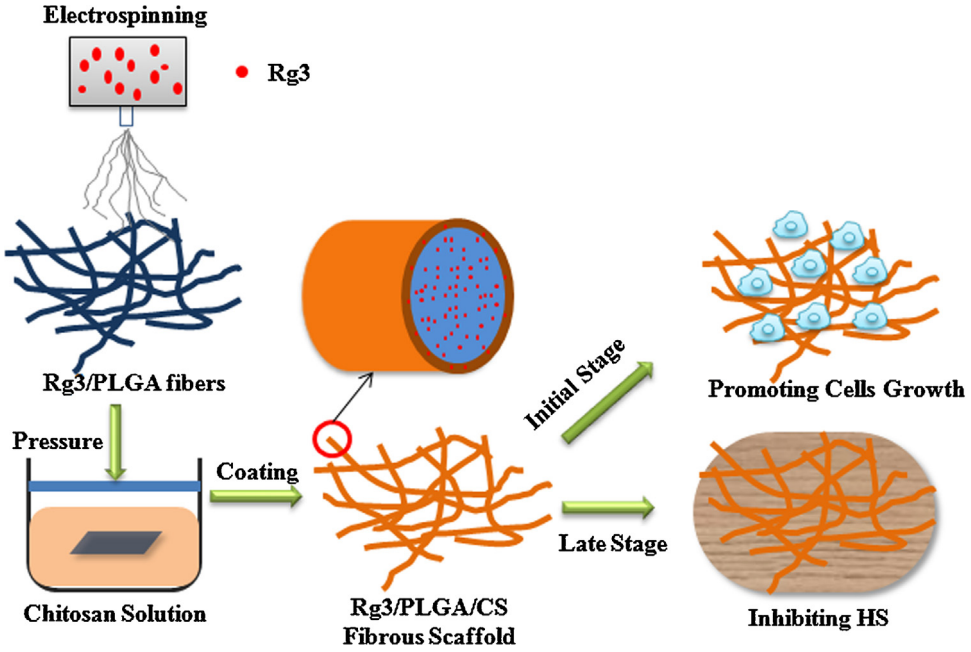

Fig. 1. Schematic illustration of Rg3 loaded PLGA electrospun fibrous membranes coating with chitosan via PDP method for promoting skin regeneration and inhibiting hypetplasia scar of skin.