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Localized controlled release of stratifin reduces implantation-induced dermal fibrosis

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ABSTRACT

Localized controlled release of anti-fibrogenic factors can potentially prevent tissue fibrosis surrounding biomedical prostheses, such as vascular stents and breast implants. We have previously demonstrated that therapeutic intervention with topically applied stratifin in a rabbit ear fibrotic model not only prevents dermal fibrosis but also promotes more normal tissue repair by regulating extracellular matrix deposition. In this work, the anti-fibrogenic effect of a controlled release form of stratifin was investigated in the prevention of fibrosis induced by dermal poly(lactic-co-glycolic acid) (PLGA) microsphere/poly(vinyl alcohol) (PVA) hydrogel implants. Pharmacodynamic effects were evaluated by histopathological examination of subcutaneous tissue surrounding implanted composites. Controlled release of stratifin from PLGA microsphere/PVA hydrogel implants significantly moderated dermal fibrosis and inflammation by reducing collagen deposition (30%), total tissue cellularity (48%) and infiltrated $CD3^+$ immune cells (81%) in the surrounding tissue compared with the stratifin-free implants. The controlled release of stratifin from implants markedly increased the level of matrix metalloproteinase-1 expression in the surrounding tissue, which resulted in less collagen deposition. These stratifin-eluting PLGA/PVA composites show promise as coatings to decrease the typical fibrosis exhibited around implanted biomedical prostheses, such as breast implants and vascular stents.

1. Introduction

Local fibrosis is one of the main factors that reduce the efficacy of implanted biomedical prostheses. Fibrous capsular contracture, appearing after implantation of breast implants, remains the greatest risk of this procedure. Various factors have been proposed to elicit capsular contracture, such as foreign body reaction, hematoma, subclinical infections and the physical–geometrical characteristics of the implant [1]. Nevertheless, the specific pathophysiology of this process remains unknown.

Localized elution of anti-fibrogenic factors and corticosteroids has been used to suppress inflammation and fibrosis associated with implantation and continuous in vivo residence of biomedical devices [2,3]. Anti-proliferative-eluting coronary stents (either sirolimus- or paclitaxel-eluting stents) in current clinical use have shown lower rates of myocardial infarction and restenosis by preventing fibrosis compared with bare-metal stents [4,5]. The advent of a locally controlled release of an anti-fibrogenic factor to prevent excessive scar formation would be an ideal strategy for the prevention of implant-induced fibrosis. The advantages of this local delivery system would be the need for only a low therapeutic dose and the avoidance of systemic application.

Stratifin (14-3-3σ protein; SFN) is a potent anti-fibrogenic factor that is involved in keratinocyte–fibroblast communication [6]. SFN is specifically expressed in stratified epithelial cells and increases the expression of matrix metalloproteinases (MMPs) in dermal fibroblasts [6,7]. It has been demonstrated that SFN enhances the expression of MMPs through the activation of a c-Fos and mitogen-activated protein kinase pathway [8]. MMPs are a group of diverse proteolytic enzymes that function to facilitate cell migration by breaking down collagen and other extracellular matrix components [9–11]. Collagen is the primary component of the extracellular matrix and is essential for tissue repair and regeneration; however, when expressed in excess, it can lead to fibrosis. The endogenous release of SFN from keratinocytes during normal wound healing appears to inhibit the net accumulation of collagen, thereby reducing scar formation. Although other agents, such as the cytotoxic drug suramin or the cytokine interferon alpha, can influence wound healing, these effects appear to be less precise and are associated with a number of potential systemic side effects even with local application, such as anticoagulation, immunosuppression [12], flu-like symptoms, headache, fever and muscle ache

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[13]. Moreover, SFN does not appear to result in non-healing wounds (within the doses examined), as has been observed with anti-proliferative agents. We have recently demonstrated that the topical application of recombinant SFN could prevent dermal fibrosis in a rabbit ear fibrotic model [14]. Thus, SFN could be an ideal biological factor for preventing fibrosis induced by implantation by regulating extracellular matrix production [15,16].

We previously developed a microsphere/hydrogel implant in which SFN-complexed chitosan particles were encapsulated in poly(lactic-co-glycolic acid) (PLGA) microspheres [17]. A hyaluronic acid hydrogel film was used as a carrier for the microspheres. This system had the advantage of controlling the release of SFN and maintaining the protein in its active, non-degraded, form [17]. The current work is an extension of our previous study toward the clinical application of a sustained release form of SFN for reduction of hypertrophic scarring. In the current study, we have improved our scaffold by using poly(vinyl alcohol) (PVA) hydrogel, which has two important advantages over the previous one: first, the use of PVA significantly reduces the swelling associated with the hyaluronic acid-based hydrogel; and second, it demonstrates a potential clinically applicable formulation for the release of SFN to reduce scarring. Furthermore, the composite implants modified using PVA are biocompatible, easy to handle and less expensive than the one developed previously. Using a rat incision model [18], we hypothesized that the controlled release of SFN from PLGA microsphere/PVA hydrogel dermal implants would reduce fibrosis following implantation.

2. Materials and methods

Ethics approval for this research was obtained from the University of British Columbia Clinical Research Ethics Board. All of the animal studies were reviewed and approved by the University of British Columbia Animal Care Committee.

2.1. Materials

Chitosan (ULTRASAN TM) (MW = 226,000, degree of deacetylation = 74.2%) (Biosyntech Inc., Canada), PLGA (85/15, IV = 0.61 dl g^{-1} (LACTEL, USA), PVA (98% hydrolyzed, low MW, 11,000–31,000) and PVA (86% hydrolyzed, medium MW, 57,000–66,000) (Alfa Aesar, USA) were used as supplied. Sodium borate was purchased from Sigma (St. Louis, USA).

PGEX-6P-1 expression vector, PreScission protease and glutathione Sepharose® 4B beads were obtained from GE Healthcare Bio-Sciences AB, Sweden. Protein-expressing bacteria, BL-21 (DE3), were purchased from Novagene, USA. EZ-Label™ fluorescein isothiocyanate (FITC) protein labeling kit (Pierce Biotechnology Inc., USA) and D-Salt™ dextran desalting columns (Pierce Biotechnology Inc., USA) were used as received.

2.2. Preparation of human recombinant SFN

Human recombinant SFN (MW = 30 Da, p*I* = 4.7) was prepared as previously described [6], with a slight modification. Briefly, the cDNA of SFN from human keratinocytes was cloned into a pGEX-6P-1 expression vector (Amersham/Pharmacia Biotech, USA) and transformed into the protein-expressing bacteria BL-21 (DE3). After lysing the bacteria, glutathione-S-transferase-fused SFN was purified by adding to glutathione Sepharose 4B beads and subsequently digested using PreScission protease. As has been shown before [6], the recombinant SFN protein was more than 95% pure.

2.3. Conjugation of SFN with FITC

For detection and quantification purposes, SFN was conjugated with fluorescein isothiocyanate. Fluorescence probe conjugation of SFN was done by EZ-Label™ FITC protein labeling kit, as previously described [17]. Analytical quantification of FITC-conjugated SFN (SFN-FITC) was done using Infinite™ F500 microplate reader (TECAN Trading AG, USA) set at excitation and emission wavelengths of 485 and 535 nm, respectively.

2.4. Preparation of PLGA microspheres containing chitosan particles

PLGA microspheres were prepared using a suspension–evaporation technique, as previously reported [17]. Briefly, for SFN-loaded microspheres, 5 mg of chitosan particles, smaller than 45 μm, was swelled in 30 μl of solution of SFN-FITC in phosphate buffer, pH 5.5 (SFN-FITC:chitosan, 1 μg:1 mg), with a molar ratio of 7.5:1.0. The suspension was vortexed and incubated at room temperature for 1 h to form ionic complexes. In order to remove the free/uncomplexed SFN from the complex, 500 μl of phosphate-buffered saline (PBS), pH 5.5, was added to the microtubes. The suspension was gently vortexed and centrifuged at 1000 rpm for 3 min. After the supernatant had been removed, the complexes were dried under nitrogen gas and the particles broken up into a fine powder using a mortar and pestle. SFN-complexed chitosan particles, smaller than 45 μm, were then encapsulated in PLGA using the suspension–solvent evaporation technique described below. Chitosan was chosen as a solid carrier to stabilize the SFN during the microencapsulation procedure in PLGA. SFN-complexed chitosan particles were dispersed in a 12.5% solution of PLGA in dichloromethane (chitosan:PLGA, 1:12.5 (w/w)). The mixture was vortexed for 30 s, then slowly pipetted into 100 ml of an aqueous solution of PVA 1% (w/v) and stirred at 400 rpm. After 2 h the microspheres were separated by gravity, washed three times with distilled water and dried under nitrogen gas for 1 h. The average size of the SFN-containing microspheres was 130 μm, and the overall encapsulation efficiency was 70%, using the method previously described [17].

2.5. Preparation of PVA composites

PVA (50:50 w/w, 98%:86% hydrolyzed) was dissolved in deionized water by heating to 80 °C to obtain a 10% (w/w) solution under aseptic conditions. Glycerol (2% w/v) was added as a plasticizer after the PVA dissolution. Composites were fabricated by casting the dispersion inside 2 × 1 cm slide chambers, then 1.2 ml of the 10% PVA solution was pipetted into the slide chambers and incubated at 37 °C for 1.5 h. To prepare the various PVA composites, following incubation, 100 mg of empty microspheres or SFN-loaded microspheres, 10 mg of unencapsulated SFN-complexed chitosan particles or 10 μl (1 μg μl^{-1}) of SFN solution was dispersed in the PVA solution. Borate solutions (4 mM) were prepared by dissolving sodium tetrahydroborate in deionized water, which was then gently added to the PVA solution in chambers without shaking. They were then incubated at −20 °C for 20 h to freeze them. Samples were then thawed at room temperature, and the excess borate buffer was decanted. Crosslinked PVA films were dried at 37 °C overnight. PVA films containing 10 μg of SFN-FITC, 10 mg of unencapsulated chitosan particles or 100 mg of PLGA microspheres were assessed for in vitro release studies.

2.6. SFN in vitro release studies

The in vitro release profiles of SFN were obtained from: (i) PVA hydrogels (PVA); (ii) chitosan particles embedded in PVA hydrogels (Chit/PVA); (iii) non-embedded PLGA microspheres (MS); and (iv)