Acta Biomaterialia 9 (2013) 4513–4524

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Controlling fibrous capsule formation through long-term down-regulation of collagen type I (COL1A1) expression by nanofiber-mediated siRNA gene silencing

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ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 22 September 2012
Accepted 24 September 2012
Available online 2 October 2012

Keywords:
RNA interference
Gene knockdown
Scaffold-mediated transfection
Cell penetrating peptides
Electrospinning

ABSTRACT

The foreign body reaction often interferes with the long-term functionality and performance of implanted biomedical devices through fibrous capsule formation. While many implant modification techniques have been adopted in attempts to control fibrous encapsulation, the outcomes remained sub-optimal. Nanofiber scaffold-mediated RNA interference may serve as an alternative approach through the localized and sustained delivery of siRNA at implant sites. In this study, we investigated the efficacy of siRNA–poly(caprolactone-co-ethylethylene phosphate) nanofibers in controlling fibrous capsule formation through the down-regulation of collagen type I (COL1A1) in vitro and in vivo. By encapsulating complexes of COL1A1 siRNA with a transfection reagent (Transit TKO) or the cell penetrating peptides CADY or MPG within the nanofibers (550–650 nm in diameter), a sustained release of siRNA was obtained for at least 28 days (loading efficiency ~60–67%). Scaffold-mediated transfection significantly enhanced cellular uptake of oligonucleotides and prolonged in vitro gene silencing duration by at least 2–3 times as compared to conventional bolus delivery of siRNA (14 days vs. 5–7 days by bolus delivery). In vivo subcutaneous implantation of siRNA scaffolds revealed a significant decrease in fibrous capsule thickness at weeks 2 and 4 as compared to plain nanofibers ($p < 0.05$). Taken together, the results demonstrated the efficacy of scaffold-mediated siRNA gene-silencing in providing effective long-term control of fibrous capsule formation.

1. Introduction

The foreign body reaction (FBR) at the tissue–implant interface frequently elicits inflammation, wound healing responses and tissue fibrosis [1–3]. In general, monocytes/macrophages are activated at implant surfaces and modulate local host fibroblast function. This often leads to excessive deposition of collagen matrix around implanted devices, a phenomenon known as fibrous encapsulation [1,2]. Consequently, the formation of fibrous capsule surrounding implants has limited their applications in the form of glial scarring around neural probes [4], fibrotic tissue formation surrounding mammary implants [5,6], loss of glucose biosensor functionality [7] and pacemaker failure [8]. While the modification of material surface chemistry/physics [9–12] and the incorporation of biological factors and proteins [13–17] have been developed to improve the biocompatibility of implanted devices, several potential drawbacks have also been reported. The use of a hydrogel-type coating, for instance, may display poor adhesion to the substrate and unacceptable mechanical properties for some applications, and can pose potential safety issues due to the use of chemical cross-linking agents [9,16]. The administration of anti-inflammatory agents, such as dexamethasone, while being able to minimize implantation-associated inflammation, can also inhibit endogenous blood vessel growth [17,18], thereby decreasing blood circulation surrounding the implant [15].

An alternative approach is to utilize RNA interference (RNAi) technology. RNAi by small-interfering RNA (siRNA) delivery has found useful applications in the treatment of cancer [19,20] and genetic diseases [21,22]. Its popularity stems from its ability to knock down virtually any gene of interest, leading to the specific down-regulation of the target protein. A potential target for modulating fibrous capsule formation by RNAi is collagen type I, the major component of fibrous tissues [1,2]. We have previously demonstrated the sustained delivery of siRNA from electrospun poly(caprolactone) [23] and poly(caprolactone-co-ethylene)

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http://dx.doi.org/10.1016/j.actbio.2012.09.029

(PCLEEP) nanofibers [24]. The encapsulation of siRNAs protected the degradation of these labile molecules over prolonged time periods and enhanced cellular uptake by seeded cells. Nanofiber scaffolds possess similar architecture as the fibrillar components of the native extracellular matrix. The biomimicking nature of these constructs may provide physical cues to direct cell fate [25,26]. In addition, nanofiber topography decreased in vivo fibrous capsule formation and enhanced host–implant integration as compared to smooth, non-porous two-dimensional surfaces [27]. We hypothesize that the sustained release of COL1A1 siRNA from these nanofibers would permit further control over in vivo fibrous capsule formation.

Recently, Takahashi et al. [28] demonstrated that delivery of siRNA against mammalian target of rapamycin (mTOR) from poly(ethylene glycol) (PEG)-based hydrogels decreased fibroblast proliferation and type I collagen mRNA expression in vitro. However, in vivo, this platform produced no significant reduction in fibrous capsule thickness and mTOR protein level. In this study, we evaluated the efficacy of COL1A1 siRNA-encapsulated PCLEEP nanofibers in reducing fibrous capsule formation through in vitro and in vivo analyses. Similar to our previous works, the transfection reagent TransIT-TKO was used to enable efficient cellular uptake. However, in order to resolve cytotoxicity issues related to TKO [24], cell penetrating peptides (CPPs) were introduced as an alternative for siRNA complexation. CPPs such as MPG and CADY are natural peptide-based molecules that mediate transfection through the formation of stable non-covalent complexes with nucleic acids, thereby improving intracellular delivery in vitro [29–33] and in vivo [29,34,35]. In addition, CPPs induced less cytotoxicity as compared to cationic lipid-based molecules [36–38] and cationic polymers [38–40]. We evaluated the functionality of PCLEEP nanofibers that encapsulated COL1A1 siRNA–CPP complexes. Such siRNA nanofibers may find useful applications as direct implantable scaffolds or surface modifications to improve tissue–implant integration of medical devices.

2. Materials and methods

Poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP, M_w: 94,000, M_n: 48,000), with 1% ethyl ethylene phosphate (EEP), was synthesized through bulk ring-opening polymerization of ε-caprolactone and EEP as reported previously [41,42]. Scrambled negative siRNA (denoted as siNEGCy5-labeled oligonucleotides (Cy5-ODN) diethylpyrocarbonate (DEPC)-treated phosphate-buffered saline (PBS, pH 7.4), DEPC-treated tris(hydroxymethyl)aminomethane–ethylenediaminetetraacetic acid (TE) buffer (pH 8.0), DEPC-treated water and CPPs (purity >90%) were purchased from 1st Base, Singapore. The CPPs MPGΔ^{NLS} (GALFLGFLGAAGSTMGAWSQPKSKRKV) and CADY (GLWRALWRLLRSLWRLLWRA) were acetylated at their N-terminus and synthesized with a cysteamide group at their C-terminus. Silencer® COL1A1 siRNA (denoted as siCOL1A1) targeting the human (NM_000088.3) and rat (NM_053304) COL1A1 genes was purchased from Ambion (ID #: s3276), USA. The transfection reagent TransIT-TKO was obtained from Mirusbio, USA. RiboGreen® reagent Quanti-IT™ RiboGreen, TRIzol® reagent, 40,6-diamidino-2-phenylindole (DAPI), Oregon Green® Phalloidin 488 and Alexa Fluor® 488 donkey anti-goat IgG were purchased from Invitrogen, USA. Ethidium bromide solution and iQ SYBR Green Supermix were purchased from Bio-Rad Laboratories, USA. Goat anti-human collagen type I was purchased from Millipore, USA. Human dermal fibroblasts (HDFs) were purchased from Lonza, Basel, Switzerland. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Hyclone, USA. Penicillin–streptomycin (10,000 U ml^{-1}), antibiotic–antimycotic and PBS (pH 7.4) were purchased from Gibco, Invitrogen, USA. RQ1 RNase-free DNase and Sensiscript® RT kit were purchased from Promega, USA, and Qiagen Germany, respectively. Poly(ε-capolactone) (PCL, M_w: 65,000), bovine serum albumin (BSA), 2,2,2-trifluoroethanol (TFE, ⩾99.0), tetrahydrofuran (⩾99.9), chloroform (⩾99.9), dimethyl sulfoxide (DMSO), Triton X-100, 10% formalin and 100% ethanol were obtained from Sigma-Aldrich, USA. Aerrane® isoflurane was obtained from Baxter Healthcare Corporation, Deerfield, IL, USA. Betadine was obtained from The Purdue Frederick Co., Stamford, CT, USA. 0.5% Marcaine solution was obtained from Abbott Laboratories, North Chicago, IL, USA. All chemicals were used as received without any further purification.

2.1. Electrospinning of siRNA-encapsulated PCLEEP nanofibers

Plain PCLEEP nanofibers (control group denoted as PCLEEP) and PCLEEP nanofibers encapsulating siRNA/TKO complexes corresponding to a volume ratio of 1/2 (denoted as siNEG/TKO and siCOL1A1/TKO when scrambled negative siRNA and siRNA targeting COL1A1 were added, respectively) were fabricated according to our previous work [24].

To obtain PCLEEP nanofibers that encapsulated siRNA/CPP complexes, a 20% w/v PCLEEP–TFE solution was prepared. siNEG or siCOL1A1 was reconstituted in RNase-free water to obtain a stock solution of 50 μM concentration. Thereafter, 15 μl of siRNA was mixed with either 30, 45 or 60 μl of CPP (350 μM of MPG in DEPC-treated water and 370 μM of CADY in 2% DMSO–DEPC-treated water) to obtain volume ratios of 1/2, 1/3 or 1/4 respectively. Thereafter, the mixture was incubated for 20 min and DEPC-treated TE buffer was then added to obtain a final volume of 100 μl. The siRNA/CPP mixture was then added into 500 μl of PCLEEP solution. The uniform siRNA/CPP–polymer mixture was dispensed using a syringe pump (New Era Pump) at a flow rate of 1.5 ml h^{-1} through a 21G needle and charged at +12 kV (GAMMA high voltage research, USA) for electrospinning. The polymer supply was set at 12 cm away from the target. A negatively charged stationary aluminum foil (−4 kV, 5 × 5 cm^2) was used as the target for randomly oriented nanofibers. In order to obtain aligned PCLEEP nanofibers for in vivo studies, the fibers were deposited directly onto a PCL film that was mounted on a grounded rotating target (2500 rpm). The spinning process was carried out at 20–23 °C and the humidity was 54–58%. The PCL film was obtained by solvent casting 0.15 g ml^{-1} of PCL–chloroform solution overnight, followed by lyophilization for 12 h to remove any residual solvents. PCLEEP nanofibers that encapsulated complexes of siNEG with MPG or CADY, and siCOL1A1 with MPG or CADY, were fabricated and denoted as siNEG/MPG, siNEG/CADY, siCOL1A1/MPG and siCOL1A1/CADY, respectively. Table 1 summarizes all electrospun nanofiber samples that were prepared for this study, along with their notations and processing parameters.

2.2. Evaluation of scaffold morphology and ODN distribution

PCLEEP, siCOL1A1/MPG, siCOL1A1/CADY and siCOL1A1/TKO nanofiber scaffolds were sputter coated with platinum and evaluated by scanning electron microscopy (SEM) (JOEL, JSM-6390LA, Japan) at 5000× magnification. The average fiber diameters were then determined using ImageJ (NIH, USA) by measuring 100 fibers per sample. In order to evaluate the distribution of siRNA inside PCLEEP nanofibers, Cy5-ODN was used. Briefly, Cy5-ODN-encapsulated (1/4 volume ratio) nanofibers were incubated with 500 μl of complete cell culture medium for 24 h. Thereafter, the scaffolds were washed three times with PBS, mounted onto glass slides using fluoromount and imaged by confocal microscopy (Zeiss, LSM 710 Meta Laser Scanning Confocal Microscope, Germany).