Local Delivery of CTGF siRNA with Poly(sorbitol-co-PEI) Reduces Scar Contraction in Cutaneous Wound Healing

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Abstract Healing process in scarring inevitably produces a considerable amount of non-organized dense collagen-rich matrix called scar thus impairing the native structure of skin. Connective tissue growth factor (CTGF) overexpression within healing tissues is known to play an imperative role in collagen production stimulated by transforming growth factor-beta in cutaneous wound healing. Undoubtedly, the knockdown of CTGF expression through siRNA-mediated gene silencing could simply impede the scarring process. However, the less stability and low transfection of siRNAs themselves urge a safe carrier to protect and transfect them into cells at a high rate avoiding toxicities. Here, we developed a degradable poly(sorbitol-co-PEI) (PSPEI), prepared by polymerization of sorbitol diacrylate with low molecular weight polyethylenimine, which has high transfection efficiency but low cytotoxicity, and utilized it in siCTGF delivery to silence the expression of CTGF in an animal model of cutaneous wound healing. Unlike contracted scar in normal healing, there was no or less contraction in the healed skin of mice treated with siCTGF using PSPEI. Histologically, the healed tissues also had distinct papillary structures and dense irregular connective tissues that were lacking in the control scar tissues. This study exemplifies a successful treatment of cutaneous wound healing using a polymer system coupled with RNA interference. Hence, the approach holds a great promise for developing new treatments with novel targets in regenerative medicines.

Keywords Connective tissue growth factor · Poly(sorbitol-co-PEI) · siRNA · Scar contraction · Wound healing

1 Introduction

Wound healing is the interaction of a complex cascade of cellular events in response to injury [1]. A simple goal of cutaneous wound healing is to restore a protective layer on

the skin to reduce risk of infection and regenerate the normal functions of tissue [2, 3]. But, the occurrence of physiological and psychological effects of scar formation after healing of wounds is a major problem of patients [4–6]. Given the major health problems that result from scarring, there has been a growing interest in scarless healing of wounded skin. While the current treatment by topical steroids for the scars provide little comfort to the patients with potential serious side-effects, surgical resection is flawed with considerable risk of infection and low success rate [7, 8].

Generally, wound healing proceed in an organized way and it consists of four stages including hemostasis, inflammation, proliferation and remodeling [9]. Among them, proliferation and remodeling are particularly important in determining the scar formation because they are related with the production of the extracellular matrix

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(ECM) and their reorganization [10–12]. But inevitably, the healing process in scarring largely produces a non-organized dense collagen-rich matrix due to high contraction by myofibroblasts thus damaging the native structure of the skin [13, 14]. Besides, scar tissue lacks many of the fundamental structures such as papillary structures, hair follicles and adipocytes found in skin impairing the healing process to revive the normal functions of skin. Hence, understanding the mechanism underlying the wound healing is of great importance to scientists involved in skin tissue engineering to make wounds heal without scars.

Over the past few decades, there have been extensive studies on discovering the close association of the upregulated expression of transforming growth factor-beta (TGF β) with the increased fibrosis in animal studies of cutaneous scarring [15]. Evidently, there are several reports now which demonstrated that the inhibition of TGF β *in vivo* reduces scar formation and impairs wound healing [16]. However, a deep look into the molecular signaling of wound healing revealed that connective tissue growth factor (CTGF) is a key mediator of scar formation through TGF β signaling to assist in stimulation of collagen which is generally overexpressed in fibrotic diseases including dermal scarring [17, 18]. Besides, CTGF has been demonstrated to play an important role in regulation of key cellular processes within wound healing such as cell proliferation, myofibroblast differentiation and ECM production [19]. Recently, several attempts have been made to reduce scar formation by targeting the expression of CTGF using anti-sense RNA therapy [20, 21]. Although these attempts have shown siRNA-specific reduction in CTGF expression leading to substantial changes in scar formation to some extent, the major drawback of these methods is the direct injection of naked siRNA which is vulnerable to rapid degradation and clearance from the target tissue in the body, thus reducing its efficacy. Hence, there is an urgent need to develop more effective method for siRNA delivery in the treatment of scarring.

In this study, we aimed to deliver CTGF siRNA by poly(sorbitol-co-PEI)(PSPEI) to reduce scar contraction during cutaneous wound healing. PSPEI is an osmotically active gene transporter that has higher transfection efficiency and gene silencing effect *in vitro* and *in vivo* due to the successful avoidance of lysosomal degradation of the cargo through stimulation of caveolae-mediated endocytosis mechanism [22]. Here, we illustrate the efficacy of PSPEI for siRNA delivery in a mouse model of cut wound healing and analyze the differences in scar formation by histological sections.

2 Materials and methods

2.1 Materials

Branched low molecular weight polyethylenimine (PEI) (molecular weight: 600 Da), methyl thiazolyl diphenyl-tetrazolium bromide (MTT) reagent and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sorbitol diacrylate was purchased from Monomer-Polymer & Dajac Labs (Trevose, PA, USA). pGL3-control vector, cell lysis buffer and luciferase assay reagent were purchased from Promega (Madison, WI, USA). HiSpeed plasmid maxi kit for plasmid purification was purchased from Qiagen (Germantown, MD, USA). TurboGFP (tGFP) encoding green fluorescent protein for monitoring protein expression was bought from Origene (Rockville, MD, USA). Roswell Park Memorial Institute (RPMI)-1640 culture medium, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were bought from HyClone (Logan, UT, USA). Opti-MEM[®] I Reduced Serum Medium and UltraPure[™] DNase/RNase-Free Distilled Water were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All siRNAs used in this study were prepared by Bioneer (Daejeon, Korea) which are shown in Table 1.

2.2 Synthesis and characterization of polymer

A polymerization reaction between sorbitol diacrylate (68 mg) and PEI (141 mg) in a 1:1 molar ratio was carried out in anhydrous DMSO (10 ml) with continuous stirring under an inert atmosphere of nitrogen at 80 °C for 24 h. The reaction product, poly(sorbitol-co-PEI) (PSPEI) was purified using a dialysis membrane (MWCO:3500) with continuous stirring in water at 4 °C for 24 h, and finally freeze-dried for further characterization. The composition of polysorbitol and PEI in PSPEI was measured by ¹H nuclear magnetic resonance spectroscopy (¹H NMR, Avance 600, Bruker, Germany). Molecular weight of PSPEI was measured by Waters Gel Permeation Chromatography (GPC) equipped with TSKgel G5000PWXL-CP columns. The column temperature was kept at 35 °C

Table 1 The sequences of siRNAs used in this study

siRNA	Sequence (5'-3')
CTGF1 sense	GUUUGAGCUUUCUGGCUGC
CTGF1 antisense	GCAGCCAGAAAGCUCAAAC
CTGF Scr sense	AUUAACGCCGAGUCGACCAUC
CTGF Scr antisense	GAUGGUCGACUCGGCGUAAU

with a flow rate of 1.0 ml/min using a mobile phase of 0.1 M NaNO₃.

2.3 Polyplex formation and dissociation

The ability of PSPEI to bind siRNA was examined by gel retardation assay. First, siRNA (0.2 mg/ml) was mixed with PSPEI at various N/P ratios (amino group of polymer per phosphate group of siRNA), and incubated at room temperature for 10 min to make polymer/siRNA complexes (polyplexes). The polyplexes were loaded into the agarose gel (0.6%, w/v) containing ethidium bromide (0.6 mg/ml) and run in gel electrophoresis with TAE buffer (40 mM Tris–acetate, 1 mM EDTA) at 100 V for 30 min. Finally, the formation of polyplexes was observed under ultraviolet (UV) illumination. To observe the dissociation of siRNA from polyplexes, heparin competitive displacement assay was performed. First, siRNA (0.2 mg/ml) was mixed with polymer at N/P ratio 10, and incubated at room temperature for 10 min to make polyplexes. The polyplexes were further incubated with various concentrations of heparin solutions for 10 min and run into agarose gel in electrophoresis with TAE buffer at 100 V for 30 min. Finally, the release of siRNA from the polyplexes was observed under UV illumination.

2.4 Particle size and zeta potential measurements

The particle sizes and zeta potentials of polyplexes were measured by an electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics, Japan). The polyplexes with siRNA were prepared at N/P ratio of 10 in 1 ml of distilled water and diluted with 1 ml of Opti-MEM medium. The particle sizes and zeta potentials of the polyplexes were measured at the scattering angle of 90° and 20°, respectively, in the ELS instrument.

2.5 Transmission electron microscopy

Polyplexes with siRNA at N/P ratio of 10 were prepared as described before. The polyplexes (10 µl) were put on formvar-coated copper grids and incubated for 60 s. The polyplexes on the grids were washed with water, stained with uranyl acetate (1%, w/v) and dried on air. Finally, images of the polyplexes were recorded with an energy-filtering transmission electron microscopy (EF-TEM) (LIBRA 120, Carl Zeiss, Germany) operated at 80 kV.

2.6 Cell culture and cell viability

Human lung adenocarcinoma epithelial cells (A549) were cultured in RPMI medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin. All cells

were cultured under standard incubation condition at 37 °C with 5% CO₂. To determine the cytotoxicity of polyplexes, cells were seeded at 5×10^4 cells/well in 24-well plates and were incubated for 24 h. The cells were treated with polyplexes prepared at various N/P ratios in Opti-MEM medium. After 4 h of treatment, the cells were incubated with fresh culture medium for 20 h under standard incubation condition. To count the number of viable cells, the incubated cells were treated with MTT at a final concentration of 0.5 mg/ml for 3 h at 37 °C. After removing the medium from the plates, the purple formazan produced due to reduction of MTT in living cells were dissolved with DMSO. The samples from each well of the plate was transferred to a 96-well plate to measure the absorbance at 570 nm using Infinite 200 PRO multimode reader (Tecan, Switzerland).

2.7 Gene silencing and luciferase assay

To determine the gene silencing efficiency of polyplexes, cells were seeded at 5×10^4 cells/well in 24-well plates and were incubated for 24 h. The cells were transfected with polyplexes (N/P = 10) prepared together with pGL3 control vector (that encodes for firefly luciferase) and Luc siRNA (that knockdowns luciferase gene expression) in Opti-MEM medium. After 4 h of transfection, the cells were incubated with fresh culture medium for 20 h under standard incubation condition. After the aspiration of the medium from each well, the cells were washed with PBS and treated with cell lysis buffer. Following the lysis of the cells, the cells were collected and centrifuged at $13,000 \times g$ to separate the luciferase proteins from the cell lysates. The amount of proteins in the cell lysates was determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL USA). The isolated proteins were analyzed for luciferase activity using a luciferase assay reagent. The luminescence produced from luciferase activity of protein was measured by a chemiluminometer of Infinite 200 PRO multimode reader. The luciferase activity is expressed as relative light units (RLUs) normalized with the amount of protein concentration in the cell lysates.

2.8 In vivo siRNA delivery

Six-week old female BALB/c mice were used for animal study. The mice provided by Samtako, Co. Ltd. (Osan, Korea) were housed and cared in accordance with the guidelines of Seoul National University for the care and use of laboratory animals. Five groups of mice (3 mice/group) were used. Prior to wounding, hairs were removed from the backs of the mice using an electric shaver. A wound of 5 mm × 5 mm size was made on the surface of the skin with the help of a blade and scissors.

Polyplexes were prepared at $N/P = 10$ with each siRNA (1 nM) for each dose. Each mouse immediately received a dose of treatment through an injection underneath the wound and the wound was covered with a dressing tape. The tapes on the wounds were changed on alternate days. Each mouse received total three treatments, one dose per week (Scheme 1). The images of wounds were taken before and after the treatment.

2.9 Histology

The wound (scar) was spotted before sacrificing the mouse. The mice were sacrificed on the fourth week and the scar tissues from healed wound were isolated for histological analysis. The tissues were fixed in 10% neutral buffered formalin, paraffin-processed, and sectioned at 5 μ m. The tissue sections were fixed on glass slides and stained with hematoxylin and eosin (H&E).

2.10 Statistical analysis

All values were represented as mean $\pm$ SEM. Statistical analysis was performed between groups using Student's *t* test and compared between multiple groups by one-way analysis of variance (ANOVA). Statistical significance is denoted by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3 Results

3.1 Synthesis and characterization of polymer

PEI 25kD is generally used as a standard non-viral vector for gene delivery due to its high efficiency of gene transfection in cells. However, the non-degradable nature of PEI 25kD results in high cytotoxicity. To overcome the

limitation of high molecular weight of non-degradable PEI, we performed a polymerization reaction between sorbitol diacrylate and low molecular weight PEI 600 to produce PSPEI with degradable ester linkages. The proportions of polysorbitol and PEI in PSPEI, as calculated by ^1H NMR measurement, were 46 and 54 mol%, respectively (Fig. 1). As measured by GPC, the molecular weight of PSPEI was 6,959 Da (~ 7 kD) with polydispersity index (Mw/Mn) of 1.3.

3.2 Complexation of polymers with siRNA

Gene delivery vectors usually bind siRNA through electrostatic interaction [23]. Therefore, the capability of PSPEI to condense siRNA was analyzed by gel retardation assay. The complexation of PSPEI with siRNA at various N/P ratios was made, run in a gel and analyzed by UV illumination (Fig. 2). The results showed that the migration of siRNA with polyplexes at $N/P = 5$ or 10 was completely retarded in the gel leading to the conclusion that PSPEI binds siRNA completely at $N/P = 10$. Thus, further experiments of PSPEI were carried out at $N/P = 10$. Another important property of gene delivery vectors is to release the siRNA from polyplexes once they reach inside cells. Heparin displacement assay was used to observe the release of siRNA from PSPEI. Initially, polyplexes were made, incubated with heparin and observed in agarose gel after electrophoresis. It was observed that about 90% of siRNA was released from PSPEI at 5 mg/ml of heparin used (Fig. 2).

3.3 Charge and size of polyplexes

The surface charge and size of the polyplexes were examined by ELS. Initially, the polyplexes at $N/P = 10$ were formulated in water and allowed to incubate for

Scheme 1 Schedule of wound healing experiment on mouse

Fig. 1 ^1H NMR of PSPEI. The respective peaks of PEI and sorbitol are shown

Fig. 2 Condensation and release of siRNA by PSPEI. Gel retardation assay shows the binding of siRNA by PSPEI at various N/P ratio (A) and release of siRNA from PSPEI after heparin treatment (B)

10 min. The polyplexes thus formed were further incubated in the transfection medium (Opti-MEM). ELS measurements showed that the polyplexes were positively charged with the zeta potential value of 4.17 mV while the sizes of the polyplexes were around 460 nm (Fig. 3). The sizes of polymeric nanoparticles by DLS measurement are usually higher than their actual sizes [24]. Besides, TEM analysis demonstrated the compact but irregular shapes of the polyplexes with sizes ranging from 200 to 300 nm. These results also indicated the stability of polyplexes in the transfection medium.

3.4 Toxicity of polyplexes

To determine the toxicity of polyplexes, A549 cells were treated with the polyplexes as described in materials and methods. MTT assay results showed that the polyplexes of PSPEI have low cytotoxicity than the polyplexes of PEI 25kD at all N/P ratios (Fig. 4). At N/P of 20, the cell viability was >80% and <30% with the treatment of PSPEI and PEI 25kD, respectively. The low toxicity of PSPEI was certainly due to its low molecular weight of the PEI after degradation of the ester linkages by hydrolysis. The results

confirmed that PSPEI was safer than PEI 25kD for gene delivery.

3.5 Silencing efficiency of polyplexes

To determine the efficiency of PSPEI for gene silencing, A549 were transfected with the polyplexes (N/P = 10) as described in materials and methods. Luciferase assay experiments showed that PSPEI had relatively higher transfection efficiency (luciferase activity) than PEI 25kD when no siRNA (siRNA 0) was treated (Fig. 5). It meant, without siRNA treatment, the transfection of luciferase vector by PSPEI led to higher expression of luciferase proteins which produced high luciferase activity. But, when siRNA (siRNA 1X) was treated during luciferase vector transfection, the luciferase activity reduced by 49 and 63% in PSPEI and PEI 25kD, respectively, due to luciferase gene silencing by siRNA used. Because the transfection efficiency of PSPEI was relatively higher than PEI 25kD, the gene silencing by PSPEI seemed to be lower than that of PEI 25kD. However, when the double amount of siRNA (siRNA 2X) was used during transfection, the luciferase activity

Fig. 3 Size and zeta potential of PSPEI polyplexes. **A** Size measurement by DLS; **B** Morphology observation by TEM, Scale bar 0.5 μm; and **C** Zeta potential measurement by ELS

reduced by 85 and 83% in PSPEI and PEI 25kD, respectively.

3.6 *In vivo* siRNA delivery

Six-week old female BALB/c mice were used for *in vivo* CTGF siRNA (siCTGF) delivery. Five groups of mice were used: (1) PSPEI/siCTGF, (2) PEI 25kD/siCTGF, (3) PSPEI/siScr, (4) siCTGF, and (5) saline (control). Three mice were used in each group. A wound of 5 mm x 5 mm size on the surface of the skin was created in each mouse. A dose of treatment, in accordance with the group, was immediately injected underneath the wound of the mice and the wound was covered with a dressing tape. A total of three treatments were given to each group of mice at the

interval of 3 weeks. The images of wounds were taken before and after the treatments (Fig. 6). The images demonstrated that there was severe skin contraction during wound healing in the control group while the skin contraction seemed reduced in the mice treated with siCTGF. Interestingly, the contraction was very less in the mice treated with PSPEI/siCTGF. The other groups of mice treated with PEI 25kD/siCTGF and PSPEI/siScr had high skin contractions. For quantification, the area of scars was measured and plotted. The area of scar on the skin of mice treated with PSPEI/siCTGF was smooth and significantly greater than the other groups with contracted surface area of scars (Fig. 7). From these results, it was evident that the low skin contraction on PSPEI/siCTGF-treated mice was due to collective effect of PSPEI and siCTGF.

Fig. 4 Cytotoxicity of PSPEI polyplexes. A549 cells were exposed to polyplexes for 4 h. The cell viability was evaluated by MTT assay. The values were expressed as percentage of control, which is set to 100%. The data was presented as mean $\pm$ SEM, n = three independent experiments

Fig. 5 Gene silencing efficiency of PSPEI polyplexes. The gene silencing effect was measured by the knockdown of luciferase expression due to siRNA treatment. Luminescence was measured after 24 h of transfection by luciferase assay. The data was presented as mean $\pm$ SEM, n = three independent experiments

Fig. 6 Representative images of wounds before and after treatment. Both scar area and lateral width were higher in PSPEI/siCTGF treated group, indicating reduced wound contraction. The data was presented as mean $\pm$ SEM, n = three independent experiments

Fig. 7 Quantification of surface area of wound scar images after 3 weeks of treatment. Scar area is clearly elevated in PSPEI/siCTGF wound compared to other groups, demonstrating significantly reduced contraction (* p < 0.05, ** p < 0.01 compared with control, siCTGF and PSPEI/siScr and # p < 0.05 compared with PEI/siCTGF). The data was presented as mean $\pm$ SEM, n = three independent experiments

3.7 Histological evaluation

After 3 weeks of treatment, the scar tissues were isolated, sectioned and stained. Histological analysis of the cutaneous scar tissues demonstrated structural differences between siCTGF-treated scar and control group (Fig. 8). The healed tissue within the all siCTGF-treated groups, irrespective of the delivery carriers, contained papillary structures, present between the epidermis and the dermis. The papillary structures were not observed in the healed tissues of control groups including PSPEI/siScr-treated group. Although the clear difference was not appeared in the epidermal layer of the scar tissues, there were noticeable differences in the dermis region of the tissue sections. While some adipose tissues were prominent in the histological sections of siCTGF- or PEI 25kD/siCTGF-treated groups, the dense irregular connective tissues were also

Fig. 8 Representative images of histology of scar tissues. Skin sections stained with H&E to analyze scar formation during wound healing. The papillary structures are shown with white arrows. Scale bar 100 μ m

distinctly visible in the histological section of PSPEI/siCTGF group. These structures were not seen in the scar sections of control and PSPEI/siScr-treated groups.

4 Discussion

Whether it is from surgery, burn, or injury, scar makes irreparable mark of wound on the body. Although time heals the wound, the scar does not just fade away. Nothing can be done for scars once they are formed, hence researchers have been figuring out the key biological factors of scar production to make wounds heal as normal skin. Although the studies have disclosed the mechanism of wound healing, the treatment for scarring has been confined due to the lack of specific targeting of these factors with chemical drugs. Alternatively, RNA interference provides a promising strategy for controlling the biological factors at gene level. However, the difficulties of site-specific delivery of siRNAs and their side effects are other challenges. While local delivery of siRNAs at the site of interest can limit the doses and off-target effects of siRNAs, the approach encounters with the major problems of low transfection efficiency of siRNAs and their rapid clearance from the body. Hence, an effective delivery system is required to overcome the instability and low transfection efficiency of siRNA.

Polymers represent a class of materials that have been extensively used for gene carriers because of their ability to condense DNA into nano-sized particles that can be easily taken up by cells. Polymers not only protect the DNA from degradation but also release the DNA inside cells maintaining its activity [25, 26]. Hence, there have been innumerable reports of successful gene delivery, both *in vitro* and *in vivo*, with a vast array of polymers [23, 27, 28]. With the advent of RNA interference, the polymers have been utilized to deliver siRNA into cells with high success rate [29, 30]. Although the polymers hold enormous potential as promising carriers for DNA and siRNA, they suffer from several limitations such as high cytotoxicity and low transfection efficiency. To overcome these defects of polymers, we have recently developed a copolymer of polysorbital and low molecular weight PEI (PSPEI). We took the advantage of sorbitol as an extender unit in the

formation of PSPEI in part to reduce the cytotoxicity of PEI while polysorbital also induces the osmotic activity of PSPEI to escape from endosomes. Unlike PEI 25kD, PSPEI is low molecular weight polymer (7 kD) and it is degradable in nature. Hence, PSPEI had comparably lower cytotoxicity than PEI 25kD when treated to cells. Notably, PSPEI had relatively higher DNA transfection efficiency than PEI 25kD as demonstrated by luciferase assay. The result of gene silencing *in vitro* also revealed the efficient delivery of siRNA by PSPEI into cells. We previously used similar PSPEI for siRNA-mediated gene silencing to suppress the tumor growth in xenograft mouse models of lung cancer [22]. The remarkable success of these experiments led us to explore PSPEI in other biomedical applications. To this end, there have been relatively few attempts to develop polymer systems for local delivery of siRNA to cutaneous wound healing. It was, therefore, a grand opportunity to study the possibility of treatment of scarring by local delivery of siRNA using our polymer system.

CTGF, among many other functions, plays a key role in scar formation during cutaneous wound healing. This role of CTGF in scarring is evidently supported by its higher expression during wound healing. Hence, we targeted to silence the expression of CTGF to reduce the scar formation as wound heals. In order to corroborate our hypothesis, we first made a mouse model with small wound of square shape by peeling the top layer of skin at the backside of the mouse. We treated a calculated dose of siCTGF on the cut wound of the group of mice for 3 weeks as per experimental design. The results of the experiment have demonstrated a prominent change in scar formation in the group of mice treated with siCTGF delivered by PSPEI, where the healed skin had very less contraction. The healed skin of mice treated with only siCTGF also had diminished contraction but the effect was not profound. A similar experiment carried out by direct bolus injection of naked siRNA resulted in significant reduction in CTGF expression leading to substantial changes in scar formation [31]. It is well documented that naked siRNA, when delivered systematically or locally, are prone to rapid degradation or clearance from the body reducing its efficacy. As stated earlier, PSPEI protects siRNA during local delivery and releases inside the cells to mediate the target gene silencing. Consistent with the assumption, the outcomes of

cutaneous wound healing by siCTGF delivered with PSPEI was remarkable and hence promising.

Histologically, scar tissue is deprived from many of the vital structures found in the skin including papillary structures, hair follicles and adipocytes preventing the healing tissue to regenerate and restore the normal functions of skin [32]. Importantly, papillary structures play a key role in providing a strong connection between the epidermis and the dermis. Our experiment on the histology of healed tissues showed that there were clear differences in the scar tissues between siCTGF-treated group and control group. A prominent difference was the presence of papillary structures between the epidermis and the dermis in all siCTGF-treated groups, regardless of carriers to deliver siCTGF. These structures were not observed in any of the control group. The absence of such clear papillary structures in the histological images of siScr-treated group further supported the role of siCTGF in wound healing. In addition to these evidences, the study of remodeling and reorganization of the healing tissues would provide deep insight into the process of wound healing and scar formation. Further investigation is required to unveil the mechanism of collagen remodeling with respect to CTGF expression and the formation of scar margin with the level of contractile forces within the tissue.

In summary, we have presented an effective approach to induce siRNA-mediated gene silencing of a key mediator in cutaneous wound healing *in vivo*. The approach consists of a degradable polymer that efficiently transfects siRNA into the target cells avoiding toxicity, to silence CTGF, a protein associated with the production of dense collagen-rich matrix during scar formation. We illustrated the efficacy of the approach using a cut wound healing model *in vivo* and demonstrated the scar tissue with less contraction, which otherwise is considerably contracted in normal tissue healing. This study exemplifies a successful treatment of cutaneous wound healing using a polymer system coupled with RNA interference. Hence, the approach holds a great promise for developing new treatments with novel targets in regenerative medicines.

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Compliance with ethical standards

Conflict of interest The authors have no potential conflicts of interest.

Ethical statement This study was conducted under the approval of animal ethics committee at Seoul National University (SNU-130520-7).

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