findings and our data imply that IL-17 and IL-27 may have opposite effects in various *in vivo* situations. The significance of our findings might be relevant to other systemic inflammatory diseases that involve TNF-α, including Crohn's disease and ulcerative colitis; both IL-17 and IL-27 are expressed in the affected mucosa, and many chemokines are suggested to be involved in the pathogenesis (17–19).

In conclusion, we found that IL-17 and IL-27 exert opposite effects on the TNF-α-mediated chemokine production in human keratinocytes. This result suggests that lesional levels of IL-17 and IL-27 may be involved in the recruitment of neutrophils, dendritic cells, monocytes, T cells or NK cells, thereby affecting inflammation in skin diseases. Although further studies using other cells are needed, the paired effects of these two cytokines might be relevant to other TNF-α-mediated inflammatory diseases.

Acknowledgements

Susumu Fujiwara performed the research; Susumu Fujiwara and Hiroshi Nagai analysed the data; Hiroshi Nagai, Shuntaro Oniki and Takayuki Yoshimoto designed the research study; and Hiroshi Nagai and Chikako Nishigori wrote the article. This work was in part supported by Grand-in Aid for Scientific Research (H.N.) and by the Support Programme for Improving Graduate School Education from the Ministry of Education, Culture, Sports, Science and Technology of Japan (S.F.).

Conflict of interests

The authors have no conflicts of interest to declare.

References

1 Giustizieri M L, Mascia F, Frezzolini A *et al.* J Allergy Clin Immunol 2001: **107**: 871–877.
2 Gottlieb A B, Chamian F, Masud S *et al.* J Immunol 2005: **175**: 2721–2729.
3 Ottaviani C, Nasorri F, Bedini C *et al.* Eur J Immunol 2006: **36**: 118–128.
4 Bradley J R. J Pathol 2008: **214**: 149–160.
5 Jurisic V, Terzic T, Colic S *et al.* Oral Dis 2008: **14**: 600–605.
6 Jurisic V, Bogdanovic G, Kojic V *et al.* Ann Hematol 2006: **85**: 86–94.
7 de Groot M, Teunissen M B, Picavet D I *et al.* Exp Dermatol 2010: **19**: 754–756.
8 Lowes M A, Kikuchi T, Fuentes-Duculan J *et al.* J Invest Dermatol 2008: **128**: 1207–1211.
9 Shibata S, Tada Y, Kanda N *et al.* J Invest Dermatol 2010: **130**: 1034–1039.
10 Albanesi C, Cavani A, Girolomni G. J Immunol 1999: **162**: 494–502.
11 Lee J W, Wang P, Kattah M G *et al.* J Immunol 2008: **181**: 6536–6545.
12 Wittmann M, Zeitvogel J, Wang D *et al.* J Allergy Clin Immunol 2009: **124**: 81–89.
13 Koga C, Kabashima K, Shiraishi N *et al.* J Invest Dermatol 2008: **128**: 2625–2630.
14 Numasaki M, Fukushi J, Ono M *et al.* Blood 2003: **101**: 2620–2627.
15 Shimizu M, Shimamura M, Owaki T *et al.* J Immunol 2006: **176**: 7317–7324.
16 Stumhofer J S, Laurence A, Wilson E H *et al.* Nat Immunol 2006: **7**: 937–945.
17 Sugihara T, Kobori A, Imaeda H *et al.* Clin Exp Immunol 2010: **160**: 386–393.
18 León A J, Gómez E, Garrote J A *et al.* Mediators Inflamm 2009: **2009**: 580450.
19 Nishimura M, Kuboi Y, Muramoto K *et al.* Ann N Y Acad Sci 2009: **1173**: 350–356.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. IL-27 suppresses TNF-α-induced production of the IL-17-enhancing group.

Figure S2. IL-17 suppresses production of the IL-27-enhancing group that was synergistically induced by TNF-α and IL-27.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

DOI:10.1111/j.1600-0625.2011.01400.x
www.blackwellpublishing.com/EXD

Letter to the Editor

Bioactive reagents used in mesotherapy for skin rejuvenation *in vivo* induce diverse physiological processes in human skin fibroblasts *in vitro* – a pilot study

Claudia Jäger[1], Christiane Brenner[2], Jüri Habicht[2] and Reinhard Wallich[2]

[1]ATOS Clinic Heidelberg, Bismarckstr, Heidelberg, Germany; [2]Institute for Immunology, University Clinics Heidelberg, Heidelberg, Germany
Correspondence: Claudia Jäger, MD, ATOS Clinic Heidelberg, Bismarckstr. 9-15, D-69115 Heidelberg, Germany, Tel.: +49-6221-983345, Fax: +49-6221-864681, e-mail: claudia.jaeger@atos.de

Abstract: The promise of mesotherapy is maintenance and/or recovery of a youthful skin with a firm, bright and moisturized texture. Currently applied medications employ microinjections of hyaluronic acid, vitamins, minerals and amino acids into the superficial layer of the skin. However, the molecular and cellular processes underlying mesotherapy are still elusive. Here we analysed the effect of five distinct medication formulas on pivotal parameters involved in skin ageing, that is collagen expression, cell proliferation and morphological changes using normal human skin fibroblast cultures *in vitro*. Whereas in the presence of hyaluronic acid, NCTF135® and NCTF135HA®, cell proliferation was comparable to control cultures; however, with higher expression of collagen type-1, matrix metalloproteinase-1 and tissue inhibitor of matrix metalloproteinase-1, addition of Soluvit® N and Meso-BK led to apoptosis and/or necrosis of human fibroblasts. The data indicate that bioactive reagents currently applied for skin rejuvenation elicit strikingly divergent physiological processes in human skin fibroblast *in vitro*.

Key words: collagen type 1 – hyaluronic acid – mesotherapy – skin rejuvenation

Accepted for publication 20 October 2011

Background

Mesotherapy is a minimally invasive technique consisting of multiple epi- or intradermal injections of nutrients, hormones, vitamins, enzymes and other reagents. The aim of mesotherapy in skin rejuvenation is maintenance or restoration of healthy and youthful texture of skin (1–9). However, optimal protocols to achieve this goal, that is the quality of medication formulas and the mode of their application as well as the proof of concept, including long-term efficiency, are still lacking (10–12). Injection of hyaluronic acid is thought to promote skin rejuvenation by increasing both hydration and fibroblast activation (13–17). In fact, hyaluronic acid injected into the skin can stimulate fibroblasts to express collagen type-1 (Col-1), matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) (18). Moreover, hyaluronic acid was shown to participate in wound healing, modulation of inflammatory cells, interaction with proteoglycans of the extracellular matrix, and scavenging of free radicals (19). Another study suggests that dermal injections of vitamins result in stimulation of collagen production in skin cells (20). It is also known that various dermatologic cosmetic procedures, such as carbon dioxide laser resurfacing, microdermabrasion and electric stimulation, can alter gene expression and protein synthesis, leading to collagen production in human skin fibroblasts (HSF) (21–26).

Questions addressed

To elucidate the cellular and molecular processes underlying mesotherapy, we have now analysed the *in vitro* effect of five medication formulas presently used in cosmetic medicine, that is hyaluronic acid, NCTF135® (Filgora, Paris, France), NCTF135HA® (Filgora), Soluvit® N (Baxter, Unterschleissheim, Germany) and Meso-BK on HSF cultures, by monitoring putative parameters of skin ageing such as morphological changes, cell proliferation and expression of Col-1, MMP-1 and TIMP-1.

Experimental design

In the light of the clinical practice, to employ undiluted formulations for intradermal injection for optimal results within some days, the five bioactive medication formulas (Table SI) were added to HSF isolated from a skin biopsy of a healthy donor at different concentrations (15–80% v/v) in Eagle's minimum essential medium supplemented with 10% foetal bovine serum and 1% glutamine for up to 11 days. Morphological changes of HSF as well as their replicative potential were monitored. In addition, expression Col-I, MMP-1 and TIMP-1 mRNA was analysed by PCR.

Results

When incubated in the presence of either hyaluronic acid or vitamin cocktails, such as NCTF135® or NCTF135HA®, HSF retain their typical elongated spindle shape (Fig. 1), with proliferation rates similar to that of control cultures (Fig. S1). In addition, HSF showed distinct mRNA expression patterns for MMP-1, TIMP-1, Col-1 and beta-2-microglobulin (β2m) at 15 min, and up to 24 h post inoculation (p.i.), when incubated with either hyaluronic acid, NCTF135®, NCTF135HA® or medium (Fig. 2). Expression of MMP-1, TIMP-1 and Col-1 was detectable for up to 11 days p.i. (Fig. S2).

In contrast, treatment of HSF with vitamin solutions Soluvit® N and Meso-BK led to a significant reduction in their replication potential, accompanied by cell shrinking, the appearance of vacuole-like structures in the cytoplasm and finally pro-apoptotic and/or necrotic cell death (Fig. S1). Note the high numbers of cell debris in the respective cell cultures (Fig. 1). In HSF treated with Soluvit® N, expression of MMP-1, Col-1 and β2m was only observed at 15 min, 1 h and 4 h but not at 24 h, whereas TIMP expression was seen at all time points, at least to some extend. In the presence of Meso-BK, expression of MMP-1, TIMP-1 and β2m in HSF was detectable at all time points tested, whereas Col-1 was drastically reduced at 24 h (Fig. 2). At day 8 p.i., expression of MMP-1, TIMP-1 and Col-1 was undetectable in HSF cultures with either Soluvit® N and Meso-BK. Lastly, a comprehensive analysis of the pH of culture media in the initial stage of 11 days of cultivation revealed normal pH values of 7.0–7.5, independent on the addition of any of the five formulations.

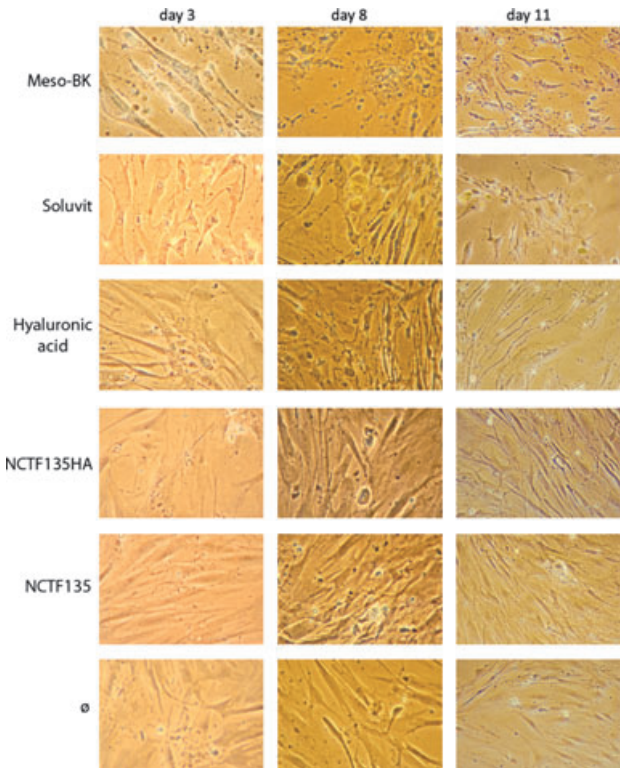

Figure 1. Phase-contrast microscopic pictures (100× magnification) of HSF cultured in the presence of the following formulations: hyaluronic acid (50%), NCTF135® (50%), NCTF135HA® (50%), Soluvit® N (15%) and Meso-BK (50%).

Discussion

It is generally assumed that many formulations used in mesotherapy improve the appearance of skin by increasing the biosynthetic capacity of HSF. Here we show that hyaluronic acid alone or combined with vitamin cocktails, for example NCTF135® and NCTF135HA®, maintain cell proliferation and induce enhanced mRNA expression of Col-1, MMP-1 and TIMP-1 in HSF for at least 11 days in culture. In contrast, the two vitamin formulations Soluvit® N and Meso-BK elicit pro-apoptotic processes and/or necrosis in HSF accompanied by impaired gene expression. These data suggest that the putative efficiency of the five formulations used in mesotherapy is achieved via the induction of divergent processes in HSF.

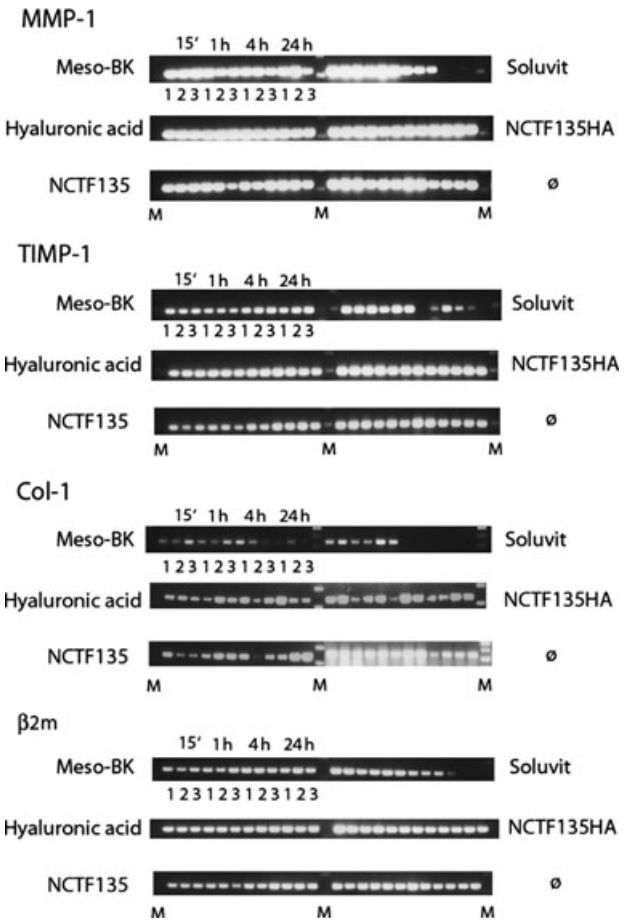

Figure 2. Expression of Col-1, MMP-1 and TIMP-1 mRNA in HSF. mRNA was prepared from fibroblasts following incubation with the indicated formulations at 20 (1), 40 (2) and 80% v/v (3) for 15 min, 1 h, 4 h and 24 h. Reverse transcription and amplification of the generated cDNA by PCR (38 cycles) were performed as described in the legend to Fig. S2. Amplified products of MMP-1, TIMP-1, Col-1 and the control gene *β*2m were visualized by ethidium bromide staining in agarose gel. The data are from one of three identical experiments.

The data support and extend previous findings suggesting that hyaluronic acid can induce Col-1 production in HSF (27). It could thus be speculated that sensitization of HSF by hyaluronic acid is elicited via interaction through various cellular receptors, including CD44 (28). Moreover, the induction of collagen degradation by MMP-1 could be counteracted by the concomitant expression of TIMP-1 allowing sustained Col-1 production.

Soluvit® N and Meso-BK are considered to stimulate wound repair-like mechanisms as previously shown for several dermatologic cosmetic procedures *in vivo*, for example microdermabrasion (29). The early stages of wound repair are characterized by inflammation and dermal collagen degradation by MMPs, followed by *de novo* synthesis of collagen (30). The present finding that Soluvit® N and Meso-BK induce apoptosis and/or necrosis in HSF *in vitro* support the notion that cytotoxic substances in general can induce, indirectly, tissue reconstruction by mechanisms similar to that observed in wound repair (31).

In conclusion, the present data demonstrate that distinct formulations currently used in mesotherapy induce strikingly divergent molecular and cellular processes in HSF *in vitro*. However, more detailed studies are warranted to elucidate whether and how the cellular and molecular processes by the five medication formulas in HSF *in vitro,* as described herein, are involved in facial skin rejuvenation *in vivo*, and whether these processes are similarly efficient, independent of the age of the recipients.

Acknowledgements

We especially thank Britta Knoll for providing substances and for insightful discussions during manuscript preparations. We also thank the Deutsche Gesellschaft für Mesotherapie (DGFM) for providing us with compounds used in this study. We thank Markus M. Simon for his helpful criticism and suggestions.

Authorship

CJ and RW conceived and designed the experiments, contributed reagents/materials/analysis tools, and wrote the article; CB, JH and RW performed the experiments; CJ, CB, JH and RW analysed the data.

Conflict of interest

The authors state no conflict of interest.

References

1 Bonnet C, Laurens D, Perrin J-J. Guide pratique de mésothérapie. Issy-les-Moulineaux Cedex: Elsevier Masson, 2008.
2 Iorizzo M, De Padova M P, Tosti A. Clin Dermatol 2008: **26**: 177–181.
3 Rotunda A M, Kolodney M S. Dermatol Surg 2006: **32**: 465–480.
4 Knoll B. Bildatlas der ästhetischen Mesotherapie: Wirkstoffe/Dosierung/Anwendung. Marburg: KVM, 2010.
5 Pistor M. Chir Dent Fr 1976: **46**: 59–60.
6 Caruso M K, Roberts A T, Bissoon L *et al.* J Plast Reconstr Aesthet Surg 2008: **61**: 1321–1324.
7 Matarasso A, Pfeifer T M. Plast Reconstr Surg 2005: **115**: 1420–1424.
8 Rotunda A M, Avram M M, Avram A S. J Cosmet Laser Ther 2005: **7**: 147–154.
9 Lacarrubba F, Tedeschi A, Nardone B *et al.* Dermatol Ther 2008: **21** (Suppl. 3): S1–S5.
10 Amin S P, Phelps R G, Goldberg D J. Dermatol Surg 2006: **32**: 1467–1472.
11 Atiyeh B S, Ibrahim A E, Dibo S A. Aesthetic Plast Surg 2008: **32**: 842–849.
12 Brown S A. Aesthet Surg J 2006: **26**: 95–98.
13 Tammi M I, Day A J, Turley E A. J Biol Chem 2002: **277**: 4581–4584.
14 Fligiel S E, Varani J, Datta S C *et al.* J Invest Dermatol 2003: **120**: 842–848.
15 Kuroda K, Shinkai H. Arch Dermatol Res 1997: **289**: 567–572.
16 Uitto J, Bernstein E F. J Investig Dermatol Symp Proc 1998: **3**: 41–44.
17 Yoneda M, Shimizu S, Nishi Y *et al.* J Cell Sci1988: **90** (Pt 2): 275–286.
18 Gao F, Liu Y, He Y *et al.* Matrix Biol 2010: **29**: 107–116.
19 Jiang D, Liang J, Noble P W. Annu Rev Cell Dev Biol 2007: **23**: 435–461.
20 Geesin J C, Hendricks L J, Falkenstein P A *et al.* Arch Biochem Biophys 1991: **290**: 127–132.
21 Orringer J S, Kang S, Johnson T M *et al.* Arch Dermatol 2004: **140**: 1326–1332.
22 Karimipour D J, Kang S, Johnson T M *et al.* J Am Acad Dermatol 2005: **52**: 215–223.
23 Sebastian A, Syed F, McGrouther D A *et al.* Exp Dermatol 2011: **20**: 64–68.
24 Ranzato E, Martinotti S, Volante A *et al.* Exp Dermatol 2011: **20**: 308–313.
25 Bujor A M, Nakerakanti S, Morris E *et al.* Exp Dermatol 2010: **19**: 347–354.
26 Tan W Q, Gao Z J, Xu J H *et al.* Exp Dermatol 2011: **20**: 119–124.
27 Wang F, Garza L A, Kang S *et al.* Arch Dermatol 2007: **143**: 155–163.
28 Weindl G, Schaller M, Schäfer-Korting M *et al.* Skin Pharmacol Physiol 2004: **17**: 207–213.
29 Karimipour D J, Karimipour G, Orringer J S. Plast Reconstr Surg 2010: **125**: 372–377.
30 Hinz B. J Invest Dermatol 2007: **127**: 526–537.
31 Shaw T J, Martin P. J Cell Sci 2009: **122**: 3209–3213.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Growth curves of human fibroblasts cultured in the presence of exogenously added formulations Soluvit, Meso-BK, HCTF135®, and medium..

Figure S2. Col-1, MMP-1, TIMP-1, and ß2m expression in skin fibroblasts for up to 11 days.

Table S1. Composition of reagents used in this study.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

DOI:10.1111/j.1600-0625.2011.01406.x
www.blackwellpublishing.com/EXD

Letter to the Editor

CXC chemokine receptor 4 is essential for Lipo-PGE1–enhanced migration of human dermal fibroblasts

Yoolhee Yang[1], Su Kyung Shim[1], Hyun-A Kim[1], Mira Seon[1], Eunjung Yang[1], Daeho Cho[2] and Sa Ik Bang[1]

[1]Department of Plastic Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Gangnam-gu, Seoul, Korea; [2]Department of Life Science, Sookmyung Women's University, Yongsan-gu, Seoul, Korea

Correspondence: Sa Ik Bang, Department of Plastic Surgery, Samsung Medical Center, Sungkyunkwan University, School of Medicine, 50 Ilwon-dong, Gangnam-gu, Seoul 135-710, Korea, Tel.: 82-2-3410-2215, Fax: 82-2-2148-7379, e-mail: si55.bang@samsung.com and Daeho Cho, Department of Life Science, Sookmyung Women's University, Hyochangwon-gil 52, Yongsan-gu, Seoul 140-742, Korea, Tel.: 82-2-710-9416, Fax: 82-2-6359-6789, e-mail: cdhkor@sookmyung.ac.kr

Abstract: Lipo-PGE1 [EGLANDIN®; a lipid microsphere-incorporated prostaglandin E1 (PGE1)] stimulates angiogenesis and promotes the healing of skin ulcers. Because the effects of Lipo-PGE1 on cutaneous wound healing are not completely understood, we investigated the ability of Lipo-PGE1 to affect *in vivo* wound healing and regulate the migration of human dermal fibroblasts (HDFs). In a murine wound model, Lipo-PGE1 reduced the wound size compared with control mice. Lipo-PGE1 significantly increased HDF migration in a dose- and time-dependent manner. Lipo-PGE1 markedly increased the expression of CXC chemokine receptor 4 (CXCR4), which controls the migration of HDFs, at the mRNA and protein levels. Small interfering RNA (siRNA)-mediated knockdown of CXCR4 inhibited Lipo-PGE1–enhanced HDF migration. Moreover, Lipo-PGE1 directly induced the phosphorylation of c-Jun N-terminal kinase (JNK), and the JNK-specific inhibitor Sp6000125 blocked Lipo-PGE1–enhanced migration and CXCR4 expression of HDFs. Our results demonstrate that Lipo-PGE1 accelerates wound healing *in vivo* and increases the CXCR4-mediated migration of HDFs through the JNK pathway.

Key words: CXC chemokine receptor 4 – human dermal fibroblasts (HDFs) – Lipo-PGE1 – migration – wound healing

Accepted for publication 26 October 2011

Background

Human dermal fibroblasts (HDFs), one of the major cell types of the skin, play an important role in cutaneous wound repair as well as in the pathophysiology of fibrotic diseases (1,2). CXC chemokine receptor 4 (CXCR4, also known as the SDF-1 receptor) is a G protein-coupled receptor (GPCR) involved in the migration of keratinocytes and fibroblasts (3). GPCRs regulate the mitogen-activated protein kinase (MAPK) signalling pathways, which control cell proliferation and apoptosis. One of the MAPKs, c-Jun N-terminal kinase (JNK), is essential for inflammation and cell migration (4–6). It has been reported that prostaglandin E1 (PGE1) promotes the healing of skin ulcers and wounds (7–10). A recent study showed that PGE1 increased the migratory activity of progenitor cells by enhancing CXCR4 expression (11). Additionally, it was shown that PGE2, an eicosanoid-like PGE1, enhances the homing of hematopoietic stem cells (HSCs) through the induction of CXCR4 (12). Lipo-PGE1 (EGLANDIN®; Mitsubishi Tanabe Pharma Corporation, Hwaseong, Gyeonggi, Korea) is a lipid emulsion of PGE1, which is used as a vasodilator in the treatment of peripheral vascular diseases. Although it is clear that PGE1 and PGE2 can affect the function of HSCs and progenitor cells by upregulating CXCR4, the effect of Lipo-PGE1 on HDF function and wound healing have yet to be determined.

Questions addressed

The role of Lipo-PGE1 in wound healing is still not completely understood. Therefore, we investigated the ability of Lipo-PGE1 to regulate HDF migration *in vitro* and to directly affect wound repair *in vivo*.

Experimental design

For Data S1, see Supporting information.

Results

To evaluate whether Lipo-PGE1 could be beneficial in wound repair, excisional wound healing experiments were performed in BALB/c-nude mice because of the advantage of their absence of hair and further application of pressure ulcer as chronic wound model (13). Lipo-PGE1 treatment promoted wound healing, as shown in Fig. 1(a). Specifically, wound closure was accelerated in the Lipo-PGE1–treated group compared to the control group (wound area in