Improved penetration of wild ginseng extracts into the skin using low-temperature atmospheric pressure plasma

Seoul Hee Nam1, Jeong Hae Choi6, Yeon Suk Song2, Hae-June Lee3, Jin-Woo Hong4,5,7,8 and Gyoo Cheon Kim6,7,8

1 Department of Dental Hygiene, College of Health Science, Kangwon National University, Samcheok-si, 25949, Republic of Korea
2 Department of Internal Medicine, School of Korean Medicine, Pusan National University, Yangsan, Republic of Korea
3 Department of Electrical Engineering, Pusan National University, Busan, 46241, Republic of Korea
4 Department of Internal Medicine, School of Korean Medicine, Pusan National University, Yangsan-si, 50612, Republic of Korea
5 Biomedical Research Institute, Pusan National University Hospital, Busan, 46241, Republic of Korea
6 Department of Oral Anatomy, School of Dentistry, Pusan National University, Yangsan-si, 50612, Republic of Korea
7 These authors contributed equally to this work.
8 Authors to whom any correspondence should be addressed.

E-mail: miss4228@naver.com, monday27@pusan.ac.kr, yccy032@hanmail.net, haejune@pusan.ac.kr, jwhong@pusan.ac.kr and ki91000m@pusan.ac.kr

Received 4 December 2017, revised 20 February 2018
Accepted for publication 7 March 2018
Published 4 April 2018

Abstract
Wild ginseng (WG) is a well-known traditional medicinal plant that grows in natural environments in deep mountains. WG has been thought to exert potent physiological and medicinal effects, and, recently, its use in skin care has attracted much interest. This study investigated the efficient penetration of WG extracts into the skin by means of low-temperature atmospheric pressure plasma (LTAPP), and its effects on the skin at the cellular and tissue levels. NIH3T3 mouse embryonic fibroblasts and HRM-2 hairless mice were used to confirm the improved absorption of WG extracts into the skin using LTAPP. The gene expression levels in NIH3T3 cells and morphological changes in skin tissues after WG treatment were monitored using both in vitro and in vivo experiments. Although WG extracts did not show any significant effects on proliferative activity and cytotoxicity, at a concentration of 1:800, it significantly increased the expression of fibronectin and vascular endothelial growth factor. In the in vivo study, the combinational treatment of LTAPP and WG markedly induced the expression of fibronectin and integrin α6, and it thickened. Our results showed that LTAPP treatment safely and effectively accelerated the penetration of the WG extracts into the skin, thereby increasing the effects of WG on the skin.

Keywords: fibronectin, integrin, low temperature atmospheric plasma, mouse skin tissue, skin cell, skin penetration, wild ginseng

1. Introduction
Ginseng is one of the most widely used herbal medicines in the world, and has been used in Asia for more than 2000 years. Wild ginseng (WG) grows naturally from seeds undisturbed in the deep mountains. It is slower in growth and more sensitive to environmental changes than cultivated
ginseng (CG), showing a preference for areas with fluctuating daily temperatures and less exposure to direct sunlight. WG is medicinally more effective and expensive compared with field-CG. These differences may account for the variation in the concentrations of active compounds between CG and WG. In both Korea and China, WG is thought to exert increased beneficial effects on health compared with CG, because WG contains higher levels of certain ginsenosides [1]. Thus, WG has been studied to examine its activity in a wide range of biological actions [2].

Several scientific findings have been documented on the components and pharmacological effects of WG [3–5]. Its physiological and medicinal benefits include positive effects on the endocrine, cardiovascular, immune, and central nervous systems, as well as the prevention of fatigue, oxidative damage, mutagenicity, and cancer [6–8]. In addition, anti-inflammatory activity has been reported in the recovery process of skin wounds, which can reduce edema and skin inflammation [9]. Choi [10] demonstrated the facilitating effects of WG on epidermal cell proliferation by upregulating the expression of proliferation-related factors. Many previous studies have reported that ginseng has anti-wrinkle effects [11], protects against ultraviolet radiation-induced skin aging [12], and exhibits anti-melanogenesis properties [13]. Some studies have also described its benefits in skin care. However, there is still insufficient information about promoting its skin penetration.

As skin care aesthetics has seen a recent increase because of concerns regarding physical appearance, many functional agents are added to cosmetics to enhance skin regeneration. Skin plays a major role in the barrier system that blocks the absorption of beneficial compounds [14]. In particular, large compounds do not penetrate intact skin [15]. Thus, safer and more efficient therapy for skin permeabilization is required. We examined the enhanced effects of WG penetration using LTAPP.

LTAPP consists of charged particles, radicals, and a strong electric field, and therefore it is a highly reactive technique [16]. For biomedical applications, low temperature is required because human tissues can be easily damaged by even weak thermal stimulation. The temperature of LTAPP is stabilized at around 37 °C, and steady-state temperature is continuously maintained throughout the application [17]. Recently, LTAPP has attracted great interest and has been widely used in biomedical applications [18–20]. Effective plasma treatment in dermatological applications has encountered challenges, and these studies have documented no plasma-induced damage to epidermal and dermal layers [14]. Previously, a safe and effective cosmetics tool was introduced through the skin into the dermis by regulating E-cadherin-mediated intercellular interactions [21]. Thus, LTAPP might be useful for skin care and increase skin penetration. The aim of this study was to achieve improved absorption of WG extracts into the skin by using LTAPP.

2. Materials and methods

2.1. Plasma device

A novel low-frequency LTAPP source was developed using a ceramic body as the dielectric with inner and outer electrodes (figure 1(A)). The outer electrode was grounded, and a sinusoidal high voltage was applied to the inner electrodes by using a high voltage source. The operating frequency adopted was 15 kHz for the plasma generator. The low-frequency LTAPP device was operated by a single plasma-ejecting module with 10 kV net input power to treat small areas [22]. Argon gas with a flow rate of 2.5 l min⁻¹ for safe and stable operation was used as the feeding gas at atmospheric pressure in air through a flow meter (KOFLOC, Ar-05). Sufficient voltage applied to argon gas ionizes argon atoms by driving off electrons. Free electrons can trigger further ionization of adjacent argon species via collision. This continuous reaction converts argon gas into a partially ionized gas called plasma. The plasma glow was retained within the electrodes, but did not extend to the ends of the electrodes. The stability of the plasma jet was increased without changes to plasma.
characteristics, and it can be touched without feeling any thermal effect. LTAPP has a dominant role in plasma biomedical applications [22].

2.2. Preparation of ethanol extracts of WG

WG was collected from the Chonbuk province in Korea and purchased from Baejjesansam Co. (Jinan-kun, Jinan-up, Yeonjung-Ri #45-1, Chonbuk, 567-807, Korea). A total of 100 g of WG was cut into small pieces and extracted with 1 L of 70% ethanol for 24 h at room temperature (25°C ± 2°C). The solutions were combined, filtered through Whatman No. 1 filter paper, concentrated using a rotary vacuum evaporator (Rotavapor R-124; Büchi Labortechnik AG, Flawil, Switzerland) under reduced pressure, and refrigerated in a recirculating chiller (EYELA CCA-1110; Tokyo Rikakikai Co., Tokyo, Japan) to obtain concentrated extracts.

2.3. Cell culture

NIH3T3 mouse embryonic fibroblast cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco BRL, Grand Island, NY, USA) with 10% heat-inactivated fetal bovine serum (Gibco BRL) and 1% penicillin/streptomycin (Gibco BRL) in a humidified atmosphere of 5% CO₂/95% air at 37°C and were refed every 2 d.

2.4. Cell cytotoxicity assay

Cells were labeled with sulforhodamine B (SRB), a fluorescent dye that labels total protein, to examine the morphological changes in the cells after WG treatment. Concentration- and time-dependent cytotoxicity was assessed at WG concentrations of 1:1600, 1:800, 1:400, and 1:200. Cells were seeded in 35 mm culture dishes and incubated for an additional 24 or 48 h in a CO₂ incubator, fixed, and stained with SRB. To eliminate non-specific staining, the cells were washed five times with 1% acetic acid. Morphological changes in the cells were examined and imaged using a light microscope outfitted with a digital camera.

2.5. Reverse transcription PCR (RT-PCR) analysis

After treating cells with WG at concentrations of 1:1600, 1:800, 1:400, and 1:200, the cells were washed and total RNA was isolated from the cultured cells using Trizol reagent (Invitrogen) at 24 h post-treatment. First-strand cDNA was synthesized using an RNA template and the specific primers, as described in the One-step PreMix kit (iNTRON Biotechnology Inc., Korea). cDNA was then amplified by PCR in a 20 μl reaction volume containing 2× PCR master mix (Doctor Protein Corp., Korea), 10 μM specific primers for type I collagen A subunit (5′-CA GCCACCTCAAGAAAG-3′ and 5′-CAGGTGG CAAGCTGTGGTT-3′), fibronectin (5′-CGGCCCGACT ACTACAAG-3′ and 5′-CCGGAGG GACCATTTCTC-3′), and vascular endothelial growth factor (VEGF) (5′-GCCACATGCAAGTGGTC-3′ and 5′- CATCTCTCATATGCTGGC-3′).

2.6. Animals model and treatment procedure

Five-week-old male HRM-2 melanin-possessing hairless mice were obtained from Central Laboratory Animal Inc. (Seoul, Korea), and housed in a controlled 12 h light/dark cycle at a temperature of 22°C ± 1°C and relative humidity of 50% ± 5%. To minimize the number of animals used and to reduce their suffering, twelve mice were divided into four groups (n = 3): control (no treatment), WG alone, LTAPP alone (phosphate-buffered saline, PBS), and LTAPP plus WG for 5 min. Animals were subjected to general anesthesia with an intramuscular ketamine HCl (50 mg kg−1, Ketalar®, Yuhan, Korea) and xylazine (10 mg kg−1, Rumpun®, Bayer, Korea) mixture prior to the treatment procedure. To test the level of skin absorption, 1 × 1 cm² 3 M paper patch containing WG extracts were left on the marked dorsal skin (approximately 10 mm in diameter). As shown in figure 1(B), the device was placed at a distance of 0.5 cm from the skin tissue during the LTAPP treatment. Immediately after 5 min of exposure, the WG extract patch was applied to the target regions. The procedure was repeated in the same manner for 2 weeks. Then, the mice were sacrificed in a CO₂ gas chamber.

2.7. Immunohistochemistry

The skin of each experimental group was placed in a labeled vial containing 4% paraformaldehyde fixative solution at room temperature for 24 h. The fixed tissues were embedded in paraffin under vacuum and sections with a thickness of 4 μm were deparaffinized and rehydrated. Endogenous peroxidases activity was inhibited in aqueous 3% hydrogen peroxide for 10 min. After permeabilization with Triton X-100 in PBS for 30 min, slides were incubated in 1% bovine serum albumin and 5% blocking serum for 30 min at room temperature. Primary antibodies were added and incubated overnight at 4°C. The slides were incubated in a 1:200 dilution of the fibronectin antibody (Santa Cruz) and in a 1:100 dilution of the integrin α6 antibody (Santa Cruz). After washing, the sections were incubated for 30 min with peroxidase-labeled polymer-horseradish peroxidase (HRP) conjugated to goat anti-rabbit immunoglobulins (Envision + System-HRP-DAB; Dako, Carpenteria, CA) for 1 h. Staining was completed by incubation with 3,3′-diaminobenzidine chromogen solution (part of Envision kit). The sections were counterstained with Harris’ hematoxylin, dehydrated, and mounted on a cover slip. In addition to histological stains, periodic acid-Schiff staining was performed to detect connective tissue structures and basal lamina. The sections were oxidized for 5 min in 1% periodic acid and later washed in distilled water for 5 min. The sections were then stained with Schiff’s regent for 15 min. Next, decolorization with 1% acid alcohol was carried out. The sections were further counterstained with hematoxylin for 1 min, dehydrated, and mounted on a cover slip. The staining was analyzed using a BX51 microscope (Olympus; Tokyo, Japan) equipped with a 40× objective. Images were photographed using a digital camera (Pixel link PL-B686 CU, Ottawa, Canada).
3. Results

3.1. Cell morphology and cytotoxicity

In concentration- and time-dependent manners, changes in NIH3T3 cell morphology were evaluated to determine cellular growth after WG treatment. As shown in figure 2(A), treatment with 1:800 WG did not have any significant effects on cell growth and cytotoxicity. Thus, WG extracts at 1:800 did not affect cell proliferation. Although cell density was reduced by the concentration of WG extract, no detached or dead cells were detected. After treatment with 1:200 WG, the proliferative activity was reduced to ∼40% compared to that of the control cells (figure 2(B)).

3.2. Gene expression in NIH3T3 cells

The effect of WG treatment on gene expression in NIH3T3 cells was monitored by RT-PCR. After 24 h of incubation, the expression of fibronectin with 1:800 WG treatment was higher than that with treatment at other concentrations (figure 3(A)). As shown in figure 3(B), the cells treated with 1:800 WG had significantly increased expression of fibronectin and VEGF. On the other hand, the mRNA level of type I collagen A was not affected by WG treatment.

3.3. In vivo mouse skin absorption

WG extracts treated with LTAPP showed higher skin penetration than did the other experimental groups. As shown in figure 4, the density of fibronectin in the target regions was markedly expressed throughout the dermis when WG was combined with LTAPP. Integrin α6 in skin treated with WG extracts and LTAPP showed a distinct stain beneath the basal layer of the epithelium (figure 5). The basement membrane was observed as a thin pink layer at the base of the epithelial layer. The skin samples treated with the WG extracts and LTAPP showed a deep pink coloration and were well defined (figure 6). The application of LTAPP did not induce any histological damage to the skin tissues. LTAPP enhanced the penetration efficiency of WG extracts.

4. Discussion

Herbal cosmetics are gaining immense popularity in the health and beauty sectors. WG is a well-known traditional medicinal plant and has long been studied for its medical efficacy [23]. This study evaluated the penetration of WG using LTAPP and monitored changes in the skin by using both in vitro and in vivo experiments.

Figure 2. The cellular proliferative activity assessed using SRB staining procedures. (A) Changes in NIH3T3 cell morphology and (B) relative growth rate after a dose response with 1:1600, 1:800, 1:400, or 1:200 WG extracts at 24 and 48 h.

Figure 3. The effects of WG extracts on mRNA expression of NIH3T3 cells by RT-PCR. (A) The level of mRNA with dose activation of WG (1:1600–1:200) for 24 h. (B) The expression pattern of genes with 1:800 WG extract at 3, 6, 9, and 24 h.
Skin provides the first line of defense against environmental stress, and consists of two layers. The epidermis is a superficial epithelial tissue and the dermis is a deep layer composed of dense connective tissue. Because of the barrier function of the epidermis, it is difficult for most materials, even water, to penetrate the dermis. In some previous studies, epidermal growth factor was delivered from the epidermis to the dermis by LTAPP treatment [14, 21]. Thus, if favorable components of WG for skin penetrate the dermis with the help of LTAPP, they could induce various useful effects on the skin. The extracellular matrix (ECM) of the dermis plays an important role in homeostasis, aging, wound healing, and disease [24]. Fibroblasts, nerves, and blood vessels are embedded in an ECM of predominantly collagens and elastins. Thus, the dermis provides the epidermis with mechanical support and nutrients [25]. Fibroblasts are cells in connective tissues that maintain the structural integrity of connective tissues by continuous secretion of precursors of ECM [26]. Fibronectin is also characteristically present in loose connective tissue, where it plays the role of a linker for binding to collagens, proteoglycans, and many ECM molecules, with important roles in wound healing, tissue regeneration, and anti-aging of skin [27]. When we performed a dose-response experiment with WG (1:1600, 1:800, 1:400, or 1:200), fibronectin mRNA was significantly increased at a dose of 1:800 in treated cells. In addition, the effect of LTAPP was validated by in vivo experiments. Immunohistochemical results showed the increased expression of fibronectin in skin tissue in accordance with that of in vitro experiments. Treatment with WG alone increased the expression of fibronectin, but it was enhanced by the combinational treatment of LTAPP and WG. This result demonstrates that the components of WG are delivered to the dermis, which then stimulate fibroblasts to secrete fibronectin. Therefore, the absorption of WG extracts after LTAPP exposure led to improvement in skin elasticity and tightening. Type I collagen in the dermis is one of the fundamental building blocks of the skin, which is composed of more than 90% organic matter [28], whereas WG extracts promoted the synthesis of type I collagen in the dermis [11]. In the present study, collagen expression was not altered by LTAPP, despite the increase in fibronectin.

Integrins play an important physiological role in cell migration, proliferation, survival, and differentiation [29]. Integrin α6β4 is mostly known for its role in the simple stratified epithelium, where it mediates strong adhesion to laminin in the basement membrane [30]. Integrin α6 has been shown to be an important regulator of angiogenesis in several in vivo models [31]. The α6 integrin is distributed along the epidermal–dermal junction and mediates the adhesion of cells.
To components of the ECM and basement membranes [32]. Because the basement membrane is an epidermal ECM, integrin expression in the basal layer cells can affect the fate of epidermal cells [24]. In this study, combinational treatment of LTAPP and WG enhanced the expression of integrin $\alpha_6$ at the basement membrane, indicating that the epidermis adhered strongly to the dermis. VEGF plays an important role in skin repair through angiogenesis [33]. Once it binds to its receptor in vascular endothelial cells, the cells promote new vessel formation. Our results clearly show that WG extracts significantly enhanced VEGF expression in cells treated with 1:800 WG for 3 h. This indicates the possibility of increased angiogenesis and epidermal cell proliferation.

The capability of LTAPP exposure to enhance the absorption of larger particles through the skin for medical or cosmetic purposes was tested. The enhanced absorption ability of the skin after LTAPP treatment was attributed to the reduction in E-cadherin-mediated cellular junctions that led to the gaps between epidermal cells [21], thereby enabling drugs and functional chemicals to be delivered to the dermis. Direct treatment of keratinocytes with LTAPP did not lead to DNA damage [34, 35]. Furthermore, repeated treatment of skin tissue with LTAPP accelerated the proliferation of keratinocytes. Therefore, the LTAPP-mediated transdermal drug delivering technique was considered safe [34]. In the present study, LTAPP effectively induced the penetration of WG extracts into the skin. Delivered WG extracts increased the expression of fibronectin, VEGF, integrin $\alpha_6$, and laminin. These results conformed with our previous studies, in which epidermal growth factor [21], hydrocortisone cream [22], and Jaun-ointment [34] were delivered to the skin by using LTAPP. Treatment with LTAPP for 5 min opened up the barrier system of skin through the inhibition of E-cadherin, which was almost restored with 3 h [21]. As the components from WG such as ginsenosid and saponin are well known to induce the activity in skin [8, 10–12], the WG extracts treated with plasma could promote healthy skin. Therefore, the WG extracts treated with plasma could promote healthy skin.

5. Conclusions

LTAPP exposure effectively enhanced the entry of WG extracts through the skin. The absorption of WG extracts into the skin was significantly increased compared to that in the other experimental groups. This indicates that dermal fibroblasts can be activated by LTAPP in combination with WG extracts, and LTAPP treatment can improve skin firmness and elasticity.
Acknowledgments

This study was supported by Biomedical Research Institute Grant (2017-08), Pusan National University Hospital.

ORCID iDs

Seoul Hee Nam https://orcid.org/0000-0001-9529-1282
Jeong Hae Choi https://orcid.org/0000-0003-4528-7618
Yeon Suk Song https://orcid.org/0000-0002-9181-6923
Hae-June Lee https://orcid.org/0000-0003-3401-3355
Gyoo Cheon Kim https://orcid.org/0000-0003-3568-3529

References

[1] Huang Y C, Chen C T, Chen S C, Lai P H, Liang H C, Chang Y, Yu L C and Sung H W 2005 A natural compound (ginsenoside re) isolated from panax ginseng as a novel angiogenic agent for tissue regeneration Pharmaceutical Res. 22 636–46
[2] McElhaney J E, Gravenstein S, Cole S K, Davidson E, O’Neill D, Petitjean S, Rumble B and Shan J J 2004 A placebo-controlled trial of a proprietary extract of north American ginseng (CVT-E002) to prevent acute respiratory illness in institutionalized older adults J. Am. Geriatr. Soc. 52 13–9
[3] Jung C H, Seog H M, Choi I W, Choi H D and Cho H Y 2005 Effects of wild ginseng (Panax ginseng C.A. Meyer) leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats J. Ethnopharmacol. 98 245–50
[4] Gum S I, Jo S J, Ahn S H, Kim J T, Shin H W and Cho M K 2007 The potent protective effect of wild ginseng (Panax ginseng C.A. Meyer) against benzo[alpha]pyrene-induced toxicity through metabolic regulation of CYP1A1 and GSTs J. Ethnopharmacol. 112 568–76
[5] Hwang J W et al 2012 Mountain ginseng extract exhibits anti-lung cancer activity by inhibiting the nuclear translocation of NF-kB Am. J. Chin. Med. 40 187–202
[6] Zhang D 1, Yasuda T, Yu Y, Zheng P, Kawabata T, Ma Y and Okada S 1996 Ginseng extract scavenges hydroxyl radicals and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation Free Radic Biol. Med. 20 145–50
[7] Yun T K, Lee Y S, Lee Y H, Kim S I and Yun H Y 2001 Anticarcinogenic effect of Panax ginseng C.A. Meyer and identification of active compounds J. Korean Med. Sci. Suppl. 16 S6–18
[8] Joo S S, Won T J and Lee D 2005 Reciprocal activity of ginsenosides in the production of a pro-inflammatory repertoire, and their potential roles in neuro-protection in vivo Planta Med. 71 476–81
[9] Navarro P, Giner R M, Recio M C, Manez S, Cerda-Nicolas M and Rios J L 2001 In vivo anti-inflammatory activity of saponins from bupleurum rotundifolium Life Sci. 68 1199–206
[10] Choi S 2002 Epidermis proliferative effect of the panax ginseng ginsenoside Rb2 Arch. Pharm. Res. 25 71–6
[11] Lee J et al 2007 Panax ginseng induces human type I collagen synthesis through activation of smad signaling J. Ethnopharmacol. 109 29–34
[12] Kim Y G, Sumiyoshi M, Sakakura M and Kimura Y 2009 Effects of ginseng saponins isolated from red ginseng on ultraviolet induced skin aging in hairless mice Eur. J. Pharmacol. 602 148–56
[13] Im S J, Kim K N, Yun Y G, Lee J C, Mun Y J, Kim J H and Woo W H 2003 Effect of radix ginseng and radix trichosanthis on the melanogenesis Biol. Pharm. Bull. 26 849–53
[14] Lademann O, Richter H, Meinke M C, Patzelt A, Kramer A, Hinz P, Weltmann K D, Hartmann B and Koh S 2011 Drug delivery through the skin barrier enhanced by treatment with tissue-tolerable plasma Exp. Dermatol. 20 488–90
[15] Andrews S N, Jeong E and Prausnitz M R 2013 Transdermal delivery of molecules is limited by full epidermis, not just stratum corneum Pharm. Res. 30 1099–109
[16] Lee H W, Kim G J, Kim J M, Park J K, Lee J K and Kim G C 2009 Tooth bleaching with non-thermal atmospheric pressure plasma J. Endod. 35 587–91
[17] Nam S H, Lee H W, Cho S H, Lee J K, Jeon Y J and Kim G C 2013 High-efficiency tooth bleaching using non-thermal atmospheric pressure plasma with low concentration of hydrogen peroxide J. Appl. Oral Sci. 21 265–70
[18] Fridman G, Friedman G, Gutsol A, Shekhter A B, Vasilets V N and Fridman A 2008 Applied plasma medicine Plasma Process Polym. 5 503–33
[19] Kim G C, Lee H W, Byun J H, Chung J, Jeon Y C and Lee J K 2013 Dental applications of low-temperature nonthermal plasmas Plasma Process Polym. 10 199–206
[20] Nam S H, Lee H J, Hong J W and Kim G C 2015 Efficacy of nonthermal atmospheric pressure plasma for tooth bleaching Sci. World J. 2015 1–5
[21] Choi J H, Nam S H, Song Y S, Lee H W K, Lee H J, Song K W, Hong J W and Kim G C 2014 Treatment with low-temperature atmospheric pressure plasma enhances cutaneous delivery of epidermal growth factor by regulating E-cadherin-mediated cell junctions Arch. Dermatol. Res. 306 635–43
[22] Choi J H, Song Y S, Lee H J, Hong J W and Kim G C 2017 Inhibition of inflammatory reactions in 2,4-Dinitrochlorobenzene induced Nc/Nga atomic dermatitis mice by non-thermal plasma Sci. Rep. 8 27376–86
[23] Falkenberg T, Mohammed A K, Henriksson B, Persson H, Winblad B and Lindefors N 1992 Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment Neurosci. Lett. 138 153–6
[24] Watt F M and Fujiwara H 2011 Cell-extracellular matrix interactions in normal and diseased skin Cold Spring Harb. Perspect. Biol. 3 a005124
[25] Aller M A, Arias J I and Arias J 2010 Pathological axes of wound repair: gastrulation revisited Theor. Biol. Med. Model. 7 37
[26] Sorrell C H and Caplan A I 2004 Fibroblast heterogeneity: more than skin deep J. Cell Sci. 117 667–75
[27] Knott A, Drenckhan A, Reuschlein K, Lucius R, Doring O, Böttger M, Stab F, Wenc H and Gallinat S 2010 Decreased fibroblast contractile activity and reduced fibronectin expression are involved in skin photoaging J. Dermatol. Sci. 58 75–7
[28] Lalande A, Taff D and Riemer K 2009 The role of collagen bioscaffolds, foamed collagen, and living skin equivalents in wound healing Clin. Podiatr. Med. Surg. 26 525–33
[29] Streuli C H 2009 Integrins and cell-fate determination J. Cell Sci. 122 171–7
[30] Margadant C, Frijns E, Wilhelmsen K and Sonnenberg A 2008 Regulation of hemidesmosome disassembly by growth factor receptors Curr. Opin. Cell Biol. 20 589–96
[31] Avraamides C J, Garmy-Susini B and Varner J A 2008 Integrins in angiogenesis and lymphangiogenesis Nat. Rev. Cancer 8 604–17
[32] Hynes R O 2002 Integrins: bidirectional, allosteric signaling machines Cell 110 673
[33] Galiano R D, Tepper O M, Pelo C R, Bhatt K A, Callaghan M, Bastidas N, Bunting S, Steinmetz H G and Gurtner G C 2004 Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells Am. J. Pathol. 164 1935–47
[34] Choi J H, Song Y S, Lee H J, Kim G C and Hong J W 2017 The topical application of low-temperature argon plasma enhances the anti-inflammatory effect of jaun-ointment on DNBC-induced NC/Nga mice BMC Complement. Altern. Med. 17 340–9
[35] Lademann J, Patzelt A, Richter H, Lademann O, Baier G, Breucker L and Landefester K 2013 Nanocapsules for drug delivery through the skin barrier by tissue-tolerable plasma Laser Phys. Lett. 10 083001