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Original Article

Effect of a nonthermal-atmospheric pressure plasma jet on wound healing: An animal study

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Abstract

Background: The use of a nonthermal plasma (NTP) jet in the treatment of living tissue has been the subject of considerable interest in the field of medical technology, and has the potential to reduce the recovery time of open wounds. We aimed to investigate the wound-healing process by clinical observation, blood tests, and expression of cell adhesion markers and reactive oxygen species in NTP jet-treated rats.
Methods: This study utilized Sprague-Dawley (SD) rats as experimental subjects, and wounds measuring 2 cm × 2 cm were produced on the animals' backs. The experimental group was treated with NTP for 5 min/d for 4 weeks. The NTP was injected in a diffused manner into the cage housing the rats. The SD rats that had not received plasma treatment were designated as the control group. Blood was drawn on Postoperative Day 2, Day 4, and at 3 months. An immunohistochemical stain of E-cadherin and 4-hydroxy-2-nonenal (4-HNE), a reactive oxygen species marker, were evaluated and quantified for analysis using a CMYK color model.
Results: A total of 35 SD rats were included in the study (25 in the NTP group and 10 in the control group). Low dose plasma treatment shortened the wound-healing time without damaging organs. In the NTP group, the white blood cell counts at Day 2 post-NTP treatment was not increased significantly more than that in the control group. After quantification of immunohistochemical staining, 4-HNE was increased at Day 14 compared with Day 7 (16.16 ± 12.81% vs. 55.11 ± 8.11%, $p < 0.001$), and E-cadherin was also increased (52.17 ± 14.96% vs. 70.46 ± 12.78%, $p = 0.04$) in the NTP group. After comparison of NTP and the control, it was observed that 4-HNE and E-cadherin were increased in the NTP group on Day 14.
Conclusion: Short-term, low-dose NTP wound treatment was demonstrated to accelerate wound healing in SD rats without vital organ toxicity.

Keywords: 4-hydroxy-2-nonenal; E-cadherin; nonthermal plasma; wound healing

1. Introduction

Caring for wounds and the process of wound healing are important issues in healthcare. A period of prolonged wound healing may cause patient discomfort, increased medical costs, and can even endanger lives. For the purpose of improving wound healing, “plasma” is a common tool that

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has the capacity to potentially benefit patient wound healing.[1] Plasma is defined as ionized gas. In terms of applications, plasma can be divided into thermal and nonthermal plasma (NTP), and is generated at atmospheric pressure with low electrical power (tens of watts). Besides the treatment of wounds, nonthermal-atmospheric pressure plasma has wide applications, such as disinfection by topical treatment of skin diseases with microbial involvement, local treatment for erosion in cancer diseases, or hemostasis for bleeding mucosa. There are several molecular mechanisms wherein NTP could improve wound healing by its antiseptic effects, by stimulation of proliferation and migration of wound relating skin cells, by activation or inhibition of integrin receptors on the cell surface, or by its proangiogenic effect.[1] In a clinical setting, NTP is widely used for a variety of purposes that include coating an implant surface with a biocompatible layer,[2] hemostasis,[3] and sterilization of surgical tools.[4] There are also several therapeutic effects of plasma including destruction of micro-organisms, acceleration of blood coagulation, and regulation of cell surfaces in the wound-healing process.[5,6] NTP may also induce neutral and charged particles, electric fields, radicals, e.g., reactive oxygen species (ROS) and other reactive molecules, such as hydrogen peroxide and nitric oxide.[7−9] Because electrons are extremely light, they can move quickly and have no heat capacity. Therefore, NTP has become a promising medical option without adverse effects.[10−14]

Wound healing requires the migration of epithelial cells. Several markers can detect cell surface adhesions, e.g., integrins, cadherins, etc.[7,15] An *in vitro* study demonstrated that plasma treatment influenced the growth and differentiation of keratinocytes and fibroblasts.[7] Cells showed a dramatic loss of E-cadherin within 24 hours after the plasma treatment, leading to loss of cell−cell contact.[7] E-cadherin was shown to be significantly reduced by the ROS-inducing treatment.[16,17] ROS changed the DNA integrity, and the effectiveness of cellular defense mechanisms characterizes the interaction of NTP and eukaryotic cells. Hence, a stimulation of eukaryotic cells using short-term NTP treatment seems possible, e.g., in the context of chronic wound care.[9] Long-term plasma treatments stopped cell proliferation and apoptosis activities, which could be relevant in controlling neoplastic conditions. It is widely believed that ROS can disrupt cell−cell adhesion, leading to various biological responses which include cell migration and proliferation.[18]

Plasma could produce ROS in cells or tissue and generate results in oxidative stress to the microenvironment.[9,19,20] One of the important products of ROS is 4-hydroxy-2-nonenal (4-HNE), an oxidized lipid, which could contribute to the disruption of the cell membrane structure and break down the protein or enzyme activity.[21−23]

The aim of this study was to investigate the effect of NTP-treated wounds. To achieve this, we compared wound size, blood data, and immunohistochemical (IHC) staining of 4-HNE and E-cadherin on rats.

2. Methods

2.1. Animals

Thirty-five Sprague-Dawley (SD) rats weighing 250−350 g were used in this study. Permission was obtained from the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital (Taichung, Taiwan; La-101937). The rats were separated into a control (10 rats) and an experimental (25 rats) group, and were anesthetized with 4% isofluorane for induction, followed by a maintenance dose (1−2%). A 2 cm × 2 cm skin wound was produced on the back of the SD rats under anesthesia (Fig. 1A and B). In the experimental group, all rats received NTP jet diffusely to the carriage 5 min/d for 4 weeks. The control group received no treatment. The size of each wound was calculated daily by multiplying the long and short axial lengths of the rats. In addition, in both the control and experimental group, 0.5 mL of blood was drawn at 2 days, 4 days, 1 week, 2 weeks, 1 month, and 3 months. The blood was sent for analysis of white blood cell counts (WBC), red blood cell counts, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, and creatinine. This study has been reviewed by our institution review board for animal study (La-101937).

2.2. Experimental equipment

The NTP apparatus generates plasma in a dielectric barrier discharge configuration, manufactured by the Taiwan Yih Dar Technologies (Changhua, Taiwan; Fig. 2). Aluminum tape electrodes were fitted onto a quartz tube with an inner diameter of 4 mm and separated by 10 mm of space. Then, argon and oxygen were released at the rate of 1.8 L/min and 0.01 L/min, respectively. High voltage mono-polar square pulses were applied using the powered electrode with a repetition rate between 0.5 kHz and 4 kHz, which provides a stable and high energy plasma.

2.3. IHC staining

In order to detect the expression of the 4-HNE and E-cadherin in the wound tissue after plasma application, antibodies were used for IHC stain. First, tissue samples were obtained from euthanized rats. The slices were fixed in 10% buffered formalin for 24 hours and then processed with conventional histopathological techniques. Thereafter, the tissue samples were stained with hematoxylin eosin and other IHC stains.

The IHC studies were performed using the Bond-Max Autostainer (Leica Microsystems, Wetzlar, Germany). Slides were stained with 4-HNE polyclonal antibody as well as E-cadherin monoclonal antibodies. These immunomarkers, including methods of pretreatment for antigen retrieval, are shown in Table 1. In short, the formalin-fixed and paraffin-embedded tissue array specimens were added to Tris-buffered saline and Tween 20, rehydrated through serial

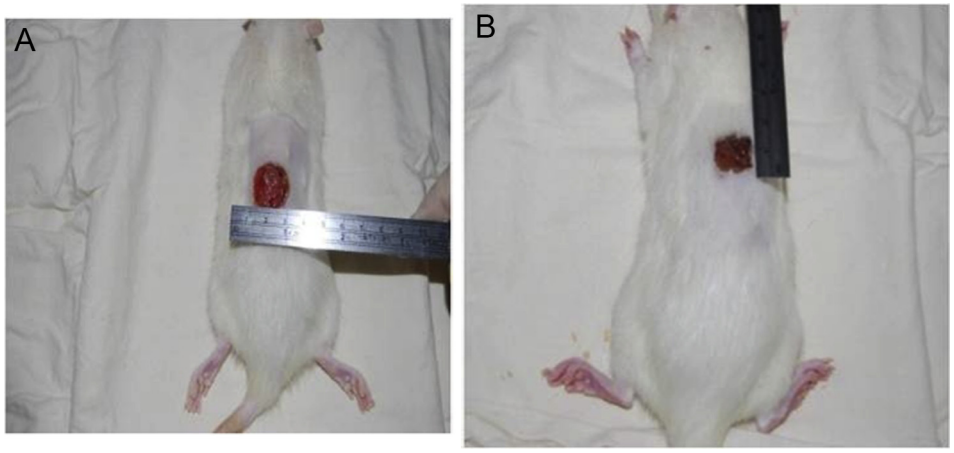

Fig. 1. Sprague-Dawley rats were the experimental animal. After shaving and sterilization of the backs of the Sprague-Dawley rats, a wound with size measuring at 2 cm × 2 cm was created: (A) experimental rat with nonthermal-plasma jet treatment after 1 day, with some exudates over the wound region; and (B) control rat with dry crust over the wound region.

dilutions of alcohol, and washed in phosphate-buffered saline (pH 7.2). This buffer was used for all subsequent washes, according to the manufacturer's recommended protocol. Slides were stained with the previously mentioned antibodies using the fully automated Bond-Max system by onboard heat-induced antigen retrieval and a Leica Refine Polymer Detection System (Leica Microsystems). Diaminobenzidine was used as the chromogen (Leica Microsystems) for all immunostainings. Negative controls were obtained by excluding the primary antibody. Appropriate positive controls were indicated to skin tissue relative to negative control throughout the study. These slides were mounted for examination and the images were captured using the Olympus BX51 microscopic/DP71 Digital Camera System (Ina-shi, Nagano, Japan).

2.4. Quatification of IHC staining of E-cadherin and 4-HNE expression

For quantification purposes, we used a computer-assisted image processing technique. To quantify the IHC stain, we adhered to those guidelines from the publication: quantitative image analysis of IHC stains using a CMYK color mode.[24] Accordingly, it was observed that the yellow channel of CMYK had improved sensitivity for IHC elevation. We found that the yellow channel had a strong and linear relationship, and was sensitive to small changes in IHC intensity. The yellow channel was unaffected by the hematoxylin counterstain.

All slides were scanned with a Nikon Eclipse E600 (Japan) using brightfield image at 200× magnification, after which the images were saved as TIFF. The IHC slide quantity was counted by using Adobe Photoshop (Version 7; Chicago, U.S.). In the negative stain (in visual view), the "Y" percentage was less than 10%. For the positive stain, the "Y" was approximately 30−100%, and increased according to intensity. In each slide (200×), we randomly chose eight points and calculated the average IHC staining percentage. The target staining of 4-HNE is the nucleus of epithelium cells. For E-cadherin expression, it expressed in the cell junctions.

2.5. Statistical analysis

All statistical analyses were performed using the SPSS (version 15.1; SPSS Inc., Chicago, IL, USA) and SAS statistical software (version 9.1.3; SAS Institute, Inc., Cary, NC). Continuous variables are expressed as means ± standard deviation. The Mann−Whitney *U* test was used for comparison of continuous variables to evaluate the differences in blood nonparametric data, including blood cell counts and creatinine. The independent sample *t* test was used for comparison for the quantification of IHC staining of 4-HNE and E-cadherin expression between the control group and the NTP group. Paired sample *t* test was used for Day 7 and Day 14 quantification of 4-HNE and E-cadherin IHC staining. A two-sided *p* value for two-group comparisons of values of < 0.05 was considered statistically significant.

3. Results

3.1. NTP improving wound healing

In this study, the wound-healing time of the experimental group was significantly shorter compared with the control group (Fig. 3A and B). The wound size was significantly smaller in the NTP group than in the control group on the 13th day post-treatment (57.8 ± 10.5 mm^2 vs. 106.8 ± 39.4 mm^2, respectively; Fig. 3C). In the experimental group, raw wounds were found covered with exudations. Following additional pathological examination, exudation of wounds suggested earlier initiation of tissue inflammatory and repairing process for hematoxylin and eosin stain (Fig. 4A and B).

3.2. Blood cells and biochemical studies

The hematological analysis showed a higher increased in WBC in the control group compared with the experimental group. On the 2nd day post-treatment, the WBC count of the control group was significant higher than the experimental group (19,280 ± 7571 mm^3 vs. 10,625 ± 2312 mm^3,

Fig. 2. Non-thermal dielectric barrier discharge plasma device manufactured by Taiwan Yih Dar Technologies, Changhua, Taiwan. Aluminum tape electrodes were fitted onto a quartz tube with an inner diameter of 4 mm and separated by 10 mm of space. Non-thermal plasma was generated by argon 1.8 L/min and oxygen 0.01 L/min under high voltage mono-polar square pulses using the powered electrode with a repetition rate between 0.5 kHz and 4 kHz.

respectively). After the 1st month, the control group showed a WBC count of 16,200 ± 2747 mm^3, while the plasma group count was 12,100 ± 3280 mm^3. However, the WBC count difference between the two groups is not statistically significant ($p = 0.063$). No obvious difference in red blood cells, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, or creatine can be observed between the control and experimental groups (Fig. 5).

3.3. NTP affects 4-HNE and E-cadherin expression

In the IHC analysis, 4-HNE was detected in both the NTP group and the control group (4-HNE control group, Fig. 6A; NTP, Fig. 6C and E; E-cadherin control, Fig. 6B; and NTP group, Fig. 6D and F). The density of 4-HNE immunopositive-cell expression was significantly higher in the NTP group than in the control group on the 14th day after the cut (Fig. 6C and E), and E-cadherin was also higher in NTP groups 14 days after cutting (Fig. 6D and F).

For further analysis, we quantified the IHC staining for 4-HNE and E-cadherin both in the control and NTP groups. In each slide (200×), we randomly chose eight points and calculated the average IHC staining percentage. The target staining of 4-HNE is the nucleus of epithelium cells. In the control group, Postcutting Day 7, the 4-HNE was 43.07 ± 4.22%; on Postcutting Day 14, this decreased to 17.50 ± 3.46% ($p < 0.001$). In the NTP group, the 4-HNE was 16.16 ± 12.81%. On Day 7, Postcutting and Postoperation Day 14, 4-HNE increased to 55.11 ± 8.11% ($p < 0.001$) (Table 2). For E-cadherin expression, it was expressed in the cell junctions. In the NTP group, Postcutting Day 7, decreased E-cadherin expression was demonstrated significantly compared with the control group (52.17 ± 14.96% vs. 84.44 ± 1.86%, $p < 0.01$). On Postoperation Day 14, the E-cadherin expression was increased in NTP groups compared with the control group (70.46 ± 12.78% vs. 49.10 ± 6.90%, $p = 0.004$).

3.4. Long-term toxicity of organs

Fig. 7 showed the organs of SD rats were normal and microscopically evaluated with hematoxylin and eosin staining: skin (Fig. 7A), kidney (Fig. 7B), lung (Fig. 7C), and liver (Fig. 7D). There were no significant differences between the experimental group and the control group.

4. Discussion

Our results demonstrated that the application of nonheating plasma to wounds resulted in shorter wound-healing times, and faster healing with fibrin/exudates covering wounds on the

Table 1
Antigens for immunohistochemical staining.

Antigen	Clone	Product code	Antibody class	Supplier	Dilution	Antigen retrieval
E-cadherin	Mouse monoclonal	NCLE-CAD	IgG1	Leica biosystems	1:100	ER2 20 min
4-HNE	Rabbit polyclonal	Orb10058	IgG	Biorbyt	1:300	ER1 20 min

4-HNE = 4-hydroxy-2-nonenal; ER1 = Bond Epitope Retrieval Solution 1 contains a citrate-based buffer and surfactant; ER2 = Bond Epitope Retrieval Solution 2 contains an ethylene diaminetetra-acetic acid-based buffer; IgG = immunoglobulin G.

2nd day postoperation. There was also a reduced increase in WBC counts in the experimental group compared with the control group, which may indicate that there was less infection/inflammation due to the disinfection effect of the NTP jet treatment.

The constantly increasing burden of wound care has been earlier reported, and conventional wound treatments are time consuming and expensive. There are several factors that are typically considered in the wound-healing process, including the host factor (wound), the bacterial factor, and the treatment factors.[1] However, new concepts and strategies controlling wound inflammation and thus improving wound care are strongly needed. Applying NTP on a wound is one of these promising strategies for improving the wound-healing process. The emphasis of NTP on improvement of wound healing may be due to direct bacteria and antiseptic effects, including proliferation, skin cell migration, or oxidative stress in the wound micro-environment. However, the effect of NTP on wound healing needs further microscopic and macroscopic evaluation. Furthermore, the potential toxicity to the host

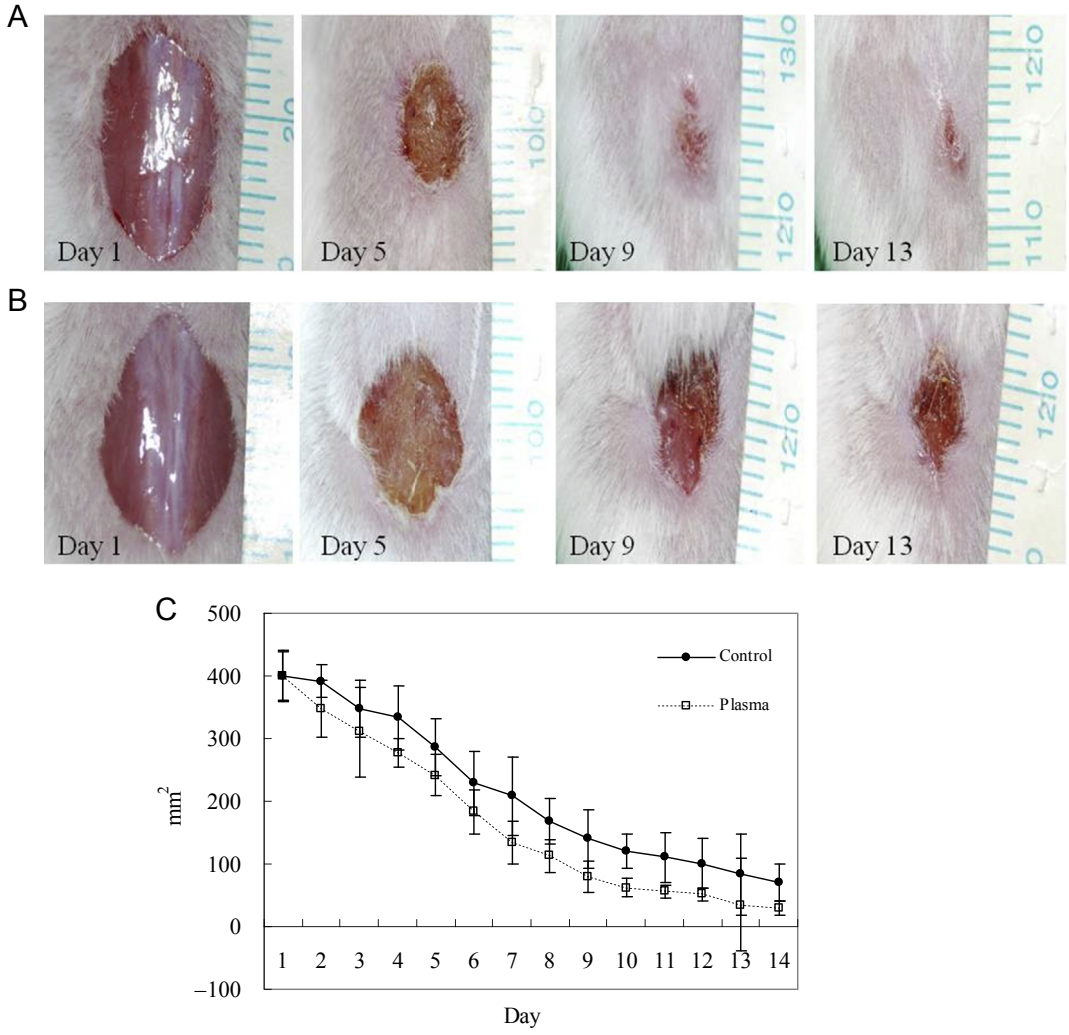

Fig. 3. Wound-healing process observation: (A) serial wound-healing process on Day 1, Day 5, Day 9, and Day 13 under gross observation in the nonthermal plasma (NTP) treatment group, and early wound healing after receiving NTP treatment; (B) gross observation on Day 1, Day 5, Day 9, and Day 13 in the control group; and (C) the wound size significantly decreased between the NTP and control group in the wound-healing process. The wound size is significantly smaller in the experimental group than in the control group on the 13th day of post-treatment (57.8 ± 10.5 mm^2 vs. 106.8 ± 39.4 mm^2, respectively).

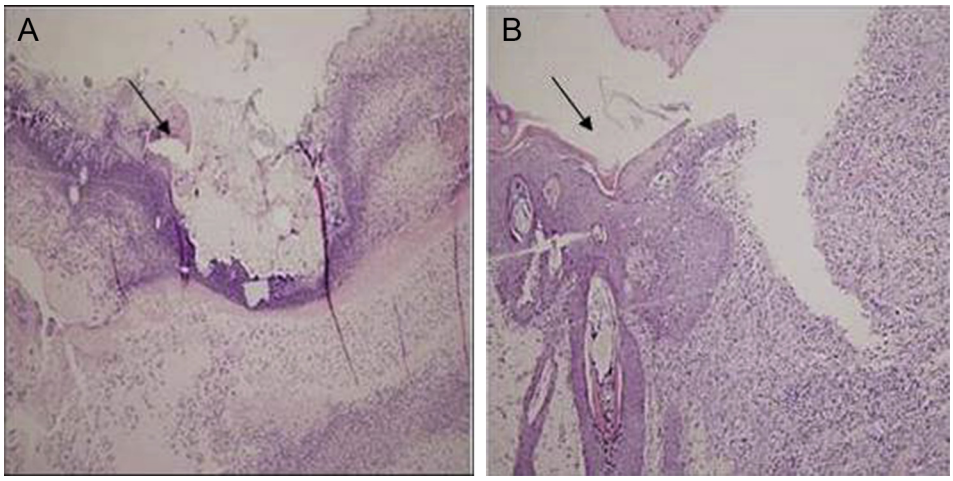

Fig. 4. Histology of epidermal tissue by hematoxylin and eosin staining post-nonthermal plasma treatment Day 2 (50×). (A) Fibrin layer covered the wound defect in the nonthermal plasma treatment group; and (B) control group, inflammatory cells covered the wound region, and no obvious exudates were present.

organ would also need to be evaluated. In the present study, we proved that NTP improved wound healing on gross observation and histological evaluation.

Cell adhesion plays an important role for fibroblasts, keratinocytes, and endothelial cells and their migration to the wound site in the wound-healing process. Cell adhesion is mediated by specialized molecules located on the cell surface, including cell–cell and cell-matrix adhesion molecules.[25] The coordinate modulation of the cellular functions of cadherins and integrins plays an essential role in fundamental physiological and pathological processes, including tissue differentiation and renewal, wound healing, immune surveillance, and inflammatory response.[18] Those molecules could be responsible for cell adhesion, detachment, cell migration, and cell growth and would be influenced by NTP. Cell detachment is often observed after treating cells with plasma. While focusing on cadherin, NTP was observed to have no effect on cell adhesion.[26] However, these studies are based on cell culture models. Our study demonstrated the effect of an NTP model in SD rats, and the expression of E-cadherin was increased expression in the plasma treated group. Loss of E-cadherin expression was more obvious at the 7th day in the experimental group than in the control group.

As previously mentioned, plasma emits several kinds of radiation and is further characterized by reactive oxygen.

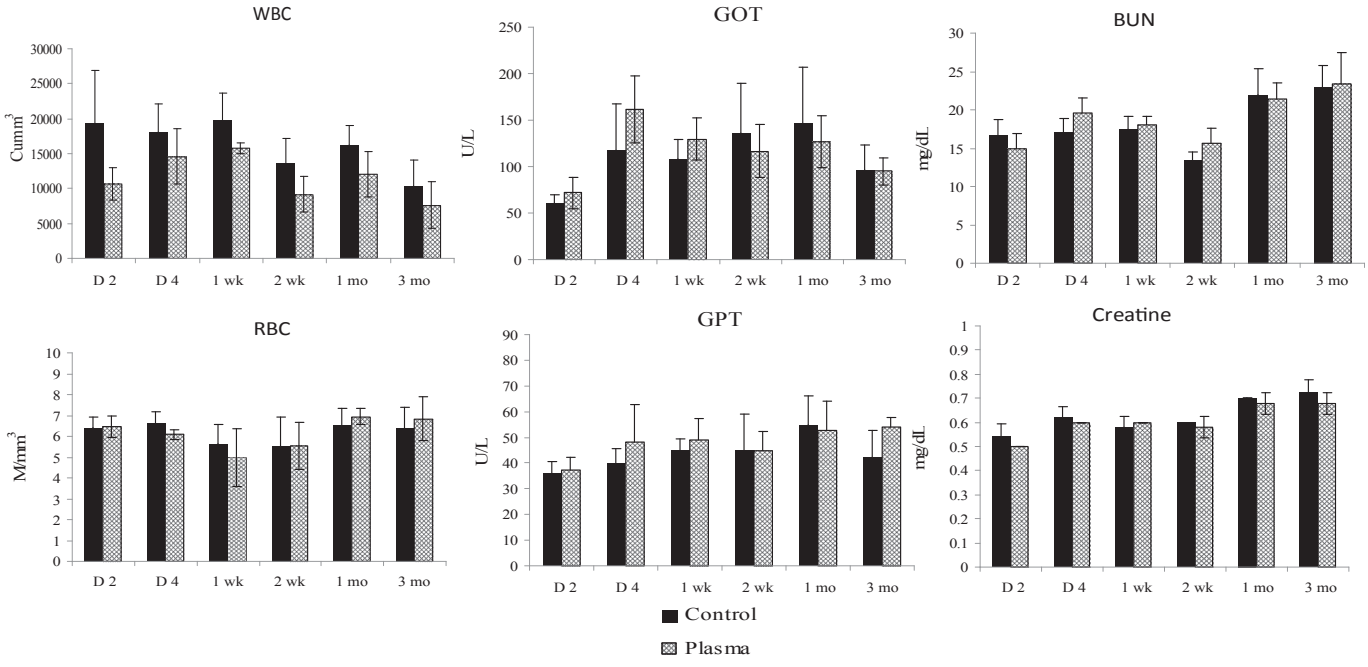

Fig. 5. In a hematological analysis of blood tests in the nonthermal plasma wound treated group and the control group, white blood cells (WBC) were increased in the control group compared with the nonthermal plasma group. There was no obvious difference in red blood cells (RBC), aspartate aminotransferase (GOT), alanine aminotransferase (GPT), blood urea nitrogen (BUN), or creatinine between the control and experimental groups.

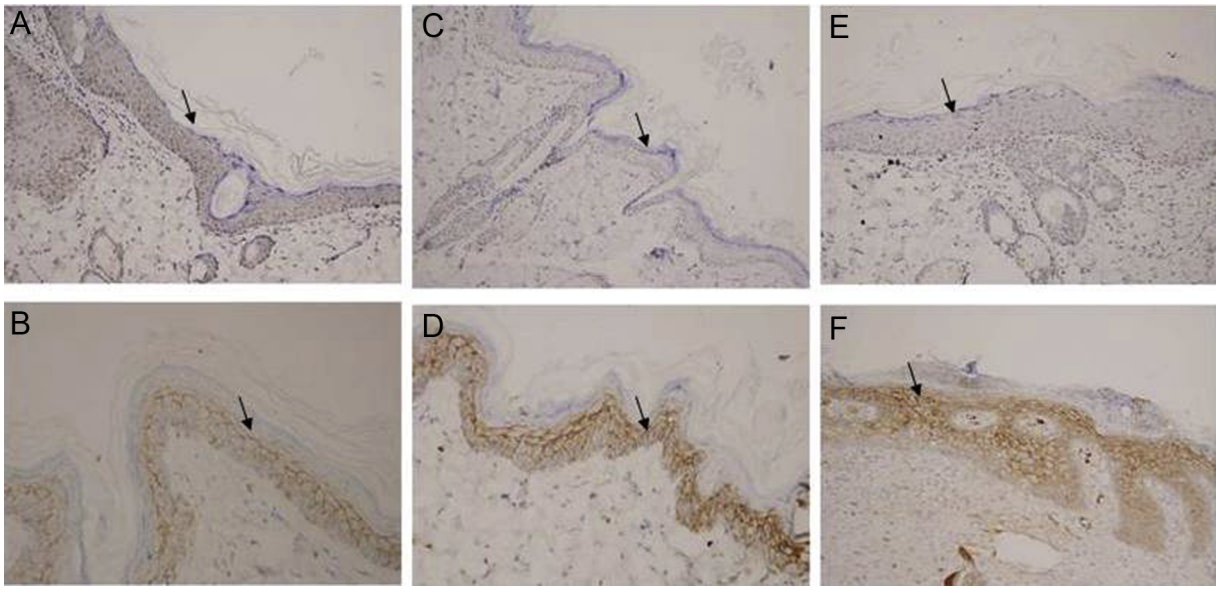

Fig. 6. Wound healing process by immunohistochemical staining for 4-hydroxy-2-nonenal (4-HNE) and E-cadherin expression in epithelium (200×): (A, B) control; (C, E) 4-HNE expression in epithelium 7 days and 14 days after nonthermal plasma treatment; (D, F) E-cadherin expression on 7 days and 14 days after nonthermal plasma treatment (arrow: 4-HNE expression in A, C, E; E-cadherin expression in B, D, F).

Table 2
Quantification of immunohistochemical staining of nonthermal plasma effects on 4-hydroxy-2-nonenal (4HNE) and E-cadherin expression.

		4-HNE		Day 7 vs. Day 14[b]	E-cadherin		Day 7 vs. Day 14[b]
		Day 7	Day 14		Day 7	Day 14	
Control group	N	7	7	<0.001	6	6	<0.001
	Mean	43.07	17.50		84.44	49.10	
	SD	4.22	3.46		1.86	6.90	
NTP group	N	7	8	<0.001	8	7	0.04
	Mean	16.16	55.11		52.17	70.46	
	SD	12.81	8.11		14.96	12.78	
Control vs NTP[a]		<0.001	<0.001		<0.001	0.004	

NTP = nonthermal plasma.
[a] p for independent sample t test.
[b] p for paired sample t test.

Mizuta et al demonstrated that ROS is produced during early-phase wound healing, and this period may be crucial for regulating ROS levels.[27] These studies indicate that the plasma-induced ROS may play a role in the process of wound inflammation. Whether plasma-induced ROS are capable of penetrating the intracellular environment still remains unknown. In our study, the plasma was not directly injected onto the wound; instead, the plasma was diffused over the carriage space. Our results also supported the notion that ROS can regulate the expression of cell surface adhesion markers. In our autopsied organs, no tissue necrosis or inflammation was observed.

The relevance of NTP for treating wounds is not only through antimicrobial effects, but is also related to the stimulation of proliferation and migration of wound-related skin cells and cell adhesion. We demonstrated that NTP could improve wound healing by the area of wound under clinical observation, histological early repair, increased oxidative stress, and adhesion protein expression. NTP did not exhibit an increased cell count and systemic organ toxicity.

This study does have several limitations. Firstly, there is a lack of more comprehensive indicators for clinical wound evaluation, histological markers for wound healing, repair, and oxidative stress evaluation. Secondly, IHC staining is typically used for microscopic observation for tissue, and is not a good method for quantifying molecular changes. Without protein quantification, such as Western blot analysis, the significant change of 4-HNE or E-cadherin between the experimental and control groups could not be well-demonstrated in the present study.

In conclusion, NTP was shown to improve the wound-healing process, increase ROS, and decrease E-cadherin expression. Short-term, low-dose NTP treatment was demonstrated to accelerate the wound-healing time without organ toxicity.

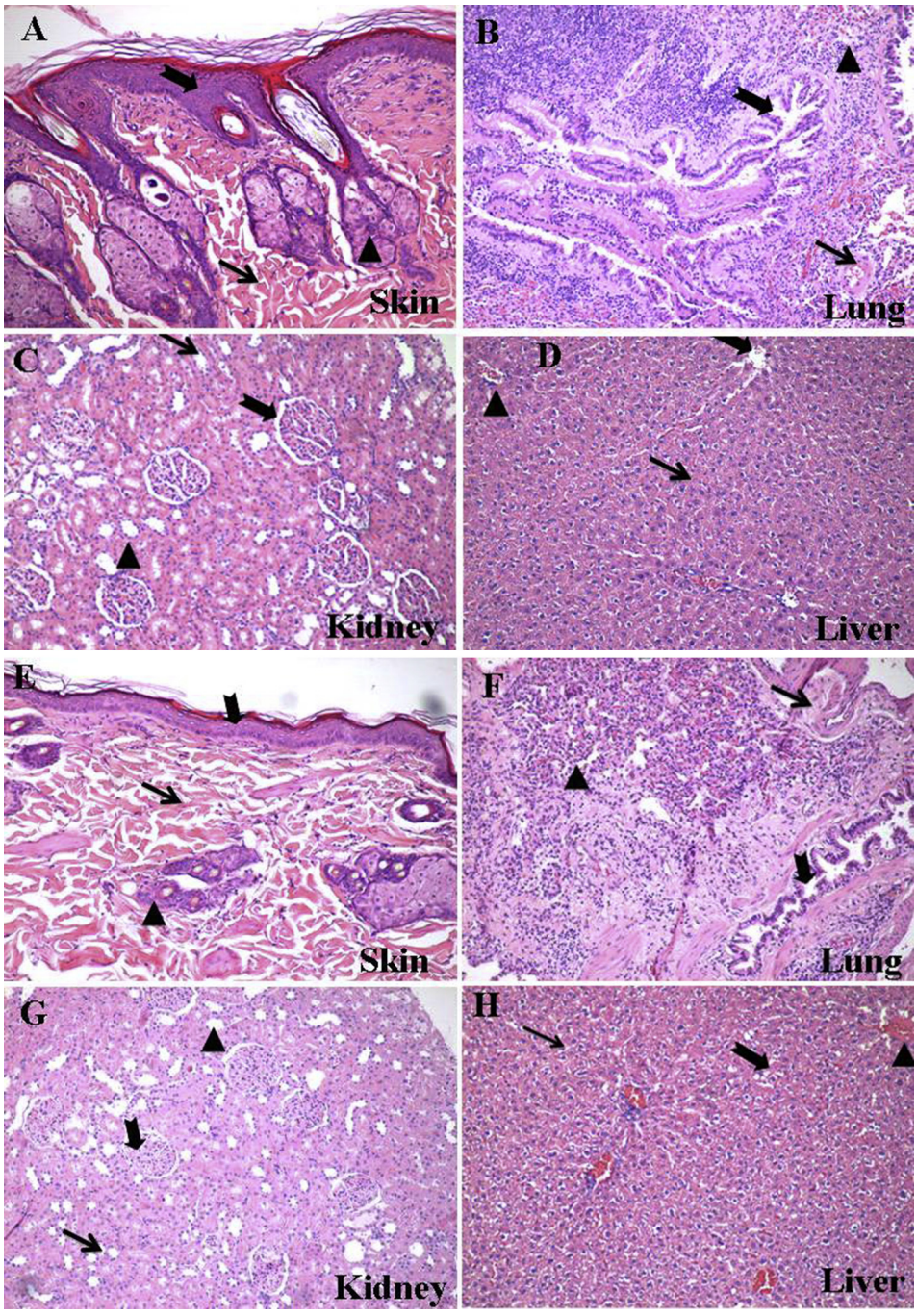

Fig. 7. Toxicity studies revealed normal organs in the nonthermal plasma treatment group and control group. Nonthermal plasma group: (A) skin: thick arrow: epidermis, arrowhead: hair follicle, arrow: dermis; (B) kidney: thick arrow: glomerulus, arrowhead: proximal tubule, arrow: distal tubule; (C) lung: thick arrow: bronchioles, arrowhead: alveolus, arrow: vessel; (D) liver: thick arrow: portal area, arrowhead: central vein, arrow: hepatocyte; and (E−H) control groups.

Acknowledgments

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References

1. Haertel B, von Woedtke T, Weltmann KD, Lindequist U. Non-thermal atmospheric-pressure plasma possible application in wound healing. *Biomol Ther* 2014;**22**:477−90.
2. Teixeira HS, Marin C, Witek L, Freitas Jr A, Silva NR, Lilin T, et al. Assessment of a chair-side argon-based non-thermal plasma treatment on the surface characteristics and integration of dental implants with textured surfaces. *J Mech Behav Biomed Mater* 2012; **9**:45−9.
3. Peng YC, Chen SW, Tung CF, Chow WK, Ho SP, Chang CS. Comparison the efficacy of intermediate dose argon plasma coagulation versus hemoclip for upper gastrointestinal non-variceal bleeding. *Hepatogastroenterology* 2013;**60**:2004−10.
4. Whittaker AG, Graham EM, Baxter RL, Jones AC, Richardson PR, Meek G, et al. Plasma cleaning of dental instruments. *J Hosp Infect* 2004; **56**:37−41.

5. Arjunan KP, Clyne AM. Non-thermal dielectric barrier discharge plasma induces angiogenesis through reactive oxygen species. *Conf Proc IEEE Eng Med Biol Soc* 2011;**2011**:2447−50.
6. Poor AE, Ercan UK, Yost A, Brooks AD, Joshi SG. Control of multi-drug-resistant pathogens with non-thermal-plasma-treated alginate wound dressing. *Surg Infect* 2014;**15**:233−43.
7. Haertel B, Hahnel M, Blackert S, Wende K, von Woedtke T, Lindequist U. Surface molecules on HaCaT keratinocytes after interaction with non-thermal atmospheric pressure plasma. *Cell Biol Int* 2012;**36**:1217−22.
8. Schmidt A, Dietrich S, Steuer A, Weltmann KD, von Woedtke T, Masur K, et al. Non-thermal plasma activates human keratinocytes by stimulation of antioxidant and phase II pathways. *J Biol Chem* 2015;**290**:6731−50.
9. Wende K, Strassenburg S, Haertel B, Harms M, Holtz S, Barton A, et al. Atmospheric pressure plasma jet treatment evokes transient oxidative stress in HaCaT keratinocytes and influences cell physiology. *Cell Biol Int* 2014;**38**:412−25.
10. Duan Y, Huang C, Yu QS. Cold plasma brush generated at atmospheric pressure. *Rev Sci Instrum* 2007;**78**:015104.
11. Fricke K, Koban I, Tresp H, Jablonowski L, Schroder K, Kramer A, et al. Atmospheric pressure plasma: a high-performance tool for the efficient removal of biofilms. *PloS ONE* 2012;**7**:e42539.
12. Heinlin J, Isbary G, Stolz W, Zeman F, Landthaler M, Morfill G, et al. A randomized two-sided placebo-controlled study on the efficacy and safety of atmospheric non-thermal argon plasma for pruritus. *J Eur Acad Dermatol Venereol* 2013;**27**:324−31.
13. Gesbert F, Larue L. Non-thermal plasmas: novel preventive and curative therapy against melanomas? *Exp Dermatol* 2014;**23**:716−7.
14. Heinlin J, Morfill G, Landthaler M, Stolz W, Isbary G, Zimmermann JL, et al. Plasma medicine: possible applications in dermatology. *J Dtsch Dermatol Ges* 2010;**8**:968−76.
15. Huo Y, Qiu WY, Pan Q, Yao YF, Xing K, Lou MF. Reactive oxygen species (ROS) are essential mediators in epidermal growth factor (EGF)-stimulated corneal epithelial cell proliferation, adhesion, migration, and wound healing. *Exp Eye Res* 2009;**89**:876−86.
16. Thews O, Lambert C, Kelleher DK, Biesalski HK, Vaupel P, Frank J. Impact of reactive oxygen species on the expression of adhesion molecules *in vivo*. *Adv Exp Med Biol* 2009;**645**:95−100.
17. Zhou S, Li Y, Huang F, Zhang B, Yi T, Li Z, et al. Live-attenuated measles virus vaccine confers cell contact loss and apoptosis of ovarian cancer cells via ROS-induced silencing of E-cadherin by methylation. *Cancer Lett* 2012;**318**:14−25.
18. Goitre L, Pergolizzi B, Ferro E, Trabalzini L, Retta SF. Molecular crosstalk between integrins and cadherins: do reactive oxygen species set the talk? *J Signal Transduct* 2012;**2012**:807682.
19. Sensenig R, Kalghatgi S, Cerchar E, Fridman G, Shereshevsky A, Torabi B, et al. Non-thermal plasma induces apoptosis in melanoma cells via production of intracellular reactive oxygen species. *Ann Biomed Eng* 2011;**39**:674−87.
20. Blackert S, Haertel B, Wende K, von Woedtke T, Lindequist U. Influence of non-thermal atmospheric pressure plasma on cellular structures and processes in human keratinocytes (HaCaT). *J Dermatol Sci* 2013;**70**:173−81.
21. Yang Y, Sharma R, Sharma A, Awasthi S, Awasthi YC. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochim Pol* 2003;**50**:319−36.
22. Ciobica A, Padurariu M, Dobrin I, Stefanescu C, Dobrin R. Oxidative stress in schizophrenia - focusing on the main markers. *Psychiatr Danub* 2011;**23**:237−45.
23. Yamaguchi T, Nagashima R, Yoneyama M, Shiba T, Ogita K. Disruption of ion-trafficking system in the cochlear spiral ligament prior to permanent hearing loss induced by exposure to intense noise: possible involvement of 4-hydroxy-2-nonenal as a mediator of oxidative stress. *PloS ONE* 2014;**9**:e102133.
24. Pham NA, Morrison A, Schwock J, Aviel-Ronen S, Iakovlev V, Tsao MS, et al. Quantitative image analysis of immunohistochemical stains using a CMYK color model. *Diagn Pathol* 2007;**2**:8.
25. Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. *Cell* 1996;**84**:359−69.
26. Haertel B, Wende K, von Woedtke T, Weltmann KD, Lindequist U. Non-thermal atmospheric-pressure plasma can influence cell adhesion molecules on HaCaT-keratinocytes. *Exp Dermat* 2011;**20**:282−4.
27. Mizuta M, Hirano S, Ohno S, Tateya I, Kanemaru S, Nakamura T, et al. Expression of reactive oxygen species during wound healing of vocal folds in a rat model. *Ann Otol Rhinol Laryngol* 2012;**121**:804−10.