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Cold plasma on full-thickness cutaneous wound accelerates healing through promoting inflammation, re-epithelialization and wound contraction

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ABSTRACT

We investigated cold plasma effects on acute wounds of mice. The mice were classified into experimental and control groups. In the former, wounds were treated using cold plasma once daily for 1 min, and then covered with hydrocolloid dressing; wounds in the control were left to heal under hydrocolloid dressing. Daily evaluation was conducted for 15 days. General and specific staining was applied to evaluate re-epithelialization, neutrophil, macrophage, myofibroblast and transforming growth factor beta. It was found that cold plasma accelerated wound healing by 1 day. Plasma may promote the late phase of inflammation, accelerate re-epithelialization and increase wound contraction.

1. Introduction

Cutaneous wound healing is a complex physiological process consisting of orchestrated events communicated by collaborative factors [1]. The utilization of various exogenous agents from natural products like Indonesian honey [2] and oleic or linoleic acid [3] to physical tools like light [4] and laser [5] has been shown to enhance the overlapping healing phases, including inflammation, proliferation and remodeling [1]. Among these, wound therapy based on cold plasma, that is, non-equilibrium plasma (with an electron temperature much higher than the gas temperature), with a low temperature of ionized gas [6], has opened the possibility of a paradigm shift in biomedical therapy [7]; it has drawn substantial attention from both plasma and wound care scientists since its feasibility to work through living tissue [8,9] and its potency for resolving problems in contemporary wound care were demonstrated [10]. As the fourth state of matter [6], plasma has the ability to produce controllable reactive species, like nitric oxide (NO) and hydroxyl radicals (OH), upon contacting the open air [11], as well as OH radicals and hydrogen peroxide (H_2O_2) upon contacting an aqueous solution [12]. Although the clinical efficacy of carefully controlled treatment with cold plasma for killing bacteria colonizing chronic wounds [13,14] and improving wound healing [15] has been demonstrated, there have been few studies about the effects of cold plasma and its mechanism of action on acute wounds in mouse models.

Re-epithelialization and wound contraction are two key events in the healing of full-thickness wounds. The former is central to wound closure, which is closely connected to granulation tissue formation in a spatiotemporal manner [16], and the latter may account for up to a 40% decrease in wound size, correlated with the expression of myofibroblasts [17]. It is well established that

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these processes are influenced by the presence of growth factors like epidermal growth factor (EGF), keratinocyte growth factor (KGF) and transforming growth factor (TGF) [15], which are likely mediated by reactive oxygen species (ROS) and NO [18–20].

Although the mechanism of the interaction between cold plasma and cells or living tissue is still unclear [21], several studies have reported the effects of cold plasma on key wound-related cells or sub-cells, included promoting the proliferation of fibroblasts [22] and endothelial cells [23], as well as the growth of epithelial cells [24], inhibiting the migration of fibroblasts [25] and their surface expression [26], and activating integrin of fibroblasts and epithelial cells [27]. Interestingly, some of these effects are likely to be similar to the activities of natural ROS and/or NO during wound healing, particularly cold plasma's effects on the proliferation of both fibroblasts and endothelial cells [19]. Therefore, the aim of this study was to assess the effects of cold plasma on acute cutaneous wound healing in an in vivo scenario with a focus on re-epithelialization and wound contraction.

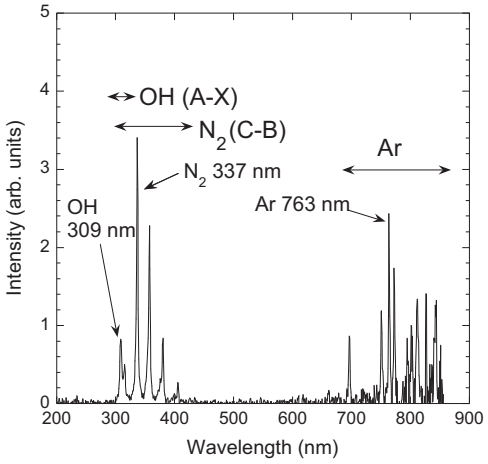

Fig. 1. Optical emission spectroscopy (OES) measurement of cold plasma jet near the wound surface (about 10 mm under the nozzle of the cold plasma reactor) during treatment. OH and nitrogen-based reactive species were identified.

2. Materials and methods

2.1. Cold plasma jet characterization and mouse wound positioning

The cold atmospheric pressure plasma jet system that we used here is similar to the device developed by Teschke et al. [28]. Two metal ring electrodes were used around the quartz tube for the cold atmospheric pressure plasma jet system provided by the Division of Electrical Engineering and Computer Science, Kanazawa University, Kanazawa, Japan. It had a quartz tube with a 1.6 mm inner diameter. A low-frequency (~20 kHz) AC high voltage, with a peak-to-peak voltage of 25 kV, was applied to the two ring electrodes when commercial argon gas (99.995% purity) at a flow rate of 5 slm was injected from one end of the quartz tube. The discharge voltage and discharge current were measured with a high-voltage probe (P6015A; Tektronix, Inc., Tokyo, Japan) and a current probe (8585C; Pearson Electronics, Palo Alto, CA, USA). The average power density at the electrode was 85 W/cm^2 in this study.

During its treatment, a mouse wound was positioned about 15 mm under the nozzle of the plasma reactor, and was not touched by its jet. Optical emission spectroscopy (OES) measurement at about 10 mm under the nozzle showed the emissions of the OH (A-X transition) transition near 309 nm, N_2 (C-B transition) (band head maximum at 337 nm) [29] and Ar I (maximum 763 nm). This observation revealed the presence of both hydroxyl radical (OH) and nitrogen-based reactive species in the gas phase during its generation (Fig. 1).

In order to evaluate its thermal effect on living tissue, a cold plasma jet was tested on normal skin of anesthetized mouse prior to its application on wound. On the basis of measurement with a non-contact infra-red digital camera (F30S; NEC Avio Infrared Technologies, Tokyo, Japan) at room temperature (24 °C), during cold plasma treatment, the temperature of the influenced skin, of non-influenced skin and of the end of the plasma nozzle were 32.3 °C, 28.8 °C and 36.6 °C, respectively. After the session, injury was not observed on treated skin.

2.2. Animals and experimental protocol

Forty BALB/c CrSlc male mice aged 8 weeks (Sankyo Lab Service Corporation, Inc., Toyama, Japan) and weighing 21.3–26.0 g were used. They were caged individually in an air-conditioned room at 25.0 ± 2.0 °C with light from 08:45 to 20:45 h. Water and laboratory chow were given freely. The experimental protocol and animal care were in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University, Japan (AP: 112243).

2.3. Wound healing model and plasma treatment

After being completely anesthetized by the injection of pentobarbital sodium (0.5 mg/10 g weight) into the peritoneal cavity, we held the skin of the dorsum including the subcutaneous tissue between thumb and finger, folded it along the apex of the median line on the dorsum in a U-shape, put both sides of the skin together, made two holes through the skin with a sterile disposable 2 mm biopsy punch (Kai Industries Co. Ltd., Gifu, Japan) and finally made two circular (2 mm in diameter) full-thickness skin wounds including the panniculus carnosus muscle and a part of the subcutaneous tissue on both sides of the dorsum of the mouse. Subsequently, the mice were randomly classified into two groups: (1) experimental group, with wounds treated once daily by a cold plasma jet during 1 min in one spot on the wound, and then covered by hydrocolloid dressing (Tegaderm; 3 M Health Care, Tokyo, Japan) to maintain its moist environment; and (2) control group, with wounds only allowed to heal under hydrocolloid dressing.

2.4. Macroscopic evaluation

The day when wounds were made was designated as day 0, and the process of wound healing was observed daily from days 0 to 15 after wounding. Before observation, the surrounding environment of wounds was cleaned with saline solution. Wounded edges were traced on polypropylene sheets and photographs were taken every day. The traces on the sheets were captured with a scanner onto a personal computer using Adobe Photoshop Elements 7.0 (Adobe System Inc., Tokyo, Japan), and the areas of wounds were calculated using image analysis software Scion Image Beta 4.02 (Scion Corporation, Frederick, Maryland, USA).

2.5. Calculation of healing day

The day of wound healing was calculated based on a graph of the ratios of areas to original areas. Initially, the overall trend of such a graph was evaluated. Wound healing day was plotted on the *y*-axis when the trend of reduction of wound size started to become flat, which was at 0.15.