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Original research article

Evaluation the effectiveness of combinative treatment of cold plasma jet, Indonesian honey, and micro-well dressing to accelerate wound healing

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A R T I C L E I N F O

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A B S T R A C T

A combinative treatment of cold plasma jet, honey solution, and micro-well dressing to accelerate acute wound healing of mouse skin was evaluated. A honey solution 1% in phosphate-buffered saline (PBS) 20 µL was dropped into micro-well dressing that is attached on an acute wound before a 2-min cold plasma treatment. An inclined treatment style plasma jet with a 45° gradient was used. An infrared thermal camera was used to monitor the fate of the solution. To evaluate its effect, macroscopic evaluation and general staining were conducted. It was revealed that a combination of that honey and plasma treatment may not be efficacious. However, the related procedure functionalising micro-well dressing may provide a new insight in how to combine plasma jets and other solutions in animal or human models for skin-oriented treatment.

1. Introduction

Generally, wound healing has been divided into 3 overlapping stages: inflammation, granulation tissue formation, and matrix formation and remodeling [1]. In wound care management, it is well known that there are many modalities, beside standard wound care, that have the ability to improve wound healing, from natural products like hormones [2,3] and honey [4,5] to physical tools, like light [6]; however, it is understood that very few can cope with conditions in the wound bed throughout all stages of healing [7]. It is therefore very important to explore a new approach in order to get fine treatment that meets the requirements of the wound. A combination treatment of cold plasma jet and honey may provide new insights.

Cold plasma is a so-called non-equilibrium plasma, in which the gas temperature is much lower than the electron temperature. In the study of biomedical application of plasma (plasma medicine), it is established that the innovative value of plasma treatment may be associated with its possibility of producing a family of exogenous biological molecules, namely reactive oxygen and nitrogen species (RONS) [8]. It is a remarkable fact that small molecules containing RONS have pivotal roles in biological systems [9]. In a biomedical sense, they not only have physiological effects, like influencing cell proliferation, cell adhesion and spreading, wound healing, and growth hormones, but also pathophysiological effects, like influencing atherosclerosis, diabetes, and cancer [10]. In recent years, cold plasma has demonstrated many possible applications in medical scope such as for disinfection of the human body or teeth [11–14], sterilization of medical equipment [15,16], and cancer therapy [17,18]. The possibility of potentiation of wound healing by cold plasma has also been the focus of attention in many recent investigations [19–23].

Even though there are a broader range of atmospheric plasma sources, cold plasma jets have been chosen as one of the most favourite sources for plasma medicine application because the plasma can be extended to regions not limited by electrodes [24]. Referring to Lu, a

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plasma jet consists of 2 main conditions with distinctive characteristics: (a) a plasma condition containing relatively short-lifetime radicals, such as N_2^*, O_2^*, OH, and N_2^+; and (b) an afterglow condition containing relative long-lifetime radicals, such as OH, O, O_3, NO, and some metastable molecules, such as O_2(a) and N_2(A) [25]. On the basis of our previous report, it was found that a non-contact treatment style of plasma jet (afterglow condition) was able to accelerate skin wound healing in animal models [20]. Moreover, such wound healing acceleration can be optimised by adding distilled water droplets during the treatment [21]. It was suspected that water is able to enhance the production of reactive species of plasma jet, like hydrogen peroxide (H_2O_2) and nitrogen based species, that, at an appropriate concentration, can improve wound healing [26,27]. Jablonowski et al. [28] stated that the interaction processes phase between plasma and liquid has been identified to be the pivotal key to understanding the detailed mechanisms of the effects atmospheric pressure plasma has on living systems. Considering that water is in a liquid phase, it is hypothesised that the other type of solution, honey, may also be compatible to be combined with a plasma jet to support wound healing.

Honey has been implemented for wound therapy since ancient times but medical-grade honey dressing was developed in the late 1990s [7]. It is now realized that honey, like Indonesian honey [4], is a biologic wound dressing with many biological activities that work in concert to support the healing processes. Honey is a potential source of natural antioxidants that become more active in dilutions [29]. Honey in dilution with a low concentration of H_2O_2 showed the least cytotoxic effect on mammalian cells [30,31]. Furthermore, its antioxidant and anti-inflammatory activity provide a favourable bioambience for wound healing.

This research was conducted to evaluate the effectiveness of a combinative treatment of cold plasma jet and micro-well dressing containing honey to accelerate acute wound healing in an animal model. A micro-concentration of Indonesian honey solution (1% in PBS) in micro-volume (about 20 µL) was dropped into a micro-well of hydrocolloid dressing that covered an acute wound before plasma treatment. Micro-well dressing is familiar dressing for clinical wound care that was modified by making a single hole (diameter ~1 mm) in it. During treatment, an infrared thermal camera was applied to monitor the fate of the honey solution.

2. Experiment

2.1. Cold plasma jet system

This research used a cold atmospheric pressure plasma jet system with an inclined style of treatment (gradient =45°) as shown in Fig. 1. This system was developed based on Teschke et al. [32] Medical-grade argon gas (99.999% purity) produced by the Samator Company (Indonesia) was used as a carrier gas. Two aluminium foil ring electrodes were used around the quartz tube for this system. It had a quartz tube with a 1.5 mm inner diameter and a 2.7 mm outer diameter. The quartz tube was produced by the Fujiwara Company (Japan). The distance between the 2 electrodes was 17 mm. The lower ring electrode was connected to the ground. A low-frequency (~20 kHz) AC high voltage, with a peak-to-peak voltage of 6.67 kV, was applied to the upper ring electrode when argon gas at a flow rate of 2 standard litres per minute (slm) was injected from one end of the quartz tube. A high-voltage probe and a current probe were applied to measure the discharge voltage and discharge current to estimate the consumed power of the power supply [20,21].

2.2. Thermal and safety evaluation of cold plasma jet on normal skin

The hair of an anaesthetized BALB/c mouse was shaved a day before treatment. It was anaesthetized via injection of ketamine-xylazine, (K) 50 mg/kg +(X) 5 mg/kg, into the peritoneal cavity [33]. The mouse was treated with a cold plasma jet under the following conditions: argon gas flow rate =2 slm; peak-to-peak voltage =6.67 kV; nozzle tip-skin distances (d) =5, 10, 15, and 20 mm as shown in Fig. 1; and treatment time =4 min. The dorsal skin of the mouse was treated with different nozzle tip-skin distances on different spots so that there were 4 spot samples. A digital camera (Panasonic Lumix FH6) was used to document the experimental conditions both during and after treatment. During treatment, the temperature distribution of the treated skin and its surroundings was measured using a low-cost non-contact infrared thermal camera (FLIR C2, Sweden). Using this device, about 5 images from each sample were produced over the 4 min treatment time. The skin condition after treatment was then visually observed.

The relationship between nozzle tip-skin surface distance and ΔT was evaluated. ΔT was calculated as $T_p - T_{ni}$, in which T_p is the peak temperature spot of the skin under plasma treatment and T_{ni} is the temperature spot on the skin with no plasma treatment. T_p and T_{ni} were obtained from thermal images processed using FLIR Software Tools. A colour palette provided by FLIR Software Tools, namely Medical, was used to express images.

2.3. Honey solution

A commercial Indonesian pure honey (Madu Murni Nusantara, Solo, Indonesia) diluted in PBS with a low concentration (1%) was applied in this research.

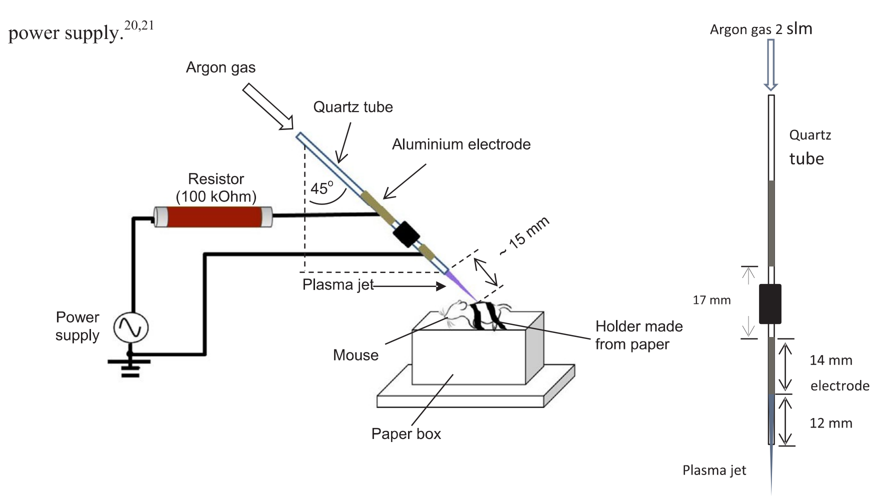

Fig. 1. Experiment setup.