Plasma activated radix arnebiae oil as innovative antimicrobial and burn wound healing agent

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Abstract: The plasma enhanced oil based drug, named plasma activated radix arnebiae oil (PARAO) is produced in the radix arnebiae oil (RAO) by a single-step plasma-enabled process. The highly reactive PARAO can not only kill the common bacterium (MRSA and P. aeruginosa) on burn wound efficiently, but also suppress inflammation, stimulate re-epithelialization, and reduce scar formation. Therefore, the PARAO treatment realizes 11.3% faster second degree burn wound healing than the RAO treatment

Keywords: plasma activated oil, C=C bond, streamer-surface discharges, burn wound

1. Introduction

Burns represent a significant morbidity and mortality among different traumatic injuries. [1] More than 50% of deaths following burn injury relate to infection, most commonly caused by MRSA and P. aeruginosa. RAO is a crimson oil compound and the active ingredient of shikonin. Over the past thousand years, RAO has been found to possess anti-inflammatory, antineoplastic, antioxidant activities. However, the RAO's potential for burn wound treatment has been hindered by limited antimicrobial activity and restricting clinical applicability.

The treatment of RAO by non-thermal plasma is a feasible and advantageous means of increasing the active species density in the RAO. These active species of the PARAO can not only kill bacteria, but also promote wound healing. In this paper, Argon+O_2 plasma jet was used to produce PARAO. PARAO killed MRSA and P. aeruginosa efficiently, suppressed inflammation, stimulated re-epithelialization, and reduced scar formation. PARAO realized faster second degree burn wound healing than the traditional RAO treatment.

2. MATERIALS AND METHODS

A schematic of the apparatus used to produce PARAO is shown in Fig.1. The discharge device consisted of a cylindrical, dielectric layer covered electrode coaxially fed into a quartz glass tube. The center electrode was coupled to the 10 kHz AC high voltage source (24 kV pk-pk). The ground electrode consisted of a coil of copper wire wrapped around the tube. Argon + O_2 gases were fed into the tubing. The gas flow injection formed an unstable cavity or pocket in the oil. The cavity was inherently unstable, resulted in the occasional breaking and subsequent bubble formation.

The peroxide value (PV), iodine value (IV), and acid value (AV) of PARAO were measured. [2]

The antimicrobial activity of PARAO was evaluated by agar well diffusion method. Agar plates were inoculated with a standardized inoculum of MRSA (10^6 CFU mL^{-1}) and P. aeruginosa (10^5 CFU mL^{-1}).

30 mice (10 mice each group) were randomly selected at the start of the burn wound healing experiment and they were used in strict accordance with the 'Animal Research: Reporting in Vivo Experiments (ARRIVE)' guidelines.

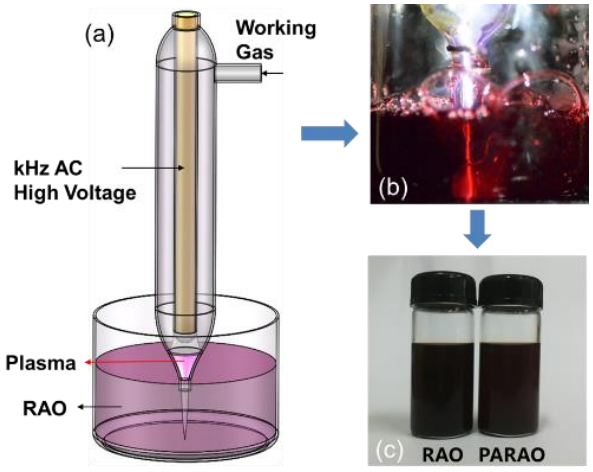

Fig.1. The PARAO produced by Ar+O2 plasma jet inside the RAO.

The protocol was approved by the Huazhong University of Science and Technology Tongji Medical College Animal Care and Ethics Committee.

3. Results and discussion

The electron density and electron energy of surface discharge on the liquid surface usually was ~ 10^{14}/cm3, and over 10 eV, respectively. For example, argon is easily ionized by the abundant electrons with the energy more than 15.8 eV, and the argon metastable atom has the energy level of 11.8 eV. This strong surface discharge results in the formation of plasma-liquid interface with a depth in the μm scale. As the basic part of RAO, the sesame oil contained significant amounts of unsaturated (C=C double bonds) fatty acids. The bond energy of C=C of unsaturated fatty acid in the oil was only 6 eV, which was below the energy levels of the significant population of the energetic plasma species. The energetic species produced by the surface discharge break C=C double bond in the plasma-oil interface. Afterwards, the atomic oxygen (with one order of magnitude higher than O_3) reacted with C-H bond directly to form H_2O_2 and carboxylic acid. The recombination of O_3 with C=C double bond produced 1,2,4-Trioxolane, which decomposed into H2O2 and carboxylic acid. The streamer discharge impacted the oil with the kHz repetition rate. The convective flow induced

by the streamer discharge facilitated transport of active species in the whole treatment volume.

The peroxide value of PARAO increased from 4 meq/kg to 300 meq/kg, 325 meq/kg, as the treatment time increased from 0 to 8 hours, 24 hours. The increasing peroxide value mainly occurred during 0~8 hours, afterwards the increasing was limited. The decreasing of shikonin caused by plasma was very limited, especially in the first 8 hours, which was consistent with the negligible color change between RAO and PARAO shown in Fig 1 (c). Therefore, the PARAO with 8 hours plasma treatment was used for burn wound healing and sterilization experiment. [3]

Because of the incidence of MRSA and P. aeruginosa infection in burn wounds, the activity of PARAO against these species was evaluated in vitro (Fig.2). PARAO exhibited a significant antimicrobial effect in comparison to both RAO and untreated control on treatment of MRSA. The growth inhibition zone produced by PARAO on MRSA has a diameter of 1.65 cm. The zoomed in Fig.2 (a) clearly indicates there were still bacteria colonies below the RAO. Although the inhibition zone produced by PARAO on P. aeruginosa (Fig.2(b)) was smaller than that on MRSA, the sterilization effect of RAO on P. aeruginosa was almost negligible.

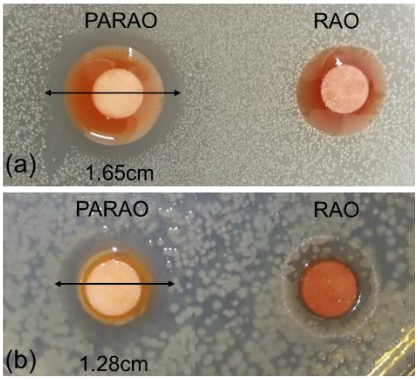

Fig.2. PARAO show higher bactericidal activity than RAO. Inhibition zones of PARAO (a), RAO (b) and Ceftazidime (CAZ) 30 μg (d) on MRSA (10^6 CFU mL^{-1}). (c) is the control group of MRSA (10^6 CFU mL^{-1}). Inhibition zones of PARAO (e), RAO (f), CAZ 30 μg (h) on P. aeruginosa (10^5 CFU mL^{-1}). (g) is the control group of P. aeruginosa (10^5 CFU mL^{-1}).

Topical administration of PARAO significantly accelerated the burn wound healing of mice as compared to untreated control and RAO groups (Fig.3). Due to the progressive tissue loss, burn wounds demonstrated an expanding zone of inflammation in early stages post injury. PARAO mitigated the wound expansion. The PARAO mitigated wound expansion. Fig.4 shows the size of the wound decreased to 84.2±5.3% of the initial wound after 5 days PARAO treatment, while the size of the wound treated by RAO for 5 days was 98.3±6.3% of the initial wound, the size of untreated control group increased to 110.2±7.3% of the initial wound. Besides accelerated closure, qualitative assessment indicated that PARAO treatment demonstrated earlier re-epithelialized and more well-formed granulation tissue than other groups (Fig.3). On the 14th day, the wound in the PARAO group closed up, while the wound closure of the control group and olive oil group was still at 77% and 89%, respectively. The wound in the RAO and control group closed up on the 16th and 19th day, respectively. The PARAO accelerated the wound healing by 26.3%, which was 11.3% faster than the RAO treatment. The decreasing of shikonin was very limited after plasma treatment, which suggests PARAO retained the wound healing ability of RAO. The high concentration of hydrogen peroxide and carboxylic acid can enhance activity of PARAO. Therefore, PARAO promoted wound healing obviously.

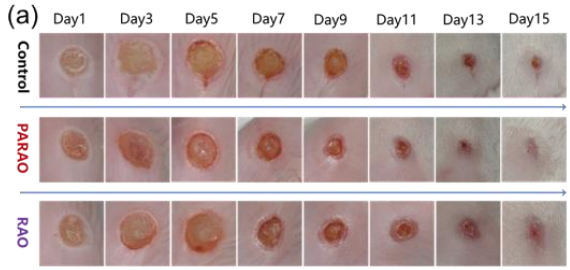

Fig.3. PARAO induces faster burn wound closure. The wound area photographed on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th after application of different treatment: control group, PARAO group and RAO group.

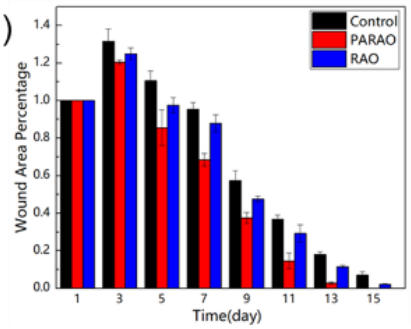

Fig.4 The percentage of wound contraction in different groups. Time points are averages of each group (10 mice, 5 measurements each mice). Statistical analysis conducted using 2-way ANOVA. Error bars denote SEM. ***P ⩽ 0.01

4. References

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