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Immediate intervention effect of dielectric barrier discharge on acute inflammation in rabbit’s ear wound

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Abstract
Acute inflammation is a protective stress response which is beneficial to the human body. However, if the duration of acute inflammation is prolonged in specific people, such as critically ill patients and people with low resistance, it will worsen the disease and increase complication rates. Most medical researchers are committed to finding an effective method to shorten the duration of acute inflammation. The purpose of our work is to shorten the duration of acute inflammation in a rabbit’s ear wound by dielectric barrier discharge (DBD) low temperature plasma. The distribution and quantity of inflammatory cells in the rabbit’s ear wound treated by plasma were studied and calculated by using a high power optical microscope and Image J software. The reactive species of plasma were characterized by optical emission spectroscopy. The results showed that the number of inflammatory cells decreased from 2312.17 ± 242.52 to 880.17 ± 89.08 after plasma treatment for 3 min. In addition, plasma has a coagulation effect on the rabbit’s ear wound. Our results indicated that DBD low temperature plasma could be an effective tool to decrease the inflammatory response time and the inflammation was further alleviated with increased treatment time.

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I. INTRODUCTION

Acute inflammation is a beneficial anti-injury stress reaction that can protect the human body against a wound, which is also extremely important for the body’s defense system. For most people, acute inflammation lasts for at most 2 or 3 days after injury. Acute inflammation is mainly in the form of deteriorating inflammation and exudative inflammation. Once the acute inflammation lasts too long, it will cause several adverse reactions such as pain, excessive tissue damage, slow wound healing, and scar hyperplasia. For some patients with low resistance, if active and effective measures are not taken on time, inflammatory cells will spread to the area surrounding the tissue gap or vascular system. Then, acute inflammation will transform to chronic inflammation, and the course of the disease is further extended. For example, continuous production of excessive inflammatory factors in the skin wound of diabetic patients is the main reason for the failure of wound healing. In short, local inflammation not only causes small range damage but can also further develop into the basis of many diseases or cause various diseases. At present, studies on chronic inflammation showed...
that appropriate plasma intervention can motivate proliferation and migration of keratinization and fibroblast, induce gene expression related to wound healing, and promote wound healing. However, there are few studies on the inflammatory reaction by plasma treatment in the acute stage of a wound. Compared with the plasma jet, dielectric barrier discharge (DBD) as a plasma source has more potential in treating different sizes of wounds. On the one hand, a large number of reactive oxygen species (ROS) produced by DBD have a good effect on killing inflammatory cells, which provides a possibility for the combination of DBD with wound treatment. On the other hand, the active substances produced by DBD enhance the absorption of extravasate and accelerate the repair of the wound. 

Plasma is the fourth state of matter, which is made up of ions, electrons, and non-ionized neutral particles. Compared with high temperature plasma, low temperature plasma has non-equilibrium characteristics. It could generate active free radicals and ions through collision with neutral gas molecules, and the temperature remains near room temperature, thus avoiding damage to tissues. DBD is one of the common plasma generating devices. It mainly produces plasma by inserting an insulated medium into the discharge space, also known as dielectric barrier corona discharge or silent discharge. DBD can produce a large amount of ROS and reactive nitrogen species (RNS) during the discharge process, including $O_2^-$, NO, and $O_3$, which is regarded as the main functional substance of DBD low temperature plasma. Their chemical properties are more active and easily react with other atoms, molecules, or other free radicals to form stable atoms and molecules. Furthermore, its coagulation function can also prevent the wound from the invasion of potentially pathogenic micro-organisms. DBD is characterized by low working temperature, high-efficiency, and non-toxicity. It has been extensively studied in the treatment of chronic trauma, tumors, anti-bacteria, dermatosis, and cancer therapy. Partecke et al. reported that the penetration depth of plasma in cancer tissues was less than 60 mm, which proved that plasma was difficult to penetrate the epidermis into deeper tissue regions. Besides, the mechanism of ROS and RNS reacting with the environment media to generate more efficient and stable substances can play a more effective role needs to be further studied.

We took rabbits, whose pathophysiological process is similar to humans, as experimental objects. The acute inflammation model was obtained by cutting a $1 \times 1 \text{ cm}^2$ wound on the rabbit’s ear and removing scabs on the surface after 24 h. Then, the DBD discharge plate was used to treat the rabbit’s ear wound. The distribution and change of inflammatory cells were observed after DBD treatment, and the alleviating effect of DBD on the acute inflammation in the rabbit’s ear wound was analyzed.

II. MATERIALS AND METHODS

A. Dielectric barrier discharge (DBD)

As shown in Fig. 1, the DBD device used in this experiment was designed. The two electrodes were obtained by micro-arc oxidation and plasma coating processes. First, the impurities on the aluminum block were cleaned up by ultrasound in acetone solution, washed in acetone with deionized water, and dried. Then, polytetrafluoroethylene was coated on the upper surface of the aluminum block. The coated aluminum block was put into electrolyte and injected with three-phase alternating current high voltage for 120 min. In this way, the unmicro-arc oxidized part of the aluminum block was considered as substance aluminum, while other uncovered parts were oxidized into insulating alumina. Next, the corrosion-resistant material coated on the upper surface was removed, and a metal film was deposited on the under surface of the aluminum block using a high-pressure cathode arc method. In the pressure of $1 \times 10^{-1}$ Pa, a voltage of 3 kV was applied to the copper cathode and a negative bias voltage was applied to a substrate for 40 min. Finally, removing the template covered on the substrate, an $\text{Al-Al}_2\text{O}_3-\text{Cu}$ electrode material was successfully prepared. As shown in Fig. 1(a), from the top to the bottom are the Al electrode, $\text{Al}_2\text{O}_3$ dielectric, and Cu electrode, respectively. Because the discharge occurs on the surface of the dielectric layer, this type of dielectric barrier discharge is also called surface discharge. Due to the existence of insulating dielectric layer, the above-mentioned atmospheric pressure dielectric barrier discharge can only work under AC voltage and the frequency of high-voltage power supply is generally tens of kilohertz.

B. Optical emission spectroscopy

The optical emission spectrum of the plasma system was collected by using the Charge Coupled Device (CCD) detector (Spec-10.100B; Princeton Instruments) over a spectral range of 200–1000 nm. 20 acquisitions of the reactive species were obtained fleetly within 1 min, and the average from 20 acquisitions was recorded as the emission spectra.

C. Establishment of the acute inflammation model

The experimental plan was approved by the Animal Protection and Use Committee of Anhui Medical University. A total of 5 mature male New Zealand white rabbits were selected (Laboratory Animal Center of Anhui Medical University, Hefei, China; age 3–4 months; weight 2–2.5 kg). All the animal experiments were conducted in accordance with the guidelines and ethical standards of the
Animal Protection and Use Committee of Anhui Medical University. Four rabbits were randomly selected and anesthetized by 4 ml kg$^{-1}$ chloral hydrate after one week rearing. As shown in Fig. 3(a), in order to create four independent wounds on the same rabbit’s ear, we measured four points from equal distance on each ear. Then, a $1 \times 1$ cm$^2$ wound was cut with a sterile scalpel and the epidermis was removed. The scab of the wound was removed after 24 h, and the acute inflammatory reaction model was built. All wounds were established under the same operation.

D. Low temperature plasma treatment

The rabbit was fixed on a fixing plate, one researcher smoothed the rabbit’s ear, another researcher placed the DBD dielectric plate at a distance of 10 mm from the rabbit’s ear wound [as shown in Fig. 3(b)], and then, another researcher turned on the power supply and recorded the time. During discharge, the DBD dielectric plate emitted blue light [Fig. 3(c)]. We designed three groups, namely, natural rabbit’s ear group (control group, C0), negative control group of acute inflammation in the rabbit’s ear wound (model group, M), and plasma intervention group (DBD group, D). Besides, the DBD group contains different treatment times 1 min, 3 min, and 5 min, which were recorded as D1, D3, and D5 groups, respectively.

E. Hematoxylin and eosin (H&E) staining

After treating the rabbit’s ear wound by using the low temperature plasma, excess pentobarbital sodium was used to kill the rabbit and the rabbit ear wound tissue was fixed overnight with 4% paraformaldehyde solution at 4 °C. The fixed sample was dehydrated in different concentrations of ethanol solution.

b. Dewaxing: The dehydrated tissue was embedded in paraffin, and 4-μm thick sections were dewaxed. Then, it was subjected to the following procedures successively: being soaked in xylene twice, each for 3 min; being cleaned by anhydrous alcohol twice, each for 3 min; being cleaned by 95% ethanol solution for 2 min and 80% ethanol solution for 2 min; and finally, being washed by distilled water twice, each for 3 min.

c. H&E staining: The sample tissue was subjected to the following procedures: successively being stained with hematoxylin solution for 3 min and washed with tap water for 10 min, using 1% HCl and 70% ethanol solution to separate color and tap water to clean, being stained with eosin solution for 10 s, and finally, being washed with tap water for 5 min.

d. Dehydration and sealing: The sample tissue further was subjected to the following procedures: being washed with 80% ethanol for 1 min and 95% ethanol twice, each for 1 min; anhydrous ethanol twice, each for 1 min; and xylene twice, each for 2 min. Finally, optical resin adhesive was used for sealing.

Finally, H&E stained sections of the rabbit’s ear tissue were observed under a 200× optical electron microscope. Six regions in different spots with the same area were randomly selected, and the number of acute inflammatory cells was calculated by using Image J.

F. Statistical processing

The experimental data were calculated and processed by MS Excel 2015 and recorded as average value ± standard deviation (SD). IBM SPSS Statistics 19 and SNK-q tests were used to compare and analyze the results of different plasma treatment groups. Origin 9.0 was used to draw figures. $P < 0.05$ was set to represent significant difference, and $P < 0.01$ was set to represent extremely significant difference.

III. RESULTS

As shown in Fig. 2, the reactive species generated by the DBD was studied by optical emission spectroscopy (OES). Figure 2(a) shows the optical emission spectrum curve of LTP in the full range of 280–1020 nm, which contained two distinct emission regions. To further understand the composition of the plasma system, two narrower wavelength ranges were plotted in Figs. 2(b) and 2(c). Region (1) [Fig. 2(b)], from 280 nm to 420 nm, was related to the emissions of the vibrations of molecular nitrogen. On the other hand, region (2) [Fig. 2(c)], over the range of 540–1020 nm, was referred to transitions of atomic oxygen and nitrogen. Both the emission spectrum of the first nitrogen negative system and $^1$OH emission can be seen. All in all, the spectrum indicated the characteristic curve relevant to H, OH, N, N$_2$, and O.

As shown in Fig. 4, the size of the “distance” represents the recovery of natural cells in different treatment groups. Figure 4(b) proves that the acute inflammation model was successfully established by our method. By comparing Fig. 4(b) with Figs. 4(c)–4(e), inflammatory cells in the rabbit’s ear wound tissue gradually dispersed and decreased with the treatment time. It could also be observed that with the increased treatment time, the plasma gradually penetrated into the rabbit’s ear tissue but did not penetrate into the cartilage.

As shown in Fig. 5, the inflammatory cells of the M group were significantly higher than the C0 group, and the inflammatory cells of the DBD low temperature plasma treatment group were significantly lower than those of the M group. In addition, with the increased plasma treatment time, the number of inflammatory cells gradually decreased. The D5 group had the best effect on the acute inflammation in the rabbit’s ear wound, compared to the M group, whose inflammatory cells were reduced to 840.33 ± 46.22.

IV. DISCUSSION

From Figs. 3(a) and 3(d), it can be seen that sticky substances can be formed on the surface of the rabbit’s ear wound after plasma intervention, which indicated that plasma could promote local coagulation. Fridman et al. stated that it will take about 15 min for the blood to coagulate. Nevertheless, it could be reduced to 1 min after being treated for 15 s by the plasma without any obvious damage on tissues. This result is consistent with the work of Kuo et al., who pointed out that plasma could stimulate the body to produce one coagulation factor, which could promote blood coagulation. In addition, many relevant reports also found that the concentration and pH value of the calcium ion remain unchanged during coagulation.

It is generally believed that shorter time and lower dose of plasma intervention can promote the body-related response, while...
longer time and higher dose of plasma treatment can induce cell apoptosis. Yan et al. proposed that plasma has a similar time and dose dependence on tumor cells. They treated various cancer cells with the plasma jet and found that plasma can inhibit the growth and proliferation of tumor cells. With the increased plasma dose, the effect of plasma on inhibiting the proliferation of cancer cells was more obvious. Plasma contains many components, such as active particles, ultraviolet rays, and positive and negative ions. These components react with organisms to destroy the properties and structures of macromolecular substances, such as proteins, organelles, DNA, RNA, and so on, thus causing cells to lose activity and inducing apoptosis. As shown in Figs. 4(b)–4(e), the penetrate depth of plasma became larger as the time increased and gradually penetrated...
into the cartilage layer. As also shown in Fig. 5, with the plasma intervention time increased, the acute inflammatory cells gradually reduced. There was no significant difference in inflammatory cells between D3 and D5 groups. However, by comparing Figs. 4(d) and 4(e), it can be seen that the distribution range of natural cells gradually widened from the epidermis to the inner layer of the rabbit’s ear wound, indicating that with the increased plasma treatment time, natural cells gradually recover and acute inflammatory cells reduced, effectively relieving acute inflammation and shortening the acute inflammatory cycle. In addition, there was no significant difference between D3 and D5 groups in inflammatory cells, which may be due to the plasma generator used in this experiment that has not penetrated enough to act on deeper tissues. 

The biological effects induced by plasma have made considerable progress in wound healing, but the molecular mechanism still needs to be studied. Our experimental results showed that low temperature plasma intervention had an immediate intervention effect on acute inflammatory response of body injury, which is of positive significance for wound healing.

AUTHOR’S CONTRIBUTIONS

C.D. and P.H. contributed equally to this paper.

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