Cold Atmospheric Plasma: A Novel Solution for Promoting Diabetic Wound Healing

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Abstract: Diabetes usually causes chronic wounds for which no satisfactory therapies currently exist. In this paper, a cold atmospheric plasma (CAP) device was developed to treat the wounds of genetically diabetic mice for 7.5 seconds per day for 8 days. Analyses of cytokines, morphometry, histology and immunohistochemistry demonstrated that the body's own inflammation-regulating function can be stimulated to reach its normal state, and angiogenesis, re-epithelialization and fibroblast repopulation can also be accelerated.

Keywords: Cold atmospheric plasma, diabetic wound healing, biomedical effects of CAP.

1. Introduction

One of the most serious and long-term unresolved problems for patients with diabetes is that as many as 25% may face a lifetime risk of developing chronic, non-healing wounds, such as diabetic foot ulcers. Unfortunately, no satisfactory therapeutic methods are currently available [1-3]. Previous studies have shown that CAP has anti-inflammatory [4], anti-tumor [5, 6], sterilizing [7–9], hemostatic [10], and tissue-regeneration-promoting [11, 12] effects on the living body. These effects are mainly attributed to numerous reactive oxygen and nitrogen species (RONS) existing in the CAPs [13]. In particular, recent experimental studies showed that CAP was effective in treating chronic wounds [14–16]. To the best of our knowledge, there is still a lack of complete understanding on the mechanisms by which CAP acts during the chronic wound healing process.

In this study, we developed a portable helium CAP jet generator that used atmospheric pressure dielectric barrier discharges. The wounds of db/db mice were treated by the CAP jet for 7.5 s per wound for 8 days. The experiments showed that the helium CAP jet treatment can significantly accelerate the diabetic wound healing process. And the possible mechanisms of the chronic wound healing at both the cellular and molecular levels have been discussed.

2. Methods

In this study, a portable CAP generation system, CAP Med-II, was developed by our group (Plasma Health Scientech Group of Tsinghua University of China). In the experiments, high purity helium (99.999%) was used as the plasma working gas. The helium flow rate (Q) was fixed at 8 slpm. The amplitudes of the discharge voltage (V_d) and current (I_d) were 3.5 kV and 36 mA, respectively. The driving frequency of the power supply (f) was fixed at 23 kHz. Under these operating conditions, a stable CAP jet can be produced. When treating the wounds of db/db mice, the quartz tube axis was kept vertical to the wound surface with an action distance of 5.0 mm. Each wound was treated by the helium CAP jet for 7.5 s each day for 8 consecutive days.

In this study, sixty db/db mice were randomly divided into the plasma treatment groups on the zeroth day (the day of modeling), and the first, second, third and seventh days [Group (D+P) (0th, 1st, 2nd, 3rd, 7th day)] and the self-healing groups on the same days [Group D (0th, 1st, 2nd, 3rd, 7th day)]. The mice in Group (D+P) were treated with the CAP jet for 7.5 s per wound per day. Four skin tissue samples, each with an area of 0.5 cm × 0.5 cm and a depth of 1.0 mm, were cut off from the backs of the mice. Based on comprehensive analyses of the inflammatory cytokines and chemokines; and morphometric, histological, and immunohistochemical analyses of the plasma-treated wounds, the regulatory process of the helium CAP jet was demonstrated in the inflammatory phase of the diabetic wound healing at both the cellular and molecular levels.

3. Results and Discussions

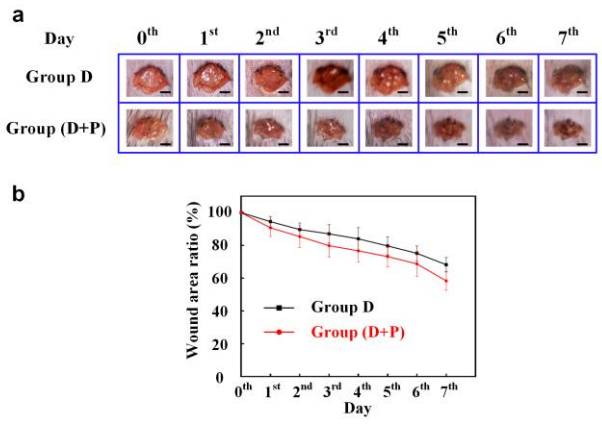

Fig. 1. Wound morphology and monitoring. (a) Photographs of the wound region on the skin of the genetically diabetic mice during the healing process with [Group (D+P)] or without (Group D) CAP treatment. (b) Variations of the wound area ratio with time after wound induction (n = 10).

Wound morphology can reflect the CAP treatment results intuitively. It can be seen from Fig. 1a that, in the early stage, the wound in Group D was wetter than that in Group (D+P), containing pus-like fluid, which is a sign of excessive inflammation. However, the wound in Group (D+P) was drier and the pus-like liquid was not found, which was attribute to the anti-inflammatory and anti-infective properties of the CAP treatment. The difference was most significant in the onset subphase of inflammation, especially on the first day. The evolution of the wound area ratio with time showed that the wound healing rate of Group (D+P) was faster than that of Group D, indicating that the CAP treatment accelerated the inflammatory-to-proliferative phase transition during the wound healing process (Fig. 1b).

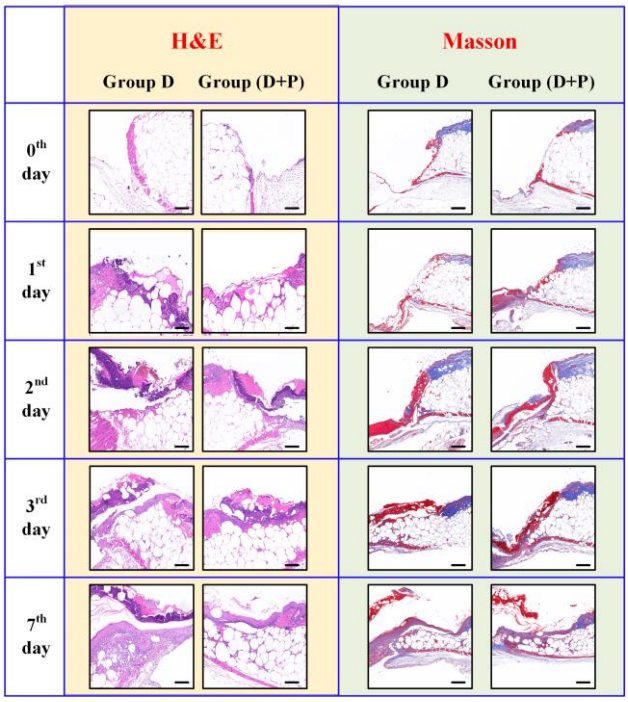

Fig. 2. Histological analysis of the wounds stained with H&E, Masson's trichrome on the 0th, 1st, 2nd, 3rd and 7th days. The photos were taken at the wound edges. Scale bar: 0.1 mm.

The H&E staining results (the first column in Fig. 2) revealed that a large number of inflammatory cells migrated to the incision area in Group D during the first week of wound healing, especially on the first day. In contrast, the inflammatory reaction in Group (D+P) was significantly reduced; further, a thin and complete scab was formed on the wound surface by the 7th day, and a complete epithelialization was observed at the edges of wounds covered by newborn epithelial tissue. This shows that the CAP treatment effectively inhibited excessive inflammatory reactions and accelerated the transition to the proliferative phase. In addition, according to the Masson's trichrome staining results (the second column in Fig. 2), when compared with Group D, Group (D+P) had more visible collagen deposition at the wound edges on the 7th day after wound induction, indicating that plasma treatment accelerated collagen generation, thereby accelerating wound healing. Furthermore, as a marker to label newborn capillaries and detect angiogenesis in granulation tissue, the expression levels of factor VIII and the von Willebrand factor (vWF) were increased in Group (D+P) compared to those in Group D on the 7th day. This indicates that the CAP treatment indeed promotes angiogenesis, leading to increased microvessel densities and formation of new capillaries in the new granulation tissues. These results show that CAP can successfully end the inflammatory phase of the diabetic wound healing, complete the natural transition to the proliferative phase, and finally, accelerate the wound healing process.

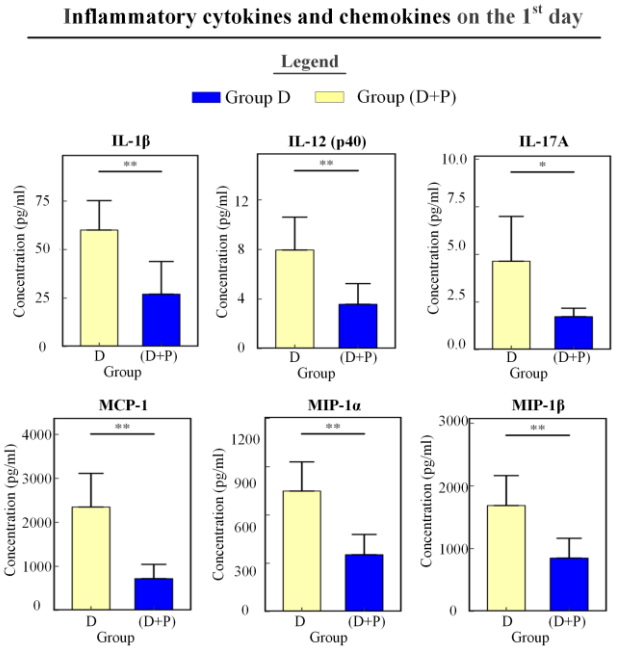

Fig. 3. Analysis of the concentrations of cytokines and chemokines on the 1st day between Groups D and (D+P). $*p < 0.05$; $**p < 0.01$.

We analysed the inflammatory cytokines and chemokines to explore the anti-inflammatory mechanisms of CAP treatment as shown in Fig. 3. In this study, downregulation of the pro-inflammatory cytokines IL-1β, IL-12 (p40) and IL-17A was observed clearly after CAP treatment on the 1st day; whereas the untreated group showed significantly higher concentrations of these cytokines. Furthermore, most of the chemokines of the CC subfamily, MCP-1, MIP-1α and MIP-1β, were significantly downregulated in Group (D+P), whereas they were overexpressed in the untreated group. The concentrations of these cytokines and chemokines increase significantly and sustain the presence of pro-inflammatory cells in the onset subphase, resulting in a persistent inflamed state of the diabetic wounds; their downregulation can interrupt inflammatory signaling pathways during diabetic wound healing. Therefore, our

research shows that the CAP jet treatment reduces the excessive immune expression by inhibiting the transitional expression of a large number of pro-inflammatory factors, which is quite different from the action mechanisms of traditional drugs that act upon just one or a few inflammatory cytokines or chemokines.

4. Conclusions

In this study, a cold atmospheric plasma jet was used to treat the wounds of the diabetic mice. This experimental results show that the helium CAP jet treatment on the wounds of the genetic diabetic mice can regulate abundant inflammatory cytokines such as IL-1β, IL-12 (p40) and IL-17A to inhibit their over-expressions at the onset phase of inflammation, thus accelerating the transition to the proliferative phase, and regulating angiogenesis, re-epithelialization and fibroblast repopulation. This research provides a theoretical basis for the CAP treatment of chronic wounds, helping to pave the way for the development into a variety of innovative and convenient CAP treatment methods in clinical applications.

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6. References

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