Microwave plasma torch for wound treatment

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Abstract. Cold atmospheric pressure plasma (CAP) sources have recently been proven to be an effective therapeutic source regarding wound healing. The most preferred and used plasma devices at this moment are the well-known dielectric barrier discharges (DBD) and free jet devices. In this work, we studied a low temperature plasma torch at atmospheric pressure sustained by a travelling electromagnetic wave excited by surfatron type wave launcher coupled to solid state microwave generator. This plasma source allows variation of discharge conditions: geometric parameters (discharge tube inner diameter and thickness), wave power and gas flow velocity which varies the main plasma parameters (length, gas temperature, concentration of charged particles and reactive species, UV and microwave radiation). Appropriate combination of the parameters lead to the low temperature plasma torch obtaining a gas temperature up to 30–37°C, suitable for in vivo treatment of BALB-C mouse models. The purpose of this research is to study the discharge conditions leading to acceleration of wound healing at short treatment times with relatively low gas flow and microwave power.

1. INTRODUCTION
The number of cold atmospheric plasma (CAP) devices used for bio-medical applications increased significantly in the last decades. Plasma assisted wound healing is a potentially effective method for enhancing natural healing processes [1, 2]. Using different gases or gas mixtures as plasma media can be created media with higher concentration of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can influence different stages of wound healing for short treatment times.

Plasmas created by a DBD [3, 4] or free jet [5–7] sources achieve contacts with the surface through the post-discharge region [8]. The active part of the discharge creates short-lived species (ROS and RNS) which are not transferred by the gas flow to the post discharge region where only long-lived species can interact with the treated surface. The recently developed plasma torch [9, 10] sustained by a high frequency (HF) surface wave combined the effects of ionized gases, HF electromagnetic fields and UV radiation [11]. The plasma torch allowed contact with the active part of the discharge; thus short-lived species could interact with the treated object. Both short- and long-lived compounds thus interacted with the cells, influencing different cellular pathways. This process is dependent of the type and concentration of active species (ROS, RNS) [12, 13].

2. MATERIALS AND METHODS
The experimental setup is presented in Figure 1. A solid-state generator (SAIREM GMS, 200 W) (1) coupled to a SAIREM SURFATRON® waveguide (3) excited an electromagnetic wave, which propagates along the plasma-dielectric interface. Microwave plasma was ignited in Argon 5.0 (6) and sustained by the traveling electromagnetic wave at 2.45 GHz inside a quartz tube with outer diameter.
8 mm, inner diameter 3 mm and dielectric permittivity $\varepsilon_d = 3.25$ (4). When the discharge tube is short enough and the wave power is not completely spent at its end the wave continues its propagation after the end of the tube sustaining in this way plasma in the open air (plasma torch). Gas flow was controlled with a thermal mass flow meter (5).

Wounds (cuts with circular shape which remove dermal and epidermal mouse skin layer) were infliction on BALB-C mice symmetrically at both side of the back and then only the left side of the back was treated for different time (8). The mouse models were anesthetized by a mixture of 90% physiological solution (0.9% NaCL) and 10% CALYSOL (Ketamine) (0.1 ml of the mixture to 10 g of the weight of the animal). The hair of the animal was removed with depilatory cream and two symmetrical cuts with area of 48–52 mm$^2$ were made on the back. The incisions on the left were treated under certain conditions and the incisions on the right were used for control. The contraction of the wound was measured with a SONY DSC-H7 camera and calculated using ImageJ software. A FLIR E30 infrared camera was used for thermal imaging during treatment.

Ten 35–45 g BALB-C male mice were separated into two groups S1 and S2 (5 models per group), and were treated once per different times (S1 for 30 s each mouse, S2 – 60 s) immediately after wound infliction.

All experiments were performed in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) regarding the use of animals in research and were approved by the Bulgarian Food Safety Agency (BFSA) in Sofia.

3. RESULTS AND DISCUSSION

The gas temperature and length of the torch is presented in Figure 2. The system configuration allowed sustaining the plasma torch at applied powers of 6–26 W (Figure 2(d)). Measurements of the temperature were done using Hg thermometer in contact with the tip of the discharge. The torch length and the plasma temperature increased with the microwave power for the given gas flow of 7 sccm (Figure 2(a–c)). For lower applied power, the temperature of the torch was below 40°C, and the length was significant, which made the torch suitable for treatment of living objects. An applied power of 9
W was used during the experiments providing length and low temperature for the given flow Figure 2(a, d).

![Plasma torch length at: (a) 9 W; (b) 15 W and (c) 20 W and temperature dependence on MW power, Ar flow was 7 sccm.](image)

**Figure 2.** Plasma torch length at: (a) 9 W; (b) 15 W and (c) 20 W and temperature dependence on MW power, Ar flow was 7 sccm.

Thermal images taken during treatment (Figure 3(a, b)) and after treatment (Figure 3(c)) were used for determination of the temperature of the skin. Only the tip of the torch was in contact with the inflicted wounds.

![Skin temperature after (a) 30 s; (b) 60 s of treatment and (c) 15 s after the end of treatment.](image)

**Figure 3.** Skin temperature after (a) 30 s; (b) 60 s of treatment and (c) 15 s after the end of treatment.

For 30 s treatment time the skin temperature of the model increased up to 45°C (Figure 3(a)), while at 60 s it was 48°C (Figure 3(b)). Immediately after treatment (Figure 3(c)) skin temperature decreased to 38°C. Data collected with the Hg thermometer did not correlate with the infrared photos. Thermal stress was probably inflicted on the wounds and further experiments are going to be done to distinguish the effects of the plasma from the effects of thermal stress.

The two groups S1 and S2 with 5 mice per group were treated for different time intervals: S1 – 30 s, S2 – 60 s. Full wound closure was achieved on the 14th day. Wound closure was followed using a digital camera. The surface area was determined by tracking the wound margins on days 0–2, 5, 7, 9 and 12 (Figure 4 (a)). Inflammation or erythema, were not observed during the experiment.
Figure 4. (a) Observation of wound healing on different days of healing period; Wound closure in percentage for both investigated groups: (b) S1 - treated once for 30 s; (c) S2 - treated once for 60 s.

The results were calculated based on averaged values of 3 images per wound and the area in percentage was given by:

\[ \text{wound closure} = \left(1 - \frac{\text{area at given day}}{\text{initial area}}\right) \times 100\% \]

The S1 treated wounds (Figure 4(b)) healed faster than the control wounds while the S2 treated wounds healed in approximately the same time as the control (Figure 4 (c)). Thermal damage to the tissue might explain why no effect was observed in the S2 group, even though faster contraction was obtained in S1. Plasma treatment led to initial fixation of the edges of the wound while the wound edges of the S1 control group expanded (Figure 4 (b)) which were due to lack of fixation on the edges.

4. CONCLUSIONS
The presented plasma device based on traveling electromagnetic wave, which propagates along the plasma–dielectric border, was used for treatment of wounds. Preliminary results confirm the potential of the discharge on triggering wound healing effects. Small acceleration in wound closure was observed for 30 s treatment times while the 60 s group showed no significant effect. Wound temperatures exceeded 40°C, which could explain the slower rate of healing. Further experiments are needed to determine the precise conditions in which the plasma torch is optimized for treatment of wounds.
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