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LETTER

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Letter

A novel cupping-assisted plasma treatment for skin disinfection

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Abstract

A novel plasma treatment method/plasma source called cupping-assisted plasma treatment/source for skin disinfection is introduced. The idea combines ancient Chinese ‘cupping’ technology with plasma sources to generate active plasma inside an isolated, pressure-controlled chamber attached to the skin. Advantages of lower pressure include reducing the threshold voltage for plasma ignition and improving the spatial uniformity of the plasma treatment. In addition, with reduced pressure inside the cup, skin pore permeability might be increased and it improves attachment of the plasma device to the skin. Moreover, at a given pressure, plasma-generated active species are restricted inside the cup, raising local reactive species concentration and enhancing the measured surface disinfection rate. A surface micro-discharge (SMD) device is used as an example of a working plasma source. We report discharge characteristics and disinfection efficiency as a function of pressure and applied voltage.

Keywords: novel plasma source, skin disinfection, Chinese cupping, low pressure

(Some figures may appear in colour only in the online journal)

Skin diseases are a significant problem all over the world and especially in low and middle income countries. In sub-Saharan Africa, some form of skin disease is estimated to affect 21–87% of the population. These afflictions constitute up to a third of outpatient visits to Pediatricians and Dermatologists. Among all skin disorders, infections are common in developing countries [1, 2]. Skin infections can be caused by various pathogenic microorganisms, such as bacteria, fungus, virus or parasites. These pathogens can cause either superficial or deep skin infections including impetigo, folliculitis, ecthyma, erysipelas, necrotizing fasciitis, dermatophytosis, cutaneous leshmaniasis, tinea versicolor, herpes simplex, herpes zoster, warts, molluscum contagiosum, etc. For some superficial skin infections, available drugs and topical treatments may be effective to kill the pathogens. However, for more complex skin infection-related diseases, these methods may be much less effective. Some possible reasons for these difficulties include limited skin-drug permeability through the stratum corneum (SC), an impaired immune system, or other causes. In some cases, many weeks of treatment using additional oral medication may result in undesirable side effects, especially in fungal infected cases [3, 4].

Atmospheric pressure room temperature plasmas have proved highly effective in safely killing different kinds of bacteria, fungus, virus or even cancer cells, with minimal or no damaging effects on adjacent tissue [5–8]. The plasma generates a suite of reactive neutral and charged species including electrons, ions and charged cluster ions [9–12]. In some cases, the electric fields generated by the plasma at treated surfaces are known to be important, leading, for example, to transdermal drug delivery and gene transfection. Plasma-generated photons can also play a significant role. Skin structure is complex, and pathogenic microorganisms have developed various ways to survive on, in or under the skin. Microorganisms

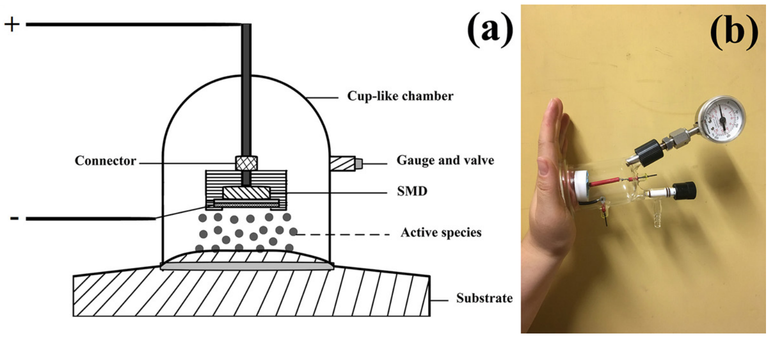

Figure 1. (a) Sketch of cupping-assisted plasma device with a SMD inside as an example; (b) proposed device shown vertically attached to a human palm.

often grow on the rough skin outer surface, but they can also penetrate pores. Furthermore, difficult to treat multi-layer biofilms are often formed instead of planktonic microbes. These aspects of skin-related infections can make skin disinfection much harder than disinfection of smooth surfaces. Many skin treatments using atmospheric pressure (atm) plasmas *in vitro* or *in vivo* have been reported [13–16]. For example, Daeschlein *et al* used an argon plasma jet device ('Kinpen') to treat a patient with *Staphylococcus aureus* colonization. A 3 min plasma treatment showed selective disinfection of *S. aureus*, while the skin flora with *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* remained undamaged. However, the pathogen was mobilized to the skin surface from deeper skin layers [17].

Existing plasma devices for skin treatment, such as the rare gas plasma jet, the surface microdischarge (SMD), the floating electrode-dielectric barrier discharge (FE-DBD) or plasma air needle are all generally operated in open air at one atmosphere [18–20]. These devices typically are associated with significant loss of active species away from the intended treated area due to diffusion and convection into the surrounding environment. This decreases the active species concentration at the site of application and will generally decrease the treatment efficiency. At the same time, operating at atmospheric pressure, spatially uniform plasma can be hard to achieve, especially if the plasma current contacts the skin. This is due to the formation of filaments and may lead to undesirably high local currents to the skin surface.

In light of these characteristics of atmospheric pressure plasmas, we propose a novel plasma treatment method and a corresponding plasma device for skin infection treatment, especially suitable for deep skin or subcutaneous infections. The proposed process and device combines plasma medicine technology with ancient Chinese 'cupping' therapy. We tested the discharge pattern under different applied voltages and pressures by using a surface micro-discharge (SMD) device as the operating plasma source. The antibacterial effectiveness under both low and higher applied voltage as a function of pressure were tested and compared. Finally, we briefly discuss the mechanism of antibacterial effect under different pressure and applied voltage.

Figures 1(a) and (b) show the basic sketch of the device and a photo of an example of the device vertically attaching to a human palm by pumping out part of the air inside, respectively. This device contains a cup chamber, a plasma source, a valve for pumping with or without air gauge. Different types of plasma devices could be mounted inside the cup chamber, such as surface discharge (SMD), floating electrode dielectric barrier discharge (FE-DBD) and plasma needle, etc. Here, a SMD device is mounted inside the chamber as an example. A valve for an air pump (automatic vacuum pump or 'smart' handheld manual pump) is fixed on top of the cup. A pressure gauge is mounted next to the valve to monitor and control the pressure inside. Similar to the practice of ancient Chinese cupping technology, when the pressure above the skin is lowered, the cup can remain attached to the body without the help of a mechanical arm, as seen in figure 1(b). In this study, the diameter of the cup is 5 cm and the height is 8 cm with a volume of ~0.16 l. The SMD device used is similar to those reported in greater detail elsewhere [21]. The diameter of the copper electrode and steel mesh are 1 cm and 1.8 cm, respectively. The dielectric between the copper electrode and the mesh is a square glass plate (2 cm × 2 cm) with thickness 1 mm. The two high voltage wires are connected to an AC power supply (Trek 10/40A) by Tungsten feedthrough mounted on the glass cup wall. A high voltage probe (Tektronix P6015A) is used to measure the applied voltage, and a 100 nF capacitor is connected to the circuit for power consumption calculation by using the Lissajous method [22].

Figure 2 shows discharge pattern images (taken from the bottom view when attached to a transparent glass plate) under different applied voltages and pressures ranging from 1 atmosphere pressure (atm) to 0.33 atm. For all the experiments presented here, the frequency of the applied voltage is 4 kHZ. The discharge area spreads from the center of the electrode to the surrounding edges as the applied voltage increases and as the inner pressure decreases [23]. As can be seen from the images, the breakdown voltage is ~3 kV (peak) at 1 atm. 4 kV readily ignites the discharge under 1 atm and the discharge becomes progressively much stronger as the pressure is reduced to 0.67 atm, 0.5 atm and 0.33 atm. Lower pressure reduces the discharge breakdown voltage, which in turn decreases the demands on the high voltage power supply. At 0.33 atm, only ~2 kV ignites the plasma. We also tested FE-DBD and plasma DC air needle devices inside the chamber, and the reduction in breakdown voltage was even more pronounced. For example, a 2 mm gap FE-DBD at 1 atm breaks down at ~6 kV but only ~3 kV is needed at 0.5 or 0.33 atm. Typically, traditional

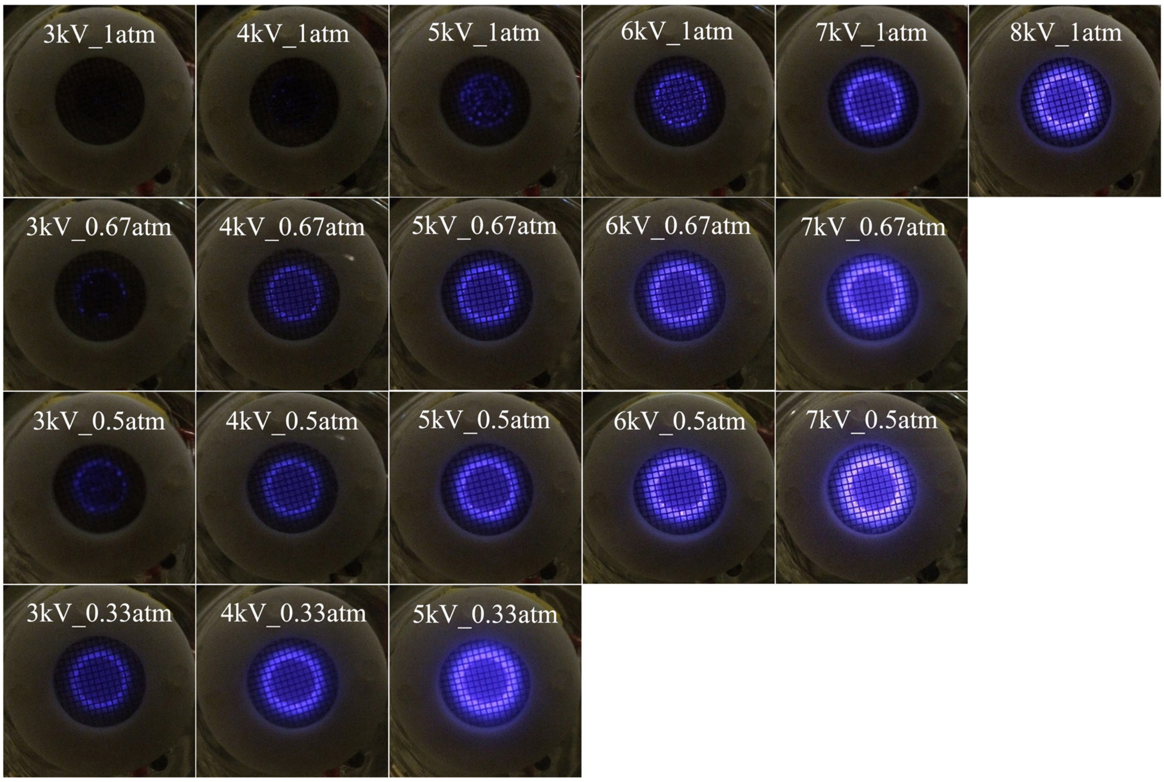

Figure 2. The discharge pattern under different applied voltage (increasing from left to right) as a function of inner pressure ranging from 1 atm (top) to 0.33 atm (bottom). As pressure is lowered, the discharge is brighter at a given applied voltage.

cupping techniques become uncomfortable below about 0.33 atm, so we limited our investigation to this pressure reduction.

The power consumption as a function of voltage and pressure is measured and is shown in figure 3. Consistent with the discharge images in figure 2, power consumption increases with applied voltage and reduced pressure. Gas molecular density should be approximately proportional to pressure assuming near-constant gas temperature. Discharge impedance is correspondingly reduced as pressure is lowered in this range, in part due to the longer collisional mean free path for both charged and neutral species [24].

In order to test the antimicrobial effects under different conditions, we deposited bacteria on the surface of nitrocellulose (NC) membranes and exposed the membranes to the SMD plasma at various pressures and applied voltages. Images of the bacteria on the membranes are shown in figures 4 and 5. Aqueous suspensions of 100 μl containing *E. coli* bacteria with concentrations of ~10^6 CFU ml^{-1} were evenly spread over the NC membranes before treatment. The diameter of the NC membrane is about 3.5 cm. The distance between the mesh and the NC membrane surface was fixed at 5 mm. In one set of experiments, no confining chamber was used. Samples were divided into 4 groups: the 1 atm without chamber group, the 1 atm with chamber group, the 0.67 atm with chamber group and the 0.33 atm with chamber group. Pretreatment of the NC membrane under all tested conditions showed no significant anti-bacteria effect.

For each group, samples were treated for 0 min, 1 min, 2 min and 5 min, respectively. For each treatment condition, at least 3 samples were repeated. In the lower pressure groups, before treatment, the inner pressure was reduced to a fixed value, and then the plasma was generated under different voltages. After treatment, the treated sample was immediately taken out and attached to the pre-prepared LB agar (Fisher Scientific) surface for growth. The inner chamber was then purged with clean air to exhaust all the residual species. All the treated samples were incubated at 37 °C for 24 h and then the results were recorded.

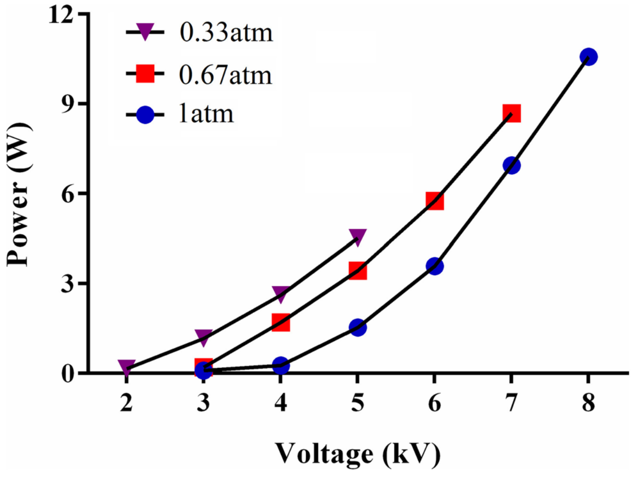

Figure 3. Power consumption with different applied voltages as a function of pressure ranging from 1 atm to 0.33 atm. Power consumption trends follow the discharge brightness trends shown in figure 2.

Figure 4. Anti-bacterial effect under different pressure with an applied voltage of 3 kV. The images show bacteria grown on nitrocellulose (NC) membrane surfaces after different times of plasma exposure. The top row is for a 1 atm application without a confining chamber. The near breakdown threshold applied voltage of 3 kV shows little antibacterial effect at 1 atm, but increasing effects at the lower pressures.

The antibacterial effects of the plasma device at different pressures and applied voltages can be seen in figures 4 and 5. We tested the effect of lowered pressure on bacteria: simply lowering pressure had no discernable affect the growth of *E. coli* in this set of experiments. At the lower voltage of 3 kV illustrated in figure 4, only at the lower pressures of 0.67 atm and 0.33 atm were any antibacterial effects observed. The effects increased with exposure time. The results in figure 5 were taken with a 5 kV applied voltage. At this higher voltage, the effects of the confining chamber can be seen clearly. With no confining chamber at 1 atm, there is no observable antibacterial effect even at 5 min exposure. This result may be partly because of the relatively long treatment distance (5 mm) and the relatively small SMD electrode structure (1 cm diameter). However, with the confining chamber present, virtually all bacteria were killed at 1 atm even for a 1 min exposure. Discharges operated at the lower pressures appear to be somewhat less effective for the same exposure times, even though figures 2 and 3 might suggest that more intense discharges are maintained at the lower pressure conditions. It is possible that even though the lower pressure discharges appear more intense, the rates of creation of active species, and therefore their concentration in the confining chamber, are reduced because the gas pressure and therefore gas densities are correspondingly lower at lower pressure.

We note that even though the apparent rate of bacterial killing does not increase at the lower pressures for a 5 kV applied voltage, it is possible that there may be other advantages in treating skin at lowered pressure. Even though there appears to be little or no direct evidence of enhanced pore opening with cupping, it is thought that lowered pressure will increase skin porosity and may promote increased bacterial killing in deeper layers of the skin, but that has not been explicitly demonstrated here [28]. Furthermore, with this design, the device can be easily attached to skin. In the cases presented here, we described the use of the SMD but with other plasma devices like the FE-DBD or plasma needle, plasma would directly touch the skin. In these cases, an electric field and current may assist skin disinfection and even contribute to transdermal transport, as well [29, 30].

It is known that SMD devices can operate in 3 different modes depending on the applied power: (1) O_3 mode at low power; (2) transition mode at medium power; and (3) NO_x mode at high power [25–27]. We are not able to measure gas composition, however, given the geometric constraints of this system. We measured the concentration of nitrite in water exposed to the plasma using Griess reagent. NO_2^- was detected in all the groups which show anti-bacterial effect, hence the active species involves in the anti-bacterial process appears to involve NO_x. Finally, the measured mesh temperature increase after 5 min exposure at 5 kV for all the pressure tested was within 5 °C. Therefore, temperature effects appear to be negligible.

In conclusion, we present a new method for air plasma-based for skin disinfection and treatment. This device combines traditional Chinese 'cupping' technology with modern cold atmospheric plasma technology. Operated at reduced pressure, the device easily attaches to skin without a supporting mechanical arm. An SMD device was used to illustrate the method for skin infection treatment. The discharge under lower pressure is easier to ignite. The power consumption was calculated and the antibacterial effect was tested under different pressures from 0.33 atm to 1 atm. A range of applied voltages and treatment times

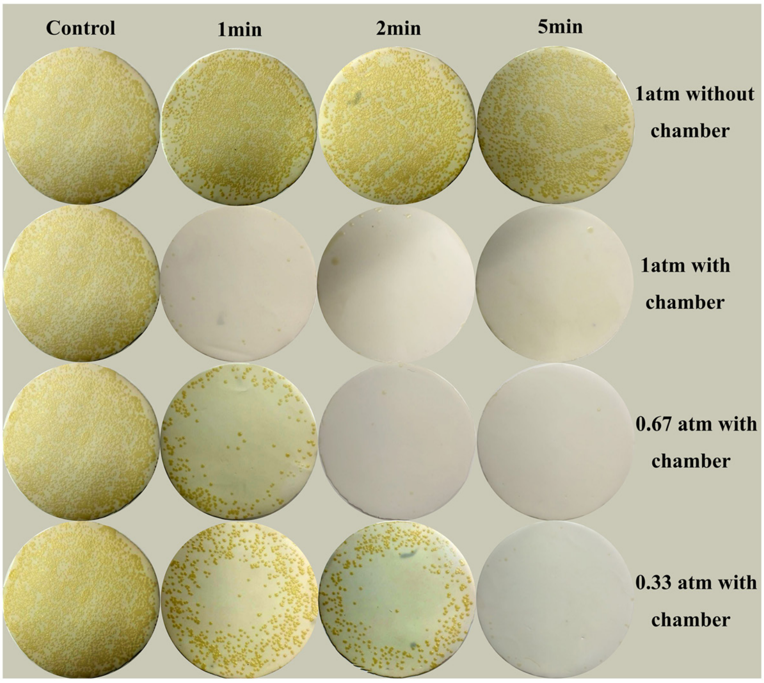

Figure 5. Anti-bacterial effect under different pressure with an applied voltage of 5 kV. At this applied voltage, the most pronounced anti-bacterial effect appears at 1 atm as virtually all bacteria are killed for even 1 min exposure. The advantage of using a confined space is clear in comparing the top row (1 atm with no confining chamber) with the second row (1 atm with confining chamber). In addition, note the declining anti-bacterial effectiveness for 1 and 2 min exposure as pressure is lowered from 1 atm to 0.33 atm. Even though figures 2 and 3 show that at 5 kV, the discharge is visually more intense and power dissipation is higher as pressure is lowered from 1 atm, the anti-bacterial effect is actually higher at 1 atm. This may be related to the possibility that at higher air density, even for a less visually intense discharge, the rate of creation of anti-bacterial reactive species may be higher.

were used in the antibacterial tests. The cup contains and concentrates the active species, avoiding loss into the environment. This dramatically enhances the antibacterial effect. Ancient Chinese cupping was thought to open skin pores and although this effect was not explicitly tested in this study, a lower pressure might aid transport of active species into deeper skin layers. Future studies will focus on skin disease treatment as well.

Acknowledgments

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