低温プラズマのchronic wounds 消毒への応用

Application of low-temperature plasma to disinfection of chronic wounds

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1. Introduction

Study of plasma sources at atmospheric pressure has been quite active recently, because they combine many advantages, such as low cost, simple design and easy handling[1]. Ever since such non-thermal atmospheric discharge sources were established, various medical applications have been investigated with growing interest as a tool for surface sterilization. Even in a room-temperature plasma at atmospheric pressure, many chemical reactions are expected because of high energy electrons that are produced. For instance, reactive oxygen and nitrogen species (ROS and RNS) are known as an agent for bactericidal effect. This can be utilized for the cleaning of medical equipment[2,3]. In addition, with atmospheric plasmas it is possible to treat substances which are not resistant to vacuum, such as living organisms[4-18]. A contact-free treatment can be achieved without any heating. Due to this reason, many low gas temperature plasma devices working at atmosphere were developed for different medical purposes. An overview of the studies in this field is given in the paper[18] by E. Stoffels.

For hospitalization of dermatological patients infectious skin diseases caused by bacteria are one of the main reasons. They cost millions of Euro each year. Leading among them are infection of wounds like chronic ulcers of the lower leg is one major part in the cost. This infection is also a major reservoir for multiresistant bacterial strains. Standard treatments for these wounds, i.e. topical and systemic antibacterial regimens, are often limited by the development of resistance of germs to antibiotics and allergic reactions.

In our group, a new plasma device (a microwave plasma torch) at atmospheric pressure has been developed and tested with a view to applying this new technique to the therapy of chronic foot and leg ulcers. We already conducted a phase I study which examined the effect of the plasma treatment on bacteria and healthy human skins. For bacteria cultures, a bactericidal effect is seen and no visible structural changes occurs for the human skins. We have also begun a phase II study, which reviews the efficiency of the plasma treatment on infectious skin diseases.

In this proceeding, our plasma torch is described. Afterwards, basic device characteristics and measurements of plasma effects on bacteria cultures are discussed. Moreover our clinical study (phase II study) is introduced. Finally we summarize this proceeding.

2. Experimental

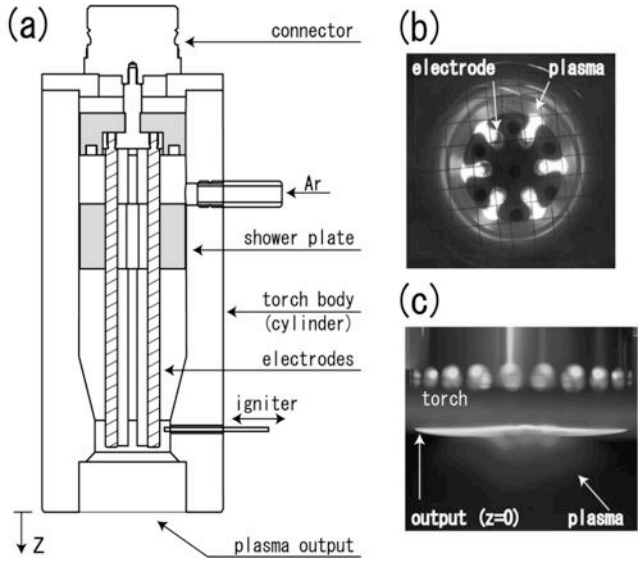

Figure 1: Plasma torch and plasma.
(a) a detailed sectioned view of plasma torch,
(b) plasma between the electrodes and the cylinder,
(c) plasma below the torch from the side.

Figure 1 (a) shows a detailed view of the plasma torch. It has 6 stainless steel electrodes placed inside an aluminum cylinder of 135 mm in length. The centers of the 6 electrodes, whose surfaces are serrated, are distributed equally at a distance of 6 mm from the inner surface of the cylinder as shown in fig. 1 (b). The diameter of the electrodes and the distance between the electrodes and the surface of the cylinder are 4 mm. It was found through a series of different designs that this geometric configuration gave the most stable plasma yield for a given input power. The material and serrated structure of the electrodes are important for the triggering and stability of the discharge. The tips of the electrodes are at 20 mm from the opening of the torch. Since the typical size of chronic wounds is relatively large, our torch's opening is also relatively large

(35 mm in diameter) in comparison with other plasma devices for medical purposes. Usually the torch is placed with the principal axis perpendicular to the ground. We already tilted the torch for flexible use and it was found that between ±45 degrees the torch could be used for bacterial disinfection. In this study, only Ar gas (purity 99.998%) is used to produce plasma in order to minimize the production of toxic gases. Moreover, Ar is relatively cheap and easy to prepare in hospitals. Ar of 2.2 slm is applied from the base of the electrodes through a Teflon shower plate which regulates gas flow around the electrodes. Microwave power at 2.45 GHz in frequency is applied to the electrodes through coaxial cables of 2 m in length via a 2 stub tuner. The microwave power is 85 W. Six plasmas are produced between each of the electrode's tips and the inner surface of the cylinder as shown in fig. 1 (b). The torch is cooled by an air flow inside the cylinder driven by an external pump. The maximum temperature of the torch-surface is 320 K during the experiments. Figure 1 (c) shows a side view of the plasma flow below the torch (the exposure time of the photo is 30 s). The shape of the plasma looks conical.

Both the gas temperature in the plasma flow below the torch and the surface temperature of the agar plates are measured by a small thermocouple (type K). To obtain plasma profiles below the torch, a mesh grid electrode (20 lpi), which covers the whole opening of the torch, is used to measure floating potential with respect to the ground. The volt meter used has 20 MΩ internal impedance. NO_2 concentration is measured by a gas detector (Dräger Multiwarn II).

Escherichia coli (ATCC No. 9637) bacteria, inoculated on agar plates (88 mm in diameter), are used for this study. The disinfection result is observed following a 16 hour incubation of the culture at 308 K.

In order to observe surface morphology of bacteria, an atomic force microscope (AFM)[19] is used because it is not necessary to conduct special and possible destructive preparation for bacteria. AFM measurements are carried out by a Dimension 3100 (Veeco, Santa Barbara, CA) in tapping mode. For imaging, silicon cantilevers with a nominal resonance frequency of 300 kHz (BS-TAP300, BudgetSensors, Sofia, Bulgaria) are used. Image processing was done using SPIP 4.5 (Image Metrology A/S, Lyngby, Denmark).

During the experiments, the ambient pressure was 950 hPa±20, the temperature was 297±2 K, and the relative humidity was 40±5 %.

3. Torch's characteristics and bactericidal effect

We have investigated the axial profile of the gas temperature and the floating potential of the mesh grid electrode. The room temperature was 298 K. Figure 2 shows the *z*-profiles of the measured gas temperature, the NO_2 concentration at the torch axis, and the floating potential of the mesh grid electrode. The gas temperature is measured on the center axis of the plasma torch. In the vicinity of the torch, the gas temperature is relatively high (over 500 K). However, from z = 5 mm, the gas temperature has decreased drastically. As z increases further, the temperature decreases more gradually. At z = 17 mm, the temperature is 301 K, low enough for 'in vivo' application.

From the measured NO_2 concentration profile the maximum (6.2 ppm) is observed at z = 10 mm. This is an indication that the plasma flow from the torch has developed a good contact with the ambient air around this position –an explanation, which corresponds well with the temperature profile.

Inside the plasma torch, there is relatively strong light emission between the electrodes and the cylinder. However, from the plasma flow exiting the torch there is only weak light, especially below z = 15 mm. In order to determine how the plasma is distributed below the torch, the floating potential of the mesh grid electrode is measured. The potential also decreases as z increases, almost in the same way as the gas temperature as shown in fig. 2. Around z = 20 mm, the measured potential is not 0. This indicates that there are charged particles.

We observed the plasma exposure effect on bacteria. For example, when an *E. coli* culture is placed 20 mm away from the output of the torch for 2 minutes, a clearly visible bactericidal effect can be found, observable

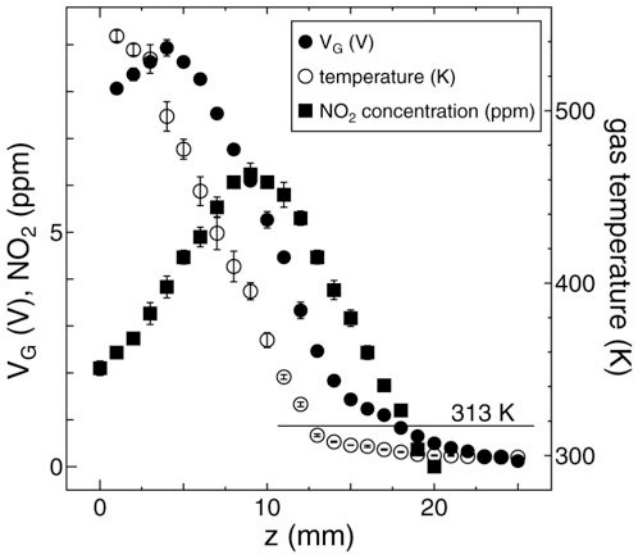

Figure 2: Gas temperature, NO_2 concentration and floating potential of mesh grid electrode as a function of distance from the torch *z*.

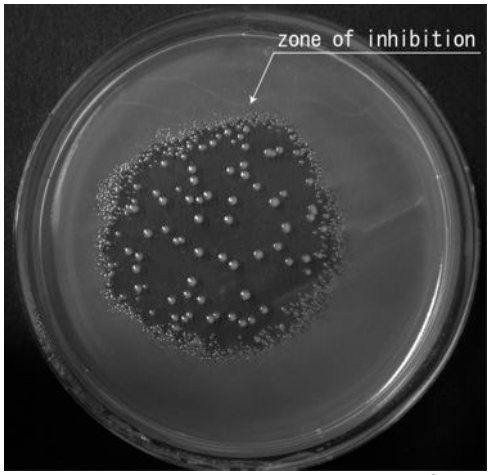

Figure 3: *E. coli* culture on an agar plate after plasma treatment for 2 minutes. The culture is placed at z = 20 mm. This image was taken after 16 hours of incubation.

by a zone of inhibition of growth on the agar plate as shown in fig. 3. In a circle of 40 mm diameter, killing of the bacteria has been observed. The boundary of the zone of inhibition is a little fuzzy and the zone of inhibition is slightly larger than the opening of the torch. We measured the surface temperature of the agar plate during the treatment. In 2 minutes, the temperature increased 4.5 degrees and reached 300 K. Thus, heat effect can be ruled out for killing bacteria in our experiments.

We have already tested our plasma torch for other kinds of bacteria relevant to wound healing. A clear bactericidal effect is observed after two minutes of plasma treatment.[20]

Figure 4: This sequence of AFM images shows the identical E. coli bacteria before (upper) and after three minutes (lower) plasma treatment. It seems that the cell wall is ruptured and cell plasma is released to the outside. The edge length of this view is approx. 5 mm.

In order to see the effect of the plasma on bacteria more in detail, morphological changes of bacteria are observed by the AFM. The upper image of fig. 4 shows two untreated E. coli bacteria. The edge length of the recorded area is 5 mm. The same two bacteria after three minutes of plasma treatment are shown in the lower image of fig. 4. Morphological changes in the bacteria are observed. After the treatment, the original shape of bacteria can be seen. However, along the bacteria the additional structure is newly visible. An explanation can be that the cell wall of the bacteria is broken and the cell plasma is released to the outside. The change in the morphology is also observed by a height decrease of approximately 25%.

One of the possible agents for bactericidal effects by plasma is UV radiation. Especially wavelengths around 255 nm (UV-C) cause dimerization of thymine molecules in the DNA which inhibits cell replication. In order to investigate the isolated effect of UV light on bacteria, a UV light emitting diode is used. This UV LED emits a light of 255 nm in wavelength and 200 $\mu W/cm^2$ in light power. At first, the bactericidal effect on bacteria by the UV light is observed. After two minutes of UV exposure on the *E. Coli.* inoculated on the agar plate, the irradiated region is completely sterilized. In the next step, the surface morphology of bacteria is observed by the AFM. The morphological change in bacteria is not observed after the 2 minutes treatment.

From the plasma, charged particles, ROS, RNS, UV light, heat, etc. can affect bacteria. The UV light is one of the important agent for the bactericidal effect by our plasma torch, however, there is other mechanisms, e.g. charged particles[21,22] in the plasma and reactive species [4,22] which are produced in the air.

4. Clinical study

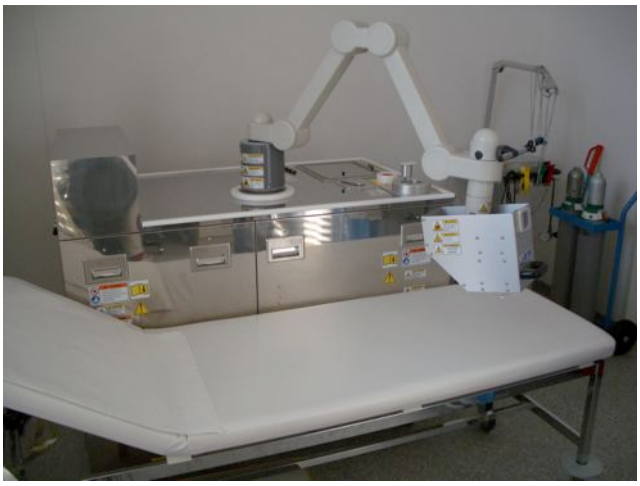

Figure 5: Experimental system (MicroPlaSter) in the clinic. All the functional units are incorporated in the system. Patients lie on the bed in front of the system.

We have begun a phase II study whose goal is to show the efficiency of the plasma treatment on different skin diseases. In order to conduct the phase II study, a plasma device (MicroPlaSter) for the clinic was designed. All the functional units, including the plasma torch, a microwave power supply, a mass flow controller, gas cylinders, etc., are incorporated in a trolley and all the experimental parameters, i.e. microwave power and gas flow, are controlled by a computer. The plasma torch is placed on a counterweighted arm in order to use it flexibly. Moreover the torch can be used both vertically and at angles.

After over 800 treatments and over 100 patients, the treatment is well tolerated in almost all cases. Until now no side effects are observed. Moreover, no painful sensation is felt by patients. Tests of the efficiency with different microbiological analysis systems show higher germ reduction in treated areas than that in non-treated areas. The bactericidal effect on gram negative germs is higher than that on gram positive germs.

5. Summary

In summary, a large opening microwave plasma torch has been developed for the purpose of "in vivo" bacterial sterilization. Using 2.2 slm of Ar gas flow and 85 W of microwave power at atmospheric pressure, we obtain a plasma with suitable characteristics for medical applications and treatment. Tests with cultures of *E. coli* (and others) have shown the bactericidal effect. The UV light is one important agent for killing bacteria in our study, however there are other mechanisms. We consider that this technique could be used for different medical application, in particular wound healing, and have started a clinical study for the therapy of chronic foot and leg ulcers. To conduct the phase II clinical study, we designed the MicroPlaSter system and tests show higher bactericidal effects on plasma-treated area than that on non treated area. In our experience, the low temperature Ar plasma at atmosphere offers a novel approach to infected chronic wounds.

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