Risk assessment of the application of a plasma jet in dermatology

Juergen Lademann  
Heike Richter  
Alena Alborova  
Daniel Humme  
Alexa Patzelt  
Charité Universitätsmedizin Berlin  
Center of Experimental and Cutaneous Physiology  
Department of Dermatology and Allergology  
Charitéplatz 1  
Berlin 10117, Germany

Axel Kramer  
University of Greifswald  
Institute of Hygiene and Environmental Medicine  
Domstrasse 11  
Greifswald 17487, Germany

Klaus-Dieter Weltmann  
Leibniz Institute for Plasma Science and Technology  
Greifswald 17487, Germany

Bernd Hartmann  
Christian Ottomann  
Traumacenter Berlin (UKB)  
Burn Center  
Wartenstrasse 7  
D-12683 Berlin, Germany

Joachim W. Fluhr  
Charité Universitätsmedizin Berlin  
Center of Experimental and Cutaneous Physiology  
Department of Dermatology and Allergology  
Charitéplatz 1  
Berlin 10117, Germany

Peter Hinz  
University of Greifswald  
Institute of Hygiene and Environmental Medicine  
Domstrasse 11  
Greifswald 17487, Germany

Georg Hübner  
Olaf Lademann  
University of Greifswald  
Institute of Hygiene and Environmental Medicine  
Greifswald, Germany

Abstract. Regardless of the fact that several highly efficient antiseptics are commercially available, the antiseptic treatment of chronic wounds remains a problem. In the past, electrical plasma discharges have been frequently used in biometrical science for disinfection and sterilization of material surfaces. Plasma systems usually have a temperature of several hundred degrees. Recently, it was reported that “cold” plasma can be applied onto living tissue. In *in vitro* studies on cell culture, it could be demonstrated that this new plasma possesses excellent antiseptic properties. We perform a risk assessment concerning the *in vivo* application of a “cold” plasma jet on patients and volunteers. Two potential risk factors, UV radiation and temperature, are evaluated. We show that the UV radiation of the plasma in the used system is an order of magnitude lower than the minimal erythema dose, necessary to produce sunburn on the skin *in vivo*. Additionally, thermal damage of the tissue by the plasma can be excluded. The results of the risk assessment stimulate the *in vivo* application of the investigated plasma jet in the treatment of chronic wounds. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3247156]

Keywords: wound healing; infections; skin; emission spectra; temperature; ultraviolet radiation.

Paper 09305 received Jul. 24, 2009; accepted for publication Aug. 10, 2009; published online Oct. 14, 2009.

1 Introduction

The life expectancy of the population in industrial countries is continuously increasing as a result of improved living conditions. Older people frequently suffer from chronic wounds caused by various diseases, for instance, venous dysfunction, diabetes mellitus, pressure ulcers, and arterial circulatory disorder. The treatment of these wounds is difficult, because of deep infections. Antisepsis is often insufficient under these conditions, due to the occurrence of scabbing and crusting of the wound.

Under these conditions, the antiseptics do not penetrate efficiently into the tissue. Furthermore, they only attach to the outer cell wall without penetrating bacterial cells. Although iodine penetrates into cells, in some conditions it shows cytotoxicity but looses its activity in presence of 20% blood.

In the past, electrical plasma discharges have frequently been used for disinfection and sterilization of nonliving materials. Such plasma systems usually have a temperature...
up to several hundred degrees. For so-called low-temperature plasma sterilization, the temperature is approximately 55 °C. This technique would also avoid the problem of inducing antibiotic resistant strains.

Recently, a plasma jet was introduced that can be applied in dermatology for antimicrobial treatment of the skin, especially in the case of chronic wounds. The plasma jet, reported in this paper, produces a "cold" plasma at a temperature of 30 to 65 °C. In primary experiments, it was demonstrated that this plasma is well suited for the destruction of bacteria and fungi in cell structures. Prior to the application of the plasma-jet system on patients in vivo, a risk assessment must be performed. Potential risk factors are the electrical charge system, thermal damage of the tissue, and formation of UV radiation on the skin surface during treatment.

In this paper, the optimal settings of plasma-jet parameters are determined; subsequently, a risk assessment is performed.

2 Materials and Methods

2.1 Plasma jet

In this study, we used a plasma jet that was developed at the Institute of Plasma Physics, Greifswald, and manufactured at neoplas GmbH (product name “kinpen09®”). The used plasma jet principally fulfills the physical demands for an ideal interventional instrument in anti-infectious dermatotherapy. However, it must prove antibacterial efficacy against clinically relevant pathogens without substantially damaging vital tissue cells. The used compact miniaturized atmospheric plasma exhibits a very promising technological potential for different surface treatments. Basically, there are two features that make this jet unique: (1) the tool-like, small-size, and lightweight plasma generation unit enables fast and almost arbitrary 3-D movements and (2) the contracted and comparably cold plasma enables focused small-spot treatments, even on heat sensitive objects, such as tissue and cells with temperature loads to the surface between 30 and 65 °C.

The “kinpen09®” device passed CE-certification (electromagnetic compatibility), thus fulfilling the standards for electrical safety in humans. Argon gas was used as a discharge medium in the plasma jet. Figure 1 is a photograph of the plasma jet under working conditions.

2.2 Tissue Samples

Our investigations were performed on pig ear skin, which is a highly suitable model for human tissue. The unscalded pig ears had been freshly obtained from the butcher. The experiments performed on these tissue samples were carried out within 4 h after slaughtering. Approval for the experiments had been obtained from the Veterinary Board, District of Berlin-Koepenick. The pig ears were washed with cold water and dried with paper towels before being used in the experiments.

2.3 Sliced Skin

Samples of sliced skin, with a thickness of 200 μm and a sample size of 3 × 3 cm², were obtained from the pig ears, using a Dermatome GA 148 (AESCLAP AG and Co. KG, Tuttingen, Germany).

2.4 Tape Stripping

Tape stripping is a well-suited method for removal of the stratum corneum of the skin. Therefore, an adhesive film (Tesa Film No. 5529, Beiersdorf AG, Hamburg, Germany) was applied and by repeating this procedure continuously on the same tissue area, the stratum corneum could be removed sequentially, cell layer by cell layer. The first tape strips were investigated microscopically to ensure that they were completely covered with one layer of corneocytes.

2.5 Spectral Analysis of the Plasma

The spectrum of the plasma was analyzed within a range of 200 to 600 nm, using a fiber-based spectrometer EPP2000 (SI Scientific Instruments GmbH, Gliching, Germany). The optical quartz fiber (Laser-und Medizintechnologie GmbH, Berlin, Germany) contained an attached scattering body, which collects the surrounding light and transfers it to the fiber. The fiber was specifically developed for measurement of the spectral distribution of light, on and in different depths of tissue samples. In this study, the scattering body was fixed onto a quartz plate and positioned into the plasma stream. Additionally, the spectral characteristics of the plasma were measured behind one cell layer of corneocytes, removed by tape stripping, and behind the sliced skin samples. This experimental arrangement is shown in Fig. 2.

2.6 Determination of the Cross Section of the Plasma-Tissue Interaction Zone

The cross section of the plasma stream was determined by means of photographs behind a quartz plate, using an EOS 400 camera (Canon, Japan).

2.7 Temperature Measurements

The temperature of the plasma stream on the skin surface was resolved by using a measuring system based on a digital thermometer GTH 1200A (Greisinger elektronik GmbH, Regenstauf, Germany). The measuring spot was approx. 1 mm² in diameter.

Fig. 1 Photograph of the plasma jet during treatment of tissue (pig ear skin).
3 Results

3.1 Optimization of the Plasma-Jet Parameters

3.1.1 Voltage

The influences of the applied voltage and the gas pressure on the parameters of the plasma stream were investigated. The emission intensity of the plasma increased continuously with increasing voltage from 30 to 60 V, which was the maximum voltage for application on the control unit (Fig. 3).

3.1.2 Pressure

The argon gas pressure was changed from 10 to 80 kPa. The length of the plasma increased with increasing gas pressure (Fig. 4). At the same time, the diameter of the plasma stream decreased, which resulted in a decrease in the light emission of the plasma intensity at 310 nm (Fig. 5).

At values higher than 50 kPa, the gas stream used resulted in an uncomfortable sensation on the skin. This effect was tested on the forearm \textit{in vivo} without a plasma discharge.

3.1.3 Spectral characteristics

Taking these results into consideration, a voltage of 60 V and a gas pressure at 40 kPa were determined as the optimal working conditions for the applied plasma jet. The spectrum of the plasma obtained under these conditions is presented in Fig. 6. The spectrum consists of a band at a high intensity of 310 nm and a series of significantly smaller bands in the spectral range from 330 to 450 nm. Emission bands below 300 nm were not observed. The plasma charge has a high stability. The emission intensity at 310 nm changed \( \leq 3\% \) during 120 s.

When the plasma stream came into contact with the quartz plate or the tissue surface, the plasma-tissue interaction zone was approximately 5 times larger than the diameter of the plasma stream. The top of the plasma stream acted in the mode of a “brush” during the treatment of material. This means that the treated area is significantly larger than the diameter of the plasma stream.
3.1.4 Optimal distance of the nozzle of the plasma jet to the tissue

Using the optimal working conditions of the plasma jet, the influence of the distance of the nozzle to the quartz plate surface, regarding the distribution of the plasma in the plasma-material interaction zone of the plasma jet, was investigated. The results are demonstrated in Figs. 7 and 8. Figure 7 shows diameter and distribution of the plasma emission in the plasma-material interaction zone. If the quartz plate was in direct contact with the nozzle of the plasma jet, an arc discharge inside the opening of the nozzle was observed. This arc discharge produced a nonhomogeneous distribution of the plasma in the cross section of the plasma stream [Fig. 7(a)]. The plasma is characterized by high emission intensity at 310 nm [Fig. 8]. However, when the distance of the plasma jet from the quartz plate surface was increased to 2 mm, a homogeneous plasma charge was obtained in the cross section of the treated quartz surface [Fig. 7(b)], with a diameter of 4 mm. This is greater than the diameter of the plasma stream itself (see Fig. 7). The increased size of the plasma-material interaction zone is caused by the "brush effect" of the plasma on the material surface. In this case, the intensity of the plasma emission was only slightly reduced in comparison to the contact regime. By further increasing the distance up to 5 mm, the diameter of the cross section remained at a size of 4 mm [Fig. 7(c)]. However, the intensity of the plasma emission at 310 nm was reduced to 30% (Fig. 8).

3.2 Safety Assessment

3.2.1 Plasma emission

The intensity of the plasma emission measured behind the sliced skin was strongly reduced. Only less than 1% of the plasma radiation at a maximum of 310 nm could be detected behind the sliced skin of the pig ear, at a depth of 200 μm. Furthermore, tape strips were removed from the forearm of volunteers. By means of microscopic measurements, it was checked that these tape strips contained one layer of corneocytes, as demonstrated in Fig. 9 for three volunteers. Subsequently, the intensity of the plasma radiation was analyzed behind these tape strips. As seen in Fig. 10, approximately 25% of the incident radiation could be detected behind the first cell layer of corneocytes.

The same spectroscopic arrangement that was applied in the plasma experiment was used to measure the intensity of the sun radiation, on a sunny day in March at noon. Figure 11 compares the spectral intensity of the sun to the spectral distribution of the plasma jet. Comparing both spectra, it must be considered that the contact time of the plasma with the skin surface is usually less than 3 s, which corresponds to a moving velocity of the plasma stream on the skin surface at 2 mm/s.

Depending on the conditions of the outdoor experiments, the skin had to be exposed to sun radiation for more than 3 h (10,800 s) to produce sunburn. This means that the radiation dose produced by the plasma jet at 310 nm was an order of magnitude below the minimal erythema dose necessary for the formation of skin damage detectable as sunburn.
Therefore, a risk assessment is essential with regard to possible side effects of in vivo plasma application prior to the initiation of a clinical trial. Three major aspects must be addressed in such a risk assessment.

One potentially dangerous aspect is the electrical properties of the discharge system, where electric shocks on the tissue must be avoided. In this study, the risk caused by electric charges could be ruled out, based on the construction of the applied plasma jet. The system was approved by the CE certification (electromagnetic compatibility) and also met the requirements for an approval by the German authorities regarding electrical safety.

The two remaining safety aspects of the plasma jet—the influence of UV radiation and the temperature effect on the skin—were investigated in the presented study.

Prior to evaluation of the safety aspects, the optimal plasma parameters concerning treatment efficacy of tissue were determined.

For reasons of practicability the plasma stream should have a length of at least 5 mm to enable the operator to use the plasma jet in a noncontact mode, i.e., in the same manner as using a brush, without the limitation of a strong fixed distance between the plasma nozzle and the tissue. On the tissue surface, the plasma should be homogeneously distributed in the induction zone, with the largest possible diameter. Our plasma-jet device has three adjustable parameters to optimize the plasma properties: (1) the applied gas, in which an electrical discharge takes place; (2) the voltage; and (3) the gas flow, determined by the applied gas pressure. The discharged electric circuit of the plasma jet was optimized for the application of argon as a discharge medium. A plasma stream was produced in the voltage range between 30 and 60 V. The highest plasma emission and the largest plasma stream were obtained at a maximum voltage of 60 V. The length of the plasma stream increased with the optimal voltage at 60 V proportionally to the gas pressure. On the other hand, the intensity of the plasma emission on the tissue surface was reduced with increasing gas pressure. This was a result of the changed discharge conditions and the reduction in the diameter of the plasma stream. Additionally, a high gas pressure (> 40 kPa) led to an uncomfortable sensation on the skin, due to the mechanical stress produced by the gas stream.

An excessive gas flow should be avoided to prevent splattering the wound fluid that is frequently present on the skin surface of chronic wounds. Wound fluid can contain a high amount of free radicals, which are able to destroy bacteria and fungi in the upper cell layers of the tissue.9

Based on the promising in vitro results, it is necessary to confirm these findings under in vivo conditions on patients.

4 Discussion

4.1 Determination of the Optimal Parameters of the Plasma Jet

Regardless of the availability of several highly efficient commercialized antiseptics, the successful treatment of chronic wounds remains a problem.15,16 The main reason for this problem is that chronic wounds usually become encrusted with scabs, which block the penetration of antiseptics into the upper layers of the tissue where microbes are located.17

Recently, it was demonstrated on excised skin and in cell cultures that the application of “cold” plasma produced in an electric discharge can be an effective tool for noninvasive antimicrobial treatment of tissue.10,18 The mode of action of cold plasma seems to be based on the formation of a high amount of free radicals, which are able to destroy bacteria and fungi in the upper cell layers of the tissue.9

Based on the promising in vitro results, it is necessary to confirm these findings under in vivo conditions on patients.

4.2 Risk Assessment

Using the optimal operation parameters of the plasma jet, the risk aspects were evaluated in terms of UV radiation and temperature effects. As seen in Fig. 7, the emission spectrum of the plasma consists of one intensive line at 310 nm and several smaller bands between 325 and 450 nm. The emission
signal at 310 nm is a nitrogen band, which results from the N₂ in the air. Using the described plasma-jet system with argon as a charge medium, no emission bands at wavelengths lower than 300 nm could be detected. The UV light at 310 nm is efficiently absorbed by the stratum corneum of the skin. As exhibited in Fig. 9, a single corneocyte layer absorbs approximately 25% of the plasma radiation. The stratum corneum of the human skin consists of 15 to 25 cell layers, depending on the body site. This means that almost no UV radiation reaches the living cells of the skin. This was also confirmed by the transmission measurements of sliced skin.

Comparing the radiation dose of the plasma jet on the tissue and the dose of sun radiation under usual conditions, we demonstrated that the UV irradiation dose of the plasma was an order of magnitude below the minimal erythema dose. Therefore, the potential risk of skin damage by UV radiation during treatment with the plasma jet under the present conditions can be ruled out.

The temperature measurements on the skin surface showed, for an interaction time of 5 s, a moderate temperature increase to approx. 45 °C. At higher plasma tissue exposure times, thermal damage of the tissue must be expected.

Bearing in mind that the usual treatment velocity is in the range of 10 mm/s, the contact time of the plasma with the tissue is less than 1 s. Under these realistic conditions, thermal damage can be disregarded.

Summarizing the results obtained with the presented plasma jet system, it can be established that no major risk potentials are expected for the patient when the system is applied in vivo. Dangerous UV radiation and temperature effects during the plasma-tissue interaction can be neglected. On the other hand, it could be demonstrated in several in vitro studies that the plasma has a strong antimicrobial efficacy.

Acknowledgments

We would like to thank the “Skin Physiology” Foundation of the Donor Association for German Science and Humanities for financial support. Also, our thanks go to the Ministry of Technology (BMBF) for their financial support.

References

3. G. Müller and A. Kramer, “Interaction of octenidine and chlorhexidine with mammalian cells and the resulting microbicidal effect (re-

manence) of the combinations,” *GMS Krankenhaushyg.* 2, 46–49 (2007).