The Plasma Jet kINPen – A Powerful Tool for Wound Healing

Article in Clinical Plasma Medicine · January 2016
DOI: 10.1016/j.cpme.2016.01.001

CITATIONS 31
READS 1,081

4 authors:

Sander Bekeschus
Leibniz Institute for Plasma Science and Tech…
40 PUBLICATIONS 479 CITATIONS
SEE PROFILE

Anke Schmidt
Leibniz Institute for Plasma Science and Tec…
72 PUBLICATIONS 747 CITATIONS
SEE PROFILE

Klaus-Dieter Weltmann
Leibniz Institute for Plasma Science and Tech…
378 PUBLICATIONS 6,570 CITATIONS
SEE PROFILE

Thomas von Woedtke
Leibniz Institute for Plasma Science and Tec…
162 PUBLICATIONS 2,466 CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

- Plasma sources View project
- Group leader: "Plasma and liquids" - part BMBF project series "Center for Innovation Competence View project

All content following this page was uploaded by Anke Schmidt on 20 May 2016.
The user has requested enhancement of the downloaded file.
1. Introduction

1.1. Plasma medicine

The innovative field of plasma medicine is emerging as a hot topic, not only in the community of physics. For over 20 years now [1], cold plasma is being investigated for biomedical applications using appropriate laboratory testing. Yet, significant progress on its molecular mechanisms has only been made in the past decade [2]. The role of oxidants and antioxidants in cells and tissues relating to the effects of plasma has increasingly been pinpointed in recent redox biological work [3–5]. Also, an ever-increasing body of information is available on controlling and tuning the plasma and its reactive species output in need to modify physical and biology targets [6–8]. Today, cold plasmas are present in a variety of applications. This includes the decontamination of heat-sensitive materials, such as, surgical equipment, without introducing alterations in their quality [9–11]. Food [12–14] and its packaging [15–17] has been decolonized using cold plasma as well. Plasma can contribute to surface functionalization in implantology [18–20]. Recently, also an imperative role for plasmas in cancer treatment has been implied [21–30]. Another highly promising area of application is treatment of chronic wounds to improve their healing which is the focus of the present work [31–33]. However, plasma sources strongly differ from each other making it challenging to summarize or even compare results from different types of sources from a biological or physical point of view. This compendium will focus on a particularly promising argon plasma jet (kINPen; Leibniz Institute for Plasma Science and Technology – INP Greifswald and neoplas tools GmbH Greifswald, Germany) and the preclinical and clinical studies obtained with this device. Potential health risks in humans as a consequence of the plasma application using the kINPen will also be discussed. Numerous studies were concerned with this risk assessment as it is pivotal for a general acceptance of cold physical plasmas among physicians, clinicians, and patients alike.

1.1.1. Cold physical plasma

Plasma is generated by energizing gas up to a critical point at which electrons dissociate from atoms. The resulting ionized gas contains charged particles, while the overall charge remains electrically neutral. In biomedical applications, plasma sources that operate at atmospheric pressure and transfer a minimal thermal output are desirable in order to be tolerated by cells and...
tissues during exposure. In plasma jets, such as the kINPen, the plasma is usually generated by applying a high-frequency alternating voltage to a gas. The electron flux exhibits a high velocity and is hot. These fast electrons then excite or ionize atoms and/or molecules of a feed gas, e.g., argon. Argon ions on the other hand are heavier than electrons and hence slower in the electric field, preventing their acceleration. Also, fast electrons inefficiently transfer their energy (heat) to the heavy particles which consequently remain cold. As the heavy particle temperature of a gas determines its overall temperature, the plasma contains highly energized particles without displaying the significant temperature increase that would usually come with them (non-equilibrium plasma). Additional cooling of plasma can be achieved using pulsed excitation patterns or high feed gas fluxes, e.g., of argon. The feed gas flux also provides a channel of easy-to-ignite gas at the outside of the jet nozzle [34]. The plasma being generated in this effluent region interacts with ambient air to create non-radical (e.g., hydrogen peroxide, ozone) or radical species (e.g., hydroxyl radical, nitrogen monoxide) [35] which then oxidize biomolecules.

1.1.2. The kINPen argon plasma jet

The kINPen is a commercially available atmospheric pressure argon plasma jet. Its concentric, light-weighted, and pen-like design was developed for biomedical applications and allows precise and arbitrary 3D movements [36]. Typical active agents being generated include ions, electrons, or reactive oxygen and nitrogen species (ROS/RNS). Electric and magnetic fields, light (visible, infrared, UV), and neutral particles are also being generated. The hand-held unit is operated using a power supply and a gas supply unit. The electrical safety of the kINPen is certified and complies with EU standards (certificate number 609.003.1). In its metal housing, a central, rod-like electrode is mounted and shielded by a dielectric quartz capillary connected to a grounded ring electrode. The plasma is generated by applying a sinusoidal voltage (2–6 kVpp) with a frequency of 1.0–1.1 MHz to the central electrode (power: <3.5 W in the hand-held unit). The typical plasma effluent length is 9–12 mm and with 1 mm in diameter. Argon is used as feed gas, but small amounts of other molecular gases can be admixed [37]. High spatial and temporal optical resolution of the plasma revealed that it propagates in a bullet-like manner [38]. If applied as recommended, temperature or UV-radiation are not harmful to humans [36,39]. Recently, a gas curtain was introduced, shielding the plasma from ambient air [40]. This allows for a more refined control of the environmental conditions surrounding the plasma and influencing its chemistry [41]. The kINPen MED (Fig. 1) is a clinic-oriented evolution of its predecessor, the kINPen 09. Both units are identical in essential respects. The kINPen MED mainly differs from the kINPen 09 by having i) an integrated mass flow controller, ii) a housing optimized for the clinical use, and iii) a pulsed plasma generation (2.5 kHz, plasma on to off ratio = 1:1). In the kINPen MED, the latter further reduces the amount of energy being transmitted, i.e., the temperature at the tip of the effluent and the UV output. Despite the lower energy output, the biological effects of both jets were found to similar in HaCaT keratinocytes in vitro [42]. Hydrogen peroxide seems to dominate the liquid chemistry of a number of plasma sources including the kINPen [43–50]. It was found to be deposited in similar concentrations in liquids by both the kINPen MED and the kINPen 09 (unpublished observation), possibly explaining their comparable effects in vitro. Yet, these preliminary results contradict expectations with regard to the different operation modes (continuous vs. pulsed) and further in depth analysis is needed to examine the similarities and differences regarding the reactive species output between the two related kINPen devices. Central properties of the kINPen 09 and the kINPen MED are summarized in Table 1. The kINPen MED (Leibniz Institute for Plasma Science and Technology – INP Greifswald and neoplas tools GmbH Greifswald, Germany) was the first cold atmospheric pressure plasma jet worldwide to be accredited as a medical device (class IIa) for the use in patients. This argon plasma jet is a reusable device with the purpose of generating a constant, non-thermal (room-temperature) plasma at atmospheric pressure. Its use is intended for the treatment of non-healing wounds and ulcers of the human skin. The compromised tissue may be infected and the plasma can be effectively applied regardless of any bacterial multidrug resistances.

1.2. Wound healing

In life, tissue injury is inevitable. Microorganisms may invade compromised tissues and cause infection. Tissues display a high plasticity and the ability to seal a gap, while bacteria are being removed by immune cells: to heal. 

1.2.1. Healing phases

The process of wound healing is characterized by four continuous, overlapping, and precisely programmed phases: hemostasis, inflammation, proliferation, and remodeling [51]. Hemostasis is characterized by vascular constriction and platelet aggregation, degradation, and fibrin formation (thrombus) [52]. In the subsequent inflammatory phase, neutrophils infiltrate the wound site, followed by monocytes and lymphocytes [53]. During the proliferation phase, epidermalization occurs, while new blood vessels are being formed (angiogenesis) and collagen is being synthesized for extracellular matrix formation [54]. Finally, collagen and the vasculature are being remodeled [55]. Many different cell types are involved in the healing process, such as, fibroblasts and...
kERs, macrophages pave the way for wound resolution by pha-
sequential immigration of immune cells helps to eradicate infectious
1.2.3. Non-healing and chronic wounds
healing, and this creates an exciting therapeutic window for cold
antioxidants in wound healing. Thus, ROS are central in wound
phases which again suggest a physiological role of oxidants and
centrations ranging between 100 and 250 \( \mu \text{M} \). During the
proliferative phase
lease of cytokines and growth factors [59], and promoting the

1.2.2. Redox control in wounds
For a long time, the dogma has been that reactive species are primarily toxic to cells. Yet, further studies revealed that at low concen-
trations these species play a pivotal role in cellular physi-
ology and signaling [61]. They are also thought to act as second
messengers [62]. During the first event in wound healing, he-
mostasis, platelet aggregation occurs, and these cells are capable
of generating reactive oxygen species (ROS) [63]. At the same time,
scavenging of ROS inhibits collagen-dependent platelet aggrega-
tion [64]. Phagocytes utilize oxidants for antimicrobial defense
[65]. Interestingly, stimulation with growth factors initiates ROS
production in many cell types [66]. Mutations in a ROS-generating
enzyme, the NADPH oxidase, causes the chronic granulomatous
disease which is an immune-deficient condition characterized by
impaired wound healing [67]. Hydrogen peroxide (H\(_2\)O\(_2\)) induces
chemotaxis in neutrophil granulocytes to the site of infection [68].
H\(_2\)O\(_2\) is especially interesting as it has a fine-tuning regulatory role
in both inflammation as well as avoidance of harmful in-
flammatory responses [69]. ROS can induce epithelial cell pro-
iferation and migration which are both important in wound re-
epithelialization [70]. To date, the highest levels of H\(_2\)O\(_2\) in the
body were found in wound fluids [71] with physiological concen-
trations ranging between 100 and 250 \( \mu \text{M} \) [72]. Interestingly,
these concentrations were modulated during different healing
phases which again suggest a physiological role of oxidants and
antioxidants in wound healing. Thus, ROS are central in wound
healing, and this creates an exciting therapeutic window for cold
physical plasma-generated reactive species in wound treatment.

1.2.3. Non-healing and chronic wounds
Non-healing wounds are a major health issue in the western
world, causing an estimated $3\ billion per year in the US [73]. By
definition, non-healing or chronic wounds “failed to progress
through the normal stages of healing and therefore enter a state of
pathologic inflammation” [74]. It is possible that different parts of
a wound may be stuck in different healing phases, having lost the ideal
synchrony of events [75]. Consequently, healing is delayed and in-
complete, resulting in poor anatomical and functional outcome.
Chronic wound etiology is heterogeneous but most ulcers are caused
by ischemia, secondary to diabetes mellitus, venous stasis, and
pressure. Additionally, wound healing can be compromised by in-
fec tion with microorganisms [76]. In healing wounds, inflammatory
processes effectively eradicate microbial invasion whereas in de-
rteriated tissues (ischemia, hypoxia, devitalized tissue, and chronic
inflammation) this process may be impeded. Consequently, in-
flammation, e.g., mediated by neutrophils, cannot be resolved. In
normal healing, neutrophil presence is self-limited to 72–96 h after
wounding [77]; in non-healing wounds they are constantly present
[78]. A continuous presence of neutrophils leads to an imbalance in
proteinase and cytokine concentrations. Proteinases degrade tissue,
and this proteolytic environment disallows the formation of suffi-
cient extracellular matrix, thus inhibiting cell migration and collagen
deposition [79]. These conditions seem to be an indirect result of an
amplified cytokine secretion. Unfortunately, to date there is no gen-
erally accepted chronic wound animal model available [80], render-
ing animal experiments difficult to interpret. This applies, for exa-
ample, to studies addressing the role of bacteria in improper wound
healing. While presence of bacteria is a well-recognized factor in
chronic wounds [81] their eradication is not necessarily associated
with improved healing responses [82]. Nonetheless, their removal is
a prerequisite for wound stabilization to allow for wound healing
phase progression.

2. Pre-clinical studies using the kINPen

2.1. In vitro activity of plasma against bacteria and other pathogens

Similar to numerous other plasma sources [83–85], the kINPen has
been shown to be effective against various bacteria in vitro
including Escherichia coli, Pseudomonas aeruginosa, Klebsiella
(group K. pneumoniae, K. oxytoca), Staphylococcus aureus, hemolyzing
Lancefield Streptococi (group A and B), Proteus group (P. mirabilis,
P. vulgaris), Acinetobacter spp., Stenotrophomonas spp., Enterococcus
faecalis, and Staphylococcus epidermidis [86–91]. Biofilms can also
be successfully removed with this argon plasma jet [5,92–94]. In a
comprehensive study, the growth of 194 clinical isolates of chronic
wounds from 13 different and partly multidrug-resistant species was
tested. All organisms very successfully inactivated using the
kINPen [95]. The plasma of the kINPen was also shown to be ef-
fective against the EHEC pathogen [96]. To test the efficacy of the
antisepsis approach of plasma on animal skin samples, freshly
enucleated porcine eyes were used as a test model. Compared to two
commonly used antisepsis agents, the plasma treatment was significant-
ly more effective against both S. aureus and P. aeruginosa [97]. Fungi
and parasites were also successfully inactivated using the plasma
of the kINPen [95]. In vitro, this includes the clinical fungal strain in-
volved in dermatomycosis, namely Trichophyton interdigitale, Tri-
chophyton rubrum, Microsporum canis, and Candida albicans [98–
100]. Plasma treatment also kills wound-causing mites [101]. Al-
together, these data suggest the plasma of the kINPen to be useful in
the treatment of infected and/or chronic wounds, especially if
multiresistant pathogens are governing the infection that are usu-
ally difficult to treat using conventional therapies.

2.2. Redox stimulation of skin and immune cells using plasma

The central function of skin cells in wounding is the wound
closure by cell division and migration [102]. Plasma treatment

Table 1
Table 1
Physical properties of the kINPen 09 and the kINPen MED.

<table>
<thead>
<tr>
<th>Properties</th>
<th>kINPen 09</th>
<th>kINPen MED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended feed gas and flux</td>
<td>Argon (5 ± 1 l/min)</td>
<td>argon (5 ± 1 l/min)</td>
</tr>
<tr>
<td>Frequency</td>
<td>1.1 MHz</td>
<td>1 MHz</td>
</tr>
<tr>
<td>Feed gas flow control</td>
<td>Via external devices</td>
<td>Yes (integrated)</td>
</tr>
<tr>
<td>Mode of operation</td>
<td>Continuous</td>
<td>Pulsed (2.5 kHz)</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>n. a.</td>
<td>Plasma on/off = 1:1</td>
</tr>
<tr>
<td>Power</td>
<td>&lt; 8 W of the whole system</td>
<td>&lt; 8 W of the whole system</td>
</tr>
<tr>
<td></td>
<td>&lt; 3.5 W of the handheld</td>
<td>&lt; 3.5 W of the handheld</td>
</tr>
<tr>
<td>device</td>
<td></td>
<td>device</td>
</tr>
<tr>
<td>Typical effluent length</td>
<td>10–12 mm</td>
<td>9–11 mm</td>
</tr>
<tr>
<td>Recommended working distance</td>
<td>9–11 mm</td>
<td>8–10 mm</td>
</tr>
<tr>
<td>Temperature at the recommended</td>
<td>46–50 °C</td>
<td>35–38 °C</td>
</tr>
<tr>
<td>working distance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-irradiation at working distance</td>
<td>UVA: 20–35 ( \mu \text{W/cm}^2 )</td>
<td>UVA: 5–15 ( \mu \text{W/cm}^2 )</td>
</tr>
<tr>
<td>(free jet)</td>
<td>UVB: 20–15 ( \mu \text{W/cm}^2 )</td>
<td>UBV: 5–15 ( \mu \text{W/cm}^2 )</td>
</tr>
<tr>
<td>Phase stabilization during</td>
<td>Manually</td>
<td>Automatic: PLL-circuit</td>
</tr>
<tr>
<td>plasma generation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please cite this article as: S. Bekeschus, et al., The plasma jet kINPen – A powerful tool for wound healing, Clinical Plasma Medicine
(2016), http://dx.doi.org/10.1016/j.cpme.2016.01.001
improved the closure of an artificially created, infected wound in a 2-D skin model [103]. Plasma exposure of keratinocytes in vitro also resulted in an enhanced production of growth factors important for wound healing and neovascularization [104]. Also, plasma affected the keratinocyte transcriptome [105] and proteome [106] strongly via antioxidant pathways [107] in vitro. Both in vitro [108] and ex vivo [109], plasma treatment enhanced the proliferation rate of skin cells which is highly important for wound closure. Therefore, the plasma of the kINPen alters the redox balance in skin cells which positively affects their growth and secretion of growth factors. During wound healing, immune cells orchestrate the wound healing phases and are decidedly required for antibacterial defenses. After plasma treatment, the ability of neutrophils to ingest and kill bacteria was not altered but overall antibacterial efficacy maybe increased due to extracellular trap formation [110] which effectively inhibits microbial growth [111]. This may decrease wound inflammation and would support healing. Plasma was not toxic in neutrophils or monocytes [112] but readily induced apoptosis in lymphocytes [113]. This was due to strong oxidation in the cell membrane and cytosol [114], culminating in an altered redox state in these cells [115]. The cytotoxic effects seen in lymphocytes are highly H₂O₂-dependent which seems to be generated in the plasma gas and liquid phase [116, 117]. Elevated numbers of lymphocytes are present in pathological wounds [118] and their removal via oxidants may be beneficial for healing. Nonetheless, lymphocytes are also important to elicit immune responses, and these cells retained their ability to proliferate after exposure to the plasma [119]. Plasma stimulated mononcyclic cells, and induced inflammatory as well as anti-inflammatory redox response at the same time [120]. These redox modifications may tip the balance of the low-grade inflammation seen with chronic wounds towards the transition to the proliferative phase required for proper wound healing.

3. Case reports and clinical results using the kINPen plasma

3.1. Antimicrobial efficacy

One-hundred and five wound-resident bacteria and fungi (11 different species with some displaying multidrug resistances) were plasma-treated on contaminated finger tips of nine healthy volunteers. Among the bacterial strains were Pseudomonas, Klebsiella, Staphylococci, and Micrococi which are often difficult to treat in clinical settings. All types of bacteria and fungi were highly susceptible to plasma treatment and regardless of them being part of the physiological or pathological skin flora [121]. The tests were carried out in accordance with the national guidelines for microbiological diagnostics, and the net reduction of bacterial burden was significant, although not similar for all types of bacteria tested. The authors suggest that the plasma of the kINPen may be an effective supplement to antiseptics in the treatment of skin and wound-resident pathogens. The antimicrobial activity of the plasma on the skin was confirmed in a study by Klebes and colleagues. Chronic wounds of 34 patients were treated with the plasma of the kINPen 09 or in combination with a wound antiseptic. The combination therapy showed the best efficacy [122]. In another study involving six psoriatic patients, an antimicrobial efficacy of plasma was found as well but remission of the disease was not observed [123]. Thus, the in vivo application of the plasma jet kINPen is effective against bacteria residing on the skin and in wounds.

3.2. Enhanced wound healing in animal studies

The efficacy of new therapeutic concepts is usually evaluated in animals first. However, it is challenging to investigate improvement in chronic wound healing as there are no valid animal models available for this pathology [124–126]. However, wound healing can be slowed for up to several weeks in a hairless mouse model [127–129]. Using this model, an animal study with 77 mice was conducted to examine wound healing after plasma-treatment. Preliminary results showed that the wound area on ears receiving plasma (3 s) at day 9 was about 30% smaller compared to untreated controls (manuscript in preparation). Moreover, healing of chronic wounds was markedly induced in domestic animals (4 dogs and 2 cats) [108]. The wound age was 2–60 months and some animals failed multiple treatments using conventional antiseptics or skin graft surgery. Within 3–24 weeks of regular plasma treatment (twice weekly), complete wound healing was achieved. Altogether, plasma treatment with the kINPen supports the healing of slow-healing or truly chronic wounds in animals.

3.3. Enhanced wound healing in clinical observational studies

The efficacy of the kINPen MED in the treatment of wounds has been tested in three observational studies with a total of 26 patients. All studies demonstrated a wound healing and/or antiseptic effect of the plasma. In a clinical case-control study, the efficacy of the kINPen for wound healing was compared to Octenisept which is one of the most effective and biocompatible antiseptics in the management of chronic wounds. Sixteen patients with chronic leg ulcers were enrolled in this study. They received plasma three times a week and over a total period of two weeks [130]. The number of bacterial colonies and the size of the wound surface and were determined thereafter. Treatment with either the kINPen MED or Octenisept achieved a comparable reduction of bacteria. The wound surface reduction was 56% in the plasma group and 19% in the Octenisept group, suggesting a benefit of plasma in wound healing [131]. A positive side effect of the plasma treatment was a slight reduction of wound exudation. Exposure to plasma was tolerated very well. In another case-report study, four sterile wounds were induced on both forearms of five volunteers and utilizing a UltraPulse CO₂ laser commonly used in esthetic surgery [132]. In a randomized, controlled experimental procedure, the wounds then either received plasma treatment (single dose 10 s, three times 10 s, or single dose 30 s) or remained untreated for three consecutive days. Wound healing progress was photographed 10 days after wounding and staged in a blinded manner by five independent physicians. They scored i) which of the four wound sites presented the best early healing, and ii) how should the esthetic outcome of this best site be scored on a numeric scale (0–10 with 10 scoring the ideal result). Three times 10 s plasma treatment on three consecutive days gave the best results followed by the single 30 s treatment. Ten seconds single treatment was similar to untreated control. Importantly, healing was final and without complications as determined in a follow-up study 1 year after plasma treatment [133]. In the third study, Vandersee and colleagues induced four artificial and sterile wounds using negative pressure on the both arms of five volunteers [134]. Initial wound size was 10–18 cm². The four wounds received either no treatment, treatment with the kINPen MED for 60 s, treatment with Octenisept, or a combination therapy involving both regimens. Wounds receiving plasma displayed an accelerated healing response while untreated wounds showed an average healing response, respectively. Using Octenisept or the combination therapy, the average grade of wound healing was above untreated control but below plasma treatment alone. Using confocal laser scanning microscopy, it was demonstrated that plasma-treated wounds displayed an earlier onset of the proliferative wound healing phase, possibly explaining the accelerated healing response observed. This also implies a shortened inflammatory phase, suggesting a plasma-induced redox modulation of immune cells that regulate inflammation to be central. It can be concluded that plasma treatment with the kINPen MED is beneficial for healing of chronic wounds.
Accelerated healing was also seen with sterile wounds, proposing a promotion of wound healing independent of the antimicrobial effects previously seen with the plasma. Vice versa, the efficacy of plasma may be enhanced in infected wounds due to its antisepptic properties. Therefore, treatment with the kINPen MED is an innovative approach to improve healing of wounds. Yet, and similar important to its efficacy, the plasma treatment needs to be safe in order to achieve a widespread clinical application in future.

4. Risk estimation of the plasma of the kINPen

4.1. Physicochemical parameters

4.1.1. Temperature

An autoclavable spacer is available for the kINPen MED to ensure a uniform plasma treatment. It facilitates a fixed distance (7–8 mm) from the jet nozzle to the surface in question. For the kINPen, an efficient oxidant delivery was shown for distances of up to 12 mm [135]. Within this distance, a thermal energy dissipation of the plasma is still measurable. To avoid thermal denaturation of proteins in tissues, the temperature output of the jet needs to be acceptable. Using a thermal camera, the average temperature at the clinically relevant distance of 7–8 mm was found to be 39 ± 1 °C [132]. On the skin of volunteers and by applying an appropriate velocity of the plasma jet over the skin (8 mm per second), no temperature increase was observed (manuscript in preparation). The lack of thermal damage was verified histologically in plasma-treated porcine skin [136].

4.1.2. UV radiation

High doses of UV radiation can induce DNA double-strand breaks and thereby pose a carcinogenic hazard. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) [137] recommends a maximum effective (weighted) exposure of 30 J/m² per day (8 h; λ = 180–400 nm) [138]. The minimum exposure needed to elicit skin erythema (sun burn) in the most sensitive human skin type is 200 J/m² [139]. By means of optical emission spectroscopy, it was found that the kINPen MED generates some UV emission in the UV-A and UV-B range, and very low emission in the UV-C range. In liquids, the latter was shown to be very quickly absorbed (100% absorption at about 220 nm of liquid depth for cell culture medium treated with the kINPen MED) [140]. For plasma treatment (60 s of 0.5 cm² at a distance of 7–8 mm), the weighted effective irradiance of a "free" kINPen MED plasma (not touching a surface) was measured and calculated to be 1.05 J/m² [141]. If the plasma jet is not being moved during the 60 s treatment time (single-spot exposure) the UV exposure is locally 8.8 J/m². Both figures are well below the recommended limits [142]. Yet, it is known that the properties of jet plasmas may change dramatically if the effluent comes in contact with a surface [143]; very likely increasing electron densities and therefore also the reactive species and UV as well as VUV output. As such, further investigations are needed to better understand potential risks of UV exposure of the kINPen plasma if in contact to a substrate, such as skin.

4.1.3. Generation of ozone

During the plasma treatment with the kINPen, ozone is being formed [144]. Ozone is part of environmental technologies for air and water disinfection, and is an unavoidable part of many other technologies, such as, laser printers in offices. The previously common MAK (maximum working concentration value) for ozone was 0.1 ppm in air, and is defined as a maximum and permanent concentration during a work day (8 h). Today, there are no binding limits of its concentration in ambient air as ozone has been classified as potentially carcinogenic. Nevertheless, no health risks are to be expected at constant (8 h) concentrations of 0.055 ppm in air [145]. Ozone concentrations in hot, European summers are sometimes 0.3 ppm locally [146] but on average between 0.03 and 0.05 ppm. Short-term concentrations of 1.5 ppm are considered to be an acute health risk. The kINPen MED expels maximum ozone concentrations of 0.10–0.13 ppm and this only in the immediate vicinity of the plasma source (distance < 10 cm) [141]. At larger distances (> 30 cm) the ozone concentration is below 0.10 ppm [39]. Because of the brief exposure during the rather short-term plasma treatment, acute health risks via ozone formation are not to be expected. The odor threshold for ozone is 0.02 ppm and may be exceeded during plasma treatment, possibly causing minor unease of odor. Formation of potentially toxic nitrogen dioxide was not detected in the plasma effluent of the kINPen [36].

4.2. Biological parameters

4.2.1. Mutagenicity

Cold plasma expels various reactive components which can oxidize biomolecules and are therefore potentially mutagenic. A common OECD test systems for genetic toxicology is the HPRT gene mutation assay that utilizes the Chinese hamster cell line V79 [147]. In this test, viable mutants are detected by the loss of activity of the HPRT enzyme which generates cytotoxic nucleotides that otherwise kill the non-mutants. Neither exposure to plasma-treated medium nor direct plasma treatment (30 s) induced an increase of mutants [148]. Micronucleus formation is assessed in another OECD test system for genetic toxicology [149]. Plasma treatment did not induce the formation of micronuclei [148] indicative of acentric DNA fragments that are common in mutations [150]. Also, non-mutated cells do not form colonies in soft agar while malignant cells have often lost the need of cell–cell contact for proliferation. During 30 days after plasma treatment, HaCaT keratinocytes did not form colonies in soft-agar which further substantiates a lack of mutagenicity of the plasma of the kINPen [148]. In ex vivo treated human skin, the plasma of the kINPen MED did not induce double strand breaks which can be indicative of mutagenic events [151]. In a long-term clinical investigation, artificial skin wounds in volunteers were plasma-treated and wound healing side effects were graded one year after treatment. In none of the five subjects a sustained tissue degeneration or cancerous lesion was observed [133]. Preliminary results of mice that have received plasma treatment of wounds did not provide any indication of a mutagenic action of plasma (manuscript in preparation). Although further research in animal models is needed to verify or exclude a mutagenic role of cold plasma, such a health risk cannot be concluded from scientific studies to date.

4.2.2. Penetration depth in the skin

The plasma treatment of the skin or wounds aims at setting topical effects on the surface of the tissue. Subcutaneous tissues, such as, muscles or fat, should not be affected by the treatment. Therefore, several studies determined the penetration depth of plasma in the skin. Plasma generates reactive species that have oxidizing properties in, e.g. β-carotenes in intact skin, which can be assessed using Raman microspectroscopy [152]. Plasma treatment of intact skin of six subjects showed oxidation to be present only up to a depth of 10 μm [153]. Similar results were obtained in other work [154]. In both studies and compared to untreated skin, no significant differences were observed in deeper layers of the skin. This implies that the oxidative challenge provided by the kINPen plasma in tissues is only superficial.

4.2.3. Subjective sensations

Subjective sensations of the plasma treatment may not per se constitute a potential health risks but decrease the acceptance of
plasma in patients. After a single-spot plasma treatment of the fingertips of nine volunteers, these were asked to indicate pain, heat, as well as other sensations (paresthesia) of the skin on a scale from 0 (no sensation) to 10 (extreme sensation) [121]. The following results were obtained for a treatment time of 240 s using the kINPen: 0.5 (pain); 1.3 (heat); 1.5 (paresthesia). No volunteer wished to discontinue the study due to any discomfort, such as, pain or heat. A similar study was conducted with artificially created wounds (suction blisters) in five volunteers [134]. On a scale of 0 (no irritation) to 5 (intolerably strong irritant effect) for sensory irritations, the volunteers reported a 1 in average. Further validating the results of ozone measurements with the kINPen, no volunteer or physician reported an unpleasant smell of any ozone created during the treatment. It was concluded that the plasma treatment is tolerated well subjectively.

4.2.4. Cytotoxicity and histocompatibility

Plasma treatment should neither irritate the skin nor cause large-scale damage or toxic effects. For ethical reasons, such studies cannot be carried out in humans but animal tissues serve as a good model to investigate the plasma’s irritation potential. As a replacement method for the Draize test in the rabbit eye, the HET-CAM test (Hen’s Egg Test on the chorioallantoic membrane) is commonly used to examine the biocompatibility of chemicals. Clinically-relevant exposure times to non-pulsed (kINPen 09) plasma caused an average hemorrhagic or intravasale thrombosis on the CAM. By contrast, plasma treatment using the pulsed mode (similar to the kINPen MED) was superior and resulted in only slight irritation [155]. Strikingly, the irritation was further reduced by a parallel application of hydrocortisone [156]. This underlines the role of plasma-mediated redox modulation in immune cells, and suggests plasma to be particularly effective in multimodal therapies. Importantly, the irritant effect was completely reversible with clinically relevant plasma treatment times (60 s). Next to the irritation potential of plasma, histological analysis was carried in different studies and after plasma treatment. The plasma of the kINPen showed identical histocompatibility compared to two commonly used skin antiseptics in an experimental model of porcine eyes [97]. Histological analysis was also carried out in surgically removed human skin which was exposed to the plasma of the kINPen MED for 60 s. Plasma treatment did not increase the number of apoptotic cells, did not induce epidermal lesions, and did not elevate extracellular concentrations of cytochrome c which is an marker for physical cell damage [109]. A lack of histological damage was also seen after 10 min of single-spot plasma treatment on human skin ex vivo [157]. In another study, no significant effect of the plasma on the water balance (moisture) of the skin was found [158]. In sum, the cold plasma treatment with the kINPen is histocompatible and not damaging to the skin per se.

5. Other sources for plasma medicine

5.1. Preclinical studies

A vast body of information is available about preclinical studies using various types of cold plasma sources with similar antimicrobial efficacy compared to the kINPen. These are effective against microorganisms in the planktonic state [159–161], on agar [162–164], in biofilms [165–167], and drug-resistance pathogens [168–170]. A hallmark of cold physical plasma is its proposed selective efficacy against unwanted microorganisms while leaving eukaryotic cells intact. Additionally, it has been observed that wound-relevant processes are supported, including activation and/or enhanced proliferation of fibroblasts [171–174], endothelial cells [175], and keratinocytes [176–178]. Importantly, several in vivo in mice studies underlined the potential of different cold plasma sources for accelerated wound healing [179–182]. Also, safety considerations were made for a number of sources using animal skin [183–185]. While first efforts have been made to rule out mutagenic effects using in vitro models [186], long-term animal studies supporting this notion have not been published yet for any plasma source.

5.2. Clinical results

Many plasma source have been developed for biomedical applications but only four (including the kINPen MED) received accreditation as a medical device for wound healing so far. Among these is the recently (August 2015) marketed SteriPlas (AdTec Ltd., Japan) which was proven to be effective in reducing bacterial burden in wounds [187,188] and supported their healing [189,190] as well as reduced local pain [191]. Another accredited source is the PlasmaDerm (Cinogy GmbH, Germany) that has been shown to be safe and beneficial in the healing of chronic leg ulcers in a randomized and controlled clinical trial [192]. Also PlasmaOne (Medical Systems GmbH, Germany) is a plasma source accredited as a medical device. There is a vast number of plasma therapy case reports available but its efficacy in supporting wound healing or conferring antimicrobial activity has not been scientifically published so far. Nonetheless, for each accredited device it has been a long way from research to market. Yet, and considering the stirring potential of cold plasma for the healing of chronic wounds in millions of patients worldwide, it is worth any effort. There is much reason to be excited about future plasma research and medical sources participating in tackling human skin pathologies.

6. Conclusion

The atmospheric pressure argon plasma jet kINPen is among the most promising sources in the field of plasma medicine. Its electrical safety is verified (CE-mark), it has received certification as a medical device class Ila, and it has been proven to be anti-septically effective. Prominently, it supports the healing of wounds. According to the available literature, its application does not pose any health risks in humans with regard to UV exposure, thermal damage, tissue toxicity, or mutagenicity. The biological and medical investigations carried out with this device may serve as role model for other plasma sources in the field of plasma medicine, and will stimulate curiosity and interest in the plasma technology altogether.

Conflict of interest

Klaus-Dieter Weltmann is a minority shareholder and the INP Greifswald is a majority shareholder of neoplas tools (Greifswald, Germany). All other authors declare no conflicts of interest.

Acknowledgments

This work was funded by the German Federal Ministry of Education and Research (Grant number 03Z2DN11) and by the Ministry of Education, Science and Culture of the State of Mecklenburg-Western Pomerania and the European Union, European Social Fund under (Grant numbers AU 11 038 and ESF/IV-BM-B35-0010/13).

Please cite this article as: S. Bekeschus, et al., The plasma jet kINPen – A powerful tool for wound healing, Clinical Plasma Medicine (2016), http://dx.doi.org/10.1016/j.cpmem.2016.01.001
References


Please cite this article as: S. Bekeschus, et al., The plasma jet kINPen – A powerful tool for wound healing, Clinical Plasma Medicine (2016), http://dx.doi.org/10.1016/j.cpme.2016.01.001