Stimulation of wound healing using cold plasma

Infection is one of the most common complications of hard-to-heal and non-healing wounds. Cold plasma is ionised gas, it has a quick and strong bactericidal effect, even on antibiotic-resistant bacteria. In this whitepaper several studies are presented about this effect and other positive effects of cold plasma treatment on wound healing.

Content:

What is cold plasma?

Figure 1: The 4 phases or states of matter

Plasma is ionised gas, the so-called fourth state of matter (see Figure 1). It is created by adding energy to a gas. Plasma is a mix of electrons and ions, reactive species, UV radiation, visible light, electromagnetic fields and warmth (see Figure 2). Nowadays it is possible to keep the temperature low, beneath 40° C. This is what we call a cold plasma, it can be used on living cells (in vivo).

Cold plasma is extremely reactive. Reactive oxygen and nitrogen species are key players in biological effects. These by cold plasma generated compounds work together with other plasma components such as UV radiation, mild warmth and electrical fields. These effects allow for an improved microenvironment by generating bactericidal conditions and they have a positive influence on microcirculation in the areas treated with plasma.

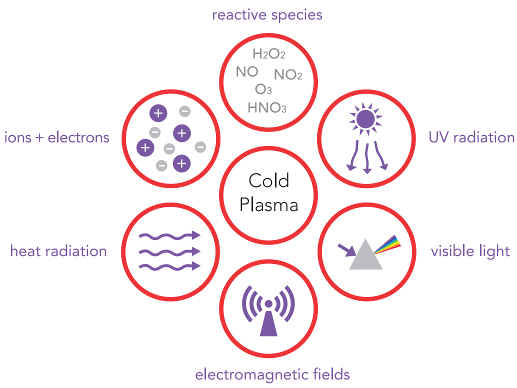

Figure 2: Cold plasma components

Cold plasma stimulates wound healing

Treatment with cold plasma is a new method with a short treatment time. Positive effects on wound healing are:

- Inactivation of a broad spectrum of microorganisms
- Stimulation of cell migration and cell proliferation
- Improvement of microcirculation

The effect of cold plasma on bacteria, biofilm and multidrug-resistant bacteria

Antiseptic treatments are extremely important for infected wounds, because in such wounds the healing process is impaired and therefore cannot proceed correctly. It is well known that microbial colonization of wounds delays the wound healing process, or stops it completely [1], resulting in the development of a chronic wound.

Numerous in vitro and in vivo studies already demonstrated the antimicrobial potential of plasma. Not only against individual pathogens, but also against resistant microorganisms as well as biofilms [1]. Both gram-negative and gram-positive bacteria, whether or not in biofilm, as well as yeasts and fungi have been successfully inactivated. For example, also the inactivation of biofilms of the methicillin-resistant Staphylococcus aureus (MRSA) or the extremophile genus Deinococcus radiodurans has been reported. Unlike antibiotics, another advantage of cold plasma is that bacteria do not show any resistance after repeated plasma treatment [2].

For example, the following study [2] shows that cold plasma will reach within two to five seconds a log 5 reduction of the number of bacteria (See Figure 3A and B). A log 5 reduction is 99,999% reduction, so 1 of 100.000 survives. This also applies to antibiotic-resistant bacteria.

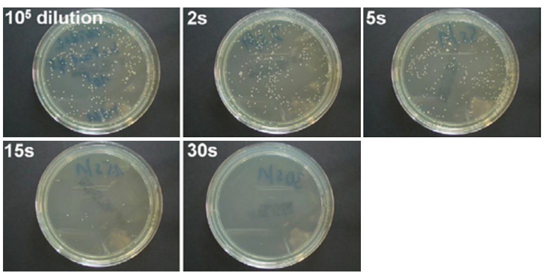

Figure 3A: Colonies formed by single surviving MRSA bacteria on agar plates after plasma treatment times of 2 - 30 s. As comparison a 10^5 dilution of untreated bacteria.

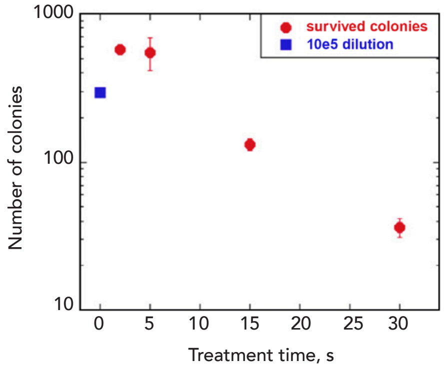

Figure 3B: The number of MRSA colonies that survived after different plasma treatment times (red data dots). As comparison the 10^5 diluted control sample (blue data dot).

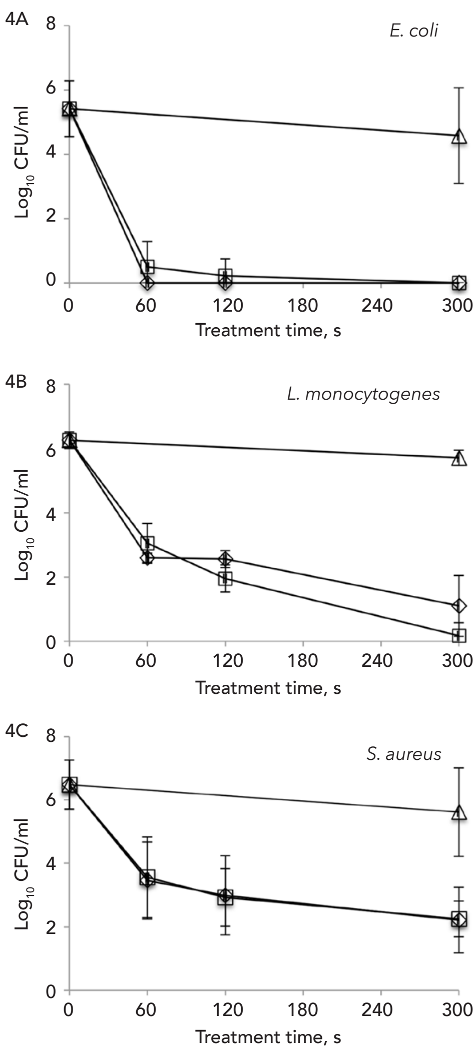

Figure 4 A, B, C: Surviving populations of bacterial biofilms after cold plasma treatment. (A) Escherichia coli, (B) Listeria monocytogenes and (C) Staphylococcus aureus: (△) untreated 24 h control, (◊) after direct and (□) indirect cold plasma treatment.

The next study [3] shows that cold plasma technology is an effective strategy for inactivation of biofilms and may play an important role in attenuation of virulence of pathogenic bacteria (See Figure 4A, 4B and 4C).

Cold plasma stimulates cell migration and cell proliferation

One of the main tasks of fibroblasts during wound healing is their migration to the wound area to synthesize new granulation tissue and to rebuild lost connective tissue. The increased proliferation of fibroblasts and keratinocytes is also an important mechanism of wound healing because it aids to rapid re-epithelialization and matrix synthesis.

Investigations indicate that plasma not only activates fibroblasts but is involved in the differentiation of fibroblasts into myofibroblasts [4]. Myofibroblasts are key players for maintaining skin homeostasis and for orchestrating physiological tissue repair and wound contraction. Studies have shown that plasma treatment induces the expression of diverse wound healing-relevant cytokines and growth factors in keratinocytes and fibroblasts and influence different cellular mechanisms important for wound healing as listed below:

- fibroblast and keratinocyte migration
- fibroblast and keratinocyte proliferation
- collagen expression and matrix formation
- cytokine and growth factor expression of fibroblasts and keratinocytes

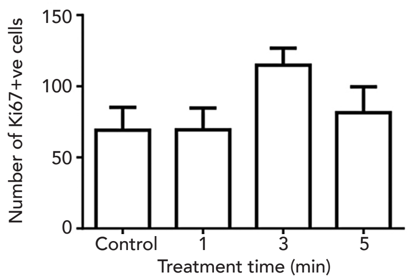

Figure 5: Quantification of Ki67-positive cells (keratinocytes) in the basal skin layer of plasma-treated and untreated samples. The number of proliferating cells was significantly increased after 3 min.

Research [5] shows a significant increase in the number of proliferating cells after a treatment of 3 minutes (see Figure 5).

Cold plasma increases microcirculation

Angiogenesis (formation of blood vessels) and sufficient blood supply are important in the second stage of wound healing. Growth factors and cytokines are important in these processes, but also reactive oxygen and nitrogen species such as ozone and nitrogen oxide that are generated by cold plasma.

The influence of plasma on local microcirculation has been demonstrated in multiple studies. A study showed that treatment of intact skin resulted in an immediate increase in the microcirculation parameters blood flow and velocity, relative hemoglobin and postcapillary oxygen saturation, lasting for at least one hour [6].

Repetitive treatment boosted as well as prolonged the microcirculatory effects, and longer treatment led to more pronounced effects. Moreover, the effects of plasma treatment are not limited to the superficial area directly reached by the plasma but are also observed in the skin layers below to a depth of ca. 8 mm [7].

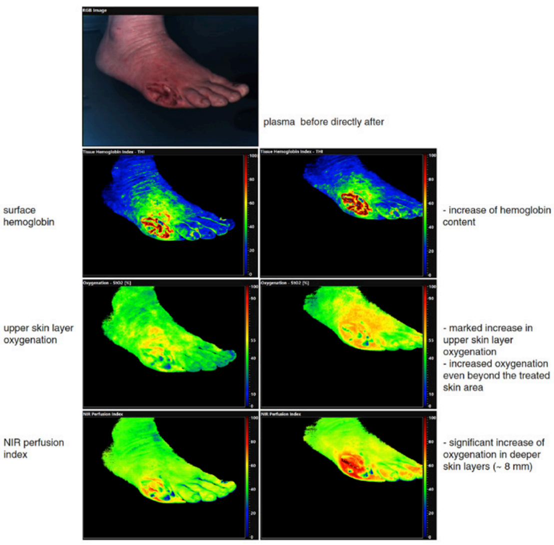

Figure 6: Hyperspectral analysis image of microcirculation changes in a diabetic foot.

Thus, plasma treatment can notably enhance microcirculation for a therapeutically relevant period, substantially surpassing the application time (treatment time 2 minutes, enhanced microcirculation 10+ minutes).
A third study [7] shows a significant increase of both hemoglobin level and oxygen saturation 10 minutes after treatment (see Figure 6).

Clinical study Amsterdam UMC with an indication of wound healing stimulation

At Amsterdam UMC, a clinical trial [8] with the Plasmacure cold plasma treatment has been conducted on 20 adult type 1 and 2 diabetes patients with hard-to-heal and non-healing diabetic foot ulcers. The ulcers were present on average 1090 days. The median was 140 days. The patients received 10 treatments in a two-week period or until their ulcer healed.

Results:
The number of Staphylococcus aureus bacteria decreased directly after treatment. That is important, as S. aureus is considered the most important pathogen in diabetic foot infection in temperate climates.

Wound size reduced significantly within the treatment period. The median wound surface decrease in 2 weeks was 55%. Two ulcers healed completely, and two ulcers increased in size. 55% of the chronic wounds appeared to have been converted from non-healing to healing wounds after 2 weeks treatment.

With this study, the first indication on the efficacy of the cold plasma treatment of Plasmacure has been obtained.

Example of a cold plasma application

PLASOMA offers a safe way to apply bactericidal cold plasma directly in the wound. It consists of a cold plasma pad and a cold plasma pulser. (See Figure 7, 8 and 9).

Figure 7: PLASOMA cold plasma treatment

Figure 8: Underneath the cold plasma pad on the ball of the foot cold plasma is formed. The pad is fixed on the wound and connected to the pulser that sends energy to the pad to create cold plasma.

Figure 9: PLASOMA cold plasma system

Conclusions

Cold plasmas contain biologically active components, mostly reactive oxygen and nitrogen species, which also play an essential role in natural bodily processes. Together with other cold plasma components such as electric fields these create an improved microenvironment by generating bactericidal conditions and they stimulate cell migration and proliferation and microcirculation in the plasma-treated areas. Therefore, cold plasmas are useful to support wound healing in hard-to-heal or non-healing wounds.

If you have any questions or would like more information about PLASOMA and cold plasma please contact us, we are happy to help.

Literature

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