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Reasons Why We Need Cold Atmospheric Plasmas in Bacteria-Related Diseases in Medicine

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ABSTRACT: Upcoming technologies for medical purposes must demonstrate their unique features and benefits compared with other methods in use. Cold atmospheric plasma technology has unique properties regarding antimicrobial purposes, owing to the numerous ways to generate and design the plasma. To date, no primary resistance against any tested microbes has been found. Furthermore, several tests and theoretical calculations show the unlikelihood of microbes acquiring resistance against cold atmospheric plasma. The latter fact is the main advantage of cold atmospheric plasma technology compared with conventional antimicrobial products (e.g., antibiotics) available in the medical sector, whose more or less uncontrolled usage has led to global resistance concerns and possibly to a lack of future treatment approaches.

KEY WORDS: plasma medicine, cold atmospheric plasma, nonthermal plasma, low-temperature plasma, plasma, chronic wounds, CAP, resistance, bacteria

I. INTRODUCTION

The World Health Organization considered the resistance of bacteria against antibiotics as one of the greatest threats to human health in 2009. This worrying statement raises the following question: "What happened to 'antibiotics'—one of the most precious resources in medicine?" The answers to this question are rather simple, but the solutions are one of the biggest challenges for scientists in the 21st century. When he won the Nobel Prize in medicine for the discovery of penicillin in 1945, Alexander Fleming warned that bacteria can become resistant against antibiotics. However, during the following so-called "golden ages" of antibiotics, the fear of resistant pathogens was not in proportion to the continuous discovery of new antibiotic substances. Decades of uncontrolled usage and a flood of antibiotic prescription, especially in nonbacteria-related diseases, led to a continuous increase in the emergence of highly resistant bacteria.[1–4] Other reasons for the development of resistance include prescription of the wrong type and insufficient doses of the antibiotic for the underlying bacterial infection, the use of a suboptimal antibiotic concentration, or an inappropriate short or too long duration of antibiotics.[3]

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This fatal trend of increasing resistance faces another inexorable trend—the lack of new antibiotic drugs.[5,6] The golden ages of antibiotics are now over and only a few pharmaceutical companies are currently attempting to develop new antibiotic substances. The reasons are again obvious: Approval for drugs for medical use is risky and costly, development of resistance to the upcoming drug is inevitable, the time frame of successful administration in patients is thus getting shorter and shorter, and side effects are unavoidable.

In 2011, World Health Day was dedicated to "combating antibiotic resistance" due to the aforementioned reasons. It is clear that we need new approaches to target these problems so that we do not end up in an era of incurable bacterial diseases.[7] Cold atmospheric plasma (CAP) is an upcoming technology that could solve at least some of the aforementioned problems, owing to its broad antibacterial properties, the probable lack of resistance against it, and the potential to preserve the functionality of the surrounding human cells.

CAP technology, however, is rather novel and the medical and hygiene communities must be convinced that CAPs are safe, controllable, and efficient. This review discusses recent problems with bacterial infections, which frequently occur in the medical community, and provides reasons where and why the usage of CAPs could be advantageous compared with other treatment approaches in use. We mainly focus on chronic wounds and not on acute wounds, which are also under current investigation, because chronic wounds are strongly involved in current bacteria-related medical issues and are the best-investigated diseases in CAP science at present.

II. WHEN SHOULD A WOUND RECEIVE AN ANTIBACTERIAL TREATMENT?

There are some inconsistencies in the medical community regarding at which point an antibacterial treatment should or should not be initiated in a chronic wound. The next obvious question concerns the appropriate way to treat a patient under certain circumstances. The bacterial load present in a wound can be of four distinct categories: contamination, colonization, critical colonization, and infection.[8] *Contamination* describes a state in which a low number of nonreplicating bacteria are present that do not damage the wound site. In other words, almost all types of wounds are contaminated (except artificial wounds formed during most surgeries under fairly sterile conditions). If the bacteria colonizing a wound start to replicate and grow in a larger amount, the state refers to *colonization*. Colonization is often associated with several different bacterial species in one wound site. These wounds can still heal, because invasion and damage of the tissue does not take place. The state switches to a *critical colonization* if the colonizing bacteria start to penetrate into superficial wound tissue layers, thereby impairing wound healing. At this point, an antibacterial regime should be initiated to prevent deeper invasions, further overgrowth, and continuous damage of tissue that corresponds to an *infection*. To distinguish between a critical colonization and an infection, Sibbald *et al*. proposed the use of the clinical bedside mnemonics NERDS (nonhealing of the wound, presence of inflammatory exudates, friable or red granulation tissue, tissue debris, and smell) and

STONEES (increased wound size, increased local wound temperature, extension of the wound to bone [os], new wound breakdown, exudate/edema/ erythema, and smell).[9] The condition of NERDS and STONEES may merge and is not always clear. In the case of a critical colonization, topical antibacterial agents such as antiseptics and topical antibiotics are suggested.[9] However, the application of topical antibiotics is neither widely accepted nor recommended in most guidelines, because of the increased risk of creating resistance and hypersensitivity as well as inappropriate antibacterial concentrations, especially in the deeper layers of the target tissue. This is why the use of systemic agents is widely performed, especially in most countries in Europe, and is not only restricted to clear clinical signs of infections. It is clear that the patient's medical history, accompanying diseases, general state, and immune status are also taken into account by the treating physician. Generally, the notion that antibacterial agents decrease the bioburden (corresponding to a critical colonization) and simultaneously promote healing processes remains controversial.[10]

III. WHAT DO WE ACTUALLY TREAT IN DAILY PRACTICE?

Another big problem that we face in daily clinical practice is the partially limited knowledge of what we actually treat. The size of this problem strongly depends on which methods are used in the clinics and which bacterial detection methods are practiced in the investigating microbiology departments. First, we can only detect what our methods allow. In most clinics, common swab techniques (flocked swabs) are utilized for detection of the colonized bacterial species. Unfortunately, there are absolutely no correlations between the qualitative and quantitative results obtained from swabs and we cannot rely on the results for measuring changes in bacterial load.[11] Newer methods, such as metagenomic analysis, revealed not only a much higher number of bacterial species located on the wounds, but also a higher amount of anaerobes than expected.[12] Furthermore, we can of course only detect bacteria that are known and can be cultured, thus representing only approximately 1% of all bacteria.[13] As such, we normally detect bacteria that are well-known and easy-to-culture strains.[14,15] At this point, the detection of a bacterial species depends again on the method utilized, the culture media used, and the chosen duration of incubation time. The latter focuses predominantly on fast-growing aerobic bacteria because long-lasting investigations are costly and may delay urgent antibiotic regimes in some patients.[13–15] Nevertheless, are the detected bacterial species relevant or not?

When we compare the different bacterial phyla (Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes) on healthy normal skin with chronic wounds, we can detect the same phyla but the bacterial load may differ.[16,17] As mentioned above, the most widely used detection method (the swab technique) is unable to detect the real bacterial load on our wound site; therefore, we have to question the obtained results. Furthermore, if we trust the obtained results, we still have no information about the current biological state of the detected bacteria. However, this has a tremendous impact on the necessary treatment modality. Schierle *et al*. demonstrated that *Staphylococcus aureus* biofilms

impair wound healing in a murine cutaneous wound model. No impairment was detected for *S. aureus* present in a planktonic state.[18] The existence of biofilms has changed the knowledge of how bacteria protect each other against external influences, as well as how a biofilm can facilitate survival and promote resistance.[13,19,20] Special focus is given to biofilms in promoting resistance. This is not only achieved by a physical barrier, but is also due to fast interbacterial communication systems (quorum sensing). Biofilms have a much larger mutant selection window.[5,19–21] As a result, bacteria in the biofilm state require a completely different treatment approach compared with their planktonic state. Depending on the biofilm location on the wound site, up to 1000 times higher concentrations of antibacterial agents are necessary to achieve the same inactivation rates as for bacteria in their planktonic state.[21]

IV. BENEFITS OF CAPS

Since the beginning of the 21st century, CAPs have gained interest in the physical, biological, and medical communities. Within the last few years, an intense effort has demonstrated the strong-targeted different antimicrobial properties of CAPs, without damaging the surrounding human cells when applied *in vivo*. These pathogenic properties are not only limited to bacteria, biofilms, and spores; CAPs also show high inactivation efficacies for fungi, viruses, and prions.[22–26]

This review focuses solely on the bactericidal properties of CAPs. CAPs have previously demonstrated that they have a very broad antibacterial potential in multiple *in vitro*, *ex vivo*, and *in vivo* animal models, which is not restricted to several species.[27–31] These experiments were carried out on agar surfaces, on porcine skin, or in liquids and demonstrated that plasma does not differentiate between several strains of the same bacterial species or their respective resistance level if treatment time/dosage is adapted.[28,32] Even extremely robust bacteria, such as *Deinococcus radiodurans*, were successfully inactivated.[33] Further important investigations demonstrated that bacteria are easily killed not only in their planktonic state, but also in biofilms.[32,34,35] However, the application time in most of the cases was much longer than for planktonic bacteria. The same applies for bacterial endospores, which are not only of medical interest but are also important for preventive aims in hygiene.[28,30,36] Depending on the desired application, CAPs can be "designed" individually, meaning that depending on the plasma production technology, the plasma parameters used, the carrier gas used, the surrounding conditions, and so forth, different plasma compositions and thus components and concentrations of these components (electrons, ions, reactive species, neutrals, ultraviolet [UV] light, etc.) are produced.[24] For hygiene purposes in which plasma is not directly applied to humans, the setting (i.e., the plasma components and their concentrations) can differ from that used for chronic wounds in which cells relevant for wound healing should be stimulated as well.[37–41]

The first clinical trials realized in patients with chronic infected wounds confirmed the findings of *in vitro* studies that the fast-growing aerobic bacteria can be easily reduced with CAPs as well. Both a 5-min and a 2-min daily single application dem-

onstrated a significantly higher reduction of the bacterial load in those wounds that received a plasma (MicroPlaSter alpha and beta) treatment as an add-on therapy in addition to standard wound care, compared with the wounds that only received standard treatment.[42,43] These studies used the reduction rate of the bacterial load as the primary end points. Unfortunately, the treatment period was too short to make conclusions about faster wound healing itself. Nevertheless, data examining the wound healing purposes of the MicroPlaSter beta are currently under investigation (unpublished data). A case report in a patient suffering from a rare disease called Hailey-Hailey disease revealed that an additional administration of a CAP treatment resulted in a quick clinical response in therapy-resistant lesions, which were secondarily infected with *Proteus mirabilis* and the yeast *Candida albicans*.[44] Another study in healthy volunteers further demonstrated that CAPs are also suitable for decolonization processes on untreated fingertips and artificially contaminated ones.[45] All of these publications underline the benefits of CAPs in inactivating bacteria *in vitro* and *in vivo* (see Fig. 1).

This antibacterial diversity of CAPs is unique among other antibacterial agents in use and is important when we consider the problems in medical care in terms of treating bacteria-related diseases. If we treat a disease that is caused by bacteria, is worsened by an infection, or simply delays the healing process due to bacteria, it is important that we have a successful treatment modality with a broad antibacterial spectrum that is not

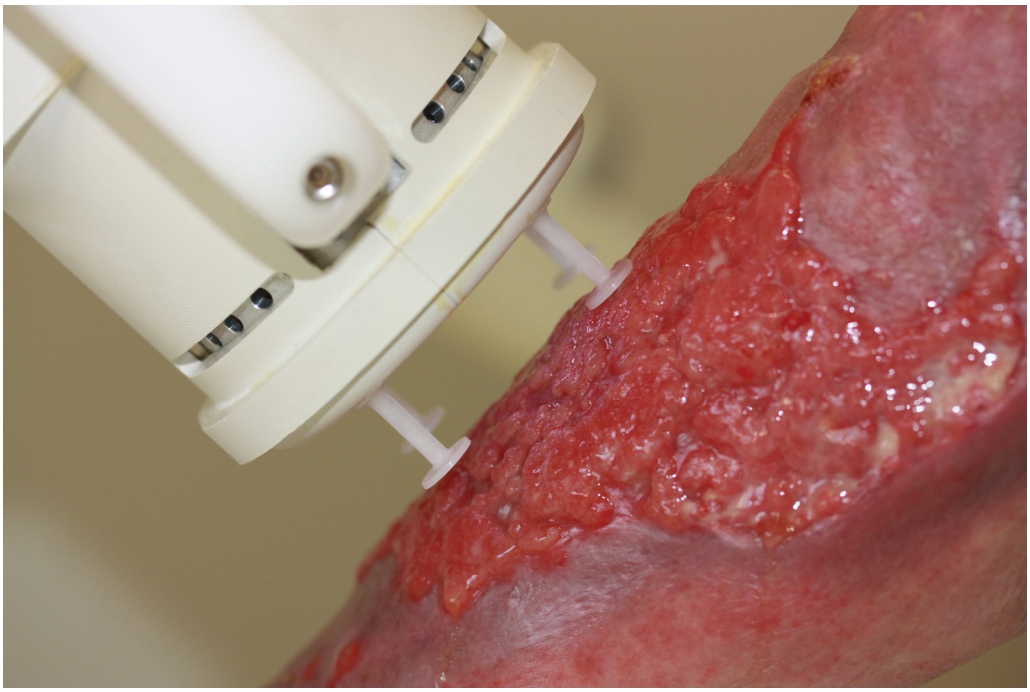

FIG. 1: MicroPlaSter beta in clinical use in a patient suffering from necrotizing cellulitis.

negatively affected by inconsistencies in the detection method (*e.g.*, bacterial swabs). CAP is one such method.

One problem occurs when data from different research groups using different plasma devices (i.e., different compositions and concentrations of plasma products) are summarized. The CAP devices and therefore their killing efficacies cannot be compared that easily with each other. On the other hand, this can also be seen as a great advantage, because the large variability of the CAP devices and their different chemical designs make it harder for bacteria to adapt to plasma than to antibiotic approaches. We next provide a brief summary on the current understanding of the antibacterial mechanisms occurring during plasma application.

If cold plasma under atmospheric pressure is produced in air, >600 chemical reactions are initiated that take place during and after plasma generation. Because air is manly composed of oxygen, nitrogen, and water vapor, reactive oxygen and nitrogen species (RONS) are produced in addition to ions and electrons, excited species, electric field, and UV radiation.[22–24,37,39,46–48] Each of these physical and chemical parameters contributes to the strong bactericidal effects. Furthermore, one may assume that in a first step, permeabilization of the bacterial cell membrane occurs due to electromechanical and chemical effects. Recombination of electrons and ions, the same as excited atoms, could make the bacterial cell wall permeable (up to 5 nm) through heating (+30°C) and local energy deposition. Leduc *et al*. previously showed that CAP can create pores of up to 4.8 and 6.5 nm in radius in the cell membrane of HeLa cells.[49] Another way to generate pores is lipid peroxidation by abstraction of H atoms from a methylene group through hydroxyl radicals. Further cell charging and disfigurement via Coulomb forces could create cell wall ruptures (E ≥600 V/m) and shear stress. Furthermore, the cell wall can be opened by simple electroporation if the electric field exceeds approximately 50 kV/cm.[23,24,37,49–54] When the prokaryotic cell becomes permeable for RONS generated by the plasma, further chemical reactions are initiated inside the cytoplasm. The missing enzyme apparatus in bacteria, which human cells developed during evolution, is not able to inactivate the toxic species and cannot protect the bacteria from the chemical attack. The toxic products generated in chemical cascades can then harm the bacterial RNA or DNA that is not protected by a nucleus and its repair systems, which is in contrast with eukaryotic cells.[23,37,49,55] These facts give us a certain therapeutic window in which we can successfully inactivate bacteria without harming surrounding cell lines.

Many RONS derived from plasma production under ambient air conditions and carrier gases used are the same as those that our bodies learned to utilize for signaling processes within the cell and for the fight against pathogens. These comprise hydroxyl radicals, hydrogen peroxide, hypochloride anions, superoxide anions, nitric oxide, nitrogen dioxide, and peroxynitrite.[48,56] However, in plasma, these RONS are provided during application for a certain time frame in possibly much higher concentrations than our bodies could generate.

The aforementioned mechanisms underline why a primary or an acquired (secondary) resistance toward the broad physical and chemical attack of a CAP application

is much less feasible than against antibiotics, which in general have only one target mechanism. In an investigation with several generations of bacteria, Zimmermann *et al*. demonstrated that the risks against CAP application are $<10^{-10}$ for primary resistance and $<10^{-30}$ for acquired resistance.[57] This fact is very important in the never-ending battle against resistance in pathogens. Because resistance does not seem to be relevant, the initiation of a CAP application can be chosen very early by protective means or as a direct treatment approach in the case of an advanced stage of bacterial colonization. Therefore, the use of CAPs can be applied very flexibly, assuming a safe application of the device, which is discussed in other publications.[23,42,58–60]

Antibiotic resistance is not the only source of problems in hospital care while treating patients with antibiotics. Serious allergic reactions are fortunately relatively rare; however, mild allergic reactions, such as type IV hypersensitivity, are quite frequent and force the treating physician to switch treatment to another antibiotic group. The reason for that is the so-called cross-reactivity, which averts the treating physician to use many other antibiotics sharing similar chemical structures.[61] Topical antibiotics have even a higher risk for type IV hypersensitivities; antiseptics that are mostly alcohol based can lead to skin desiccations such as skin irritations and intolerances.[62–64] Most clinical experience based on CAPs was obtained with the argon gas–driven MicroPlaSter devices, in which no side effects occurred during the painless application in >300 patients (with >3000 plasma treatments).[58]

A further great advantage of CAPs is the possibility to have a contact-free and self-sterilizing technology that penetrates as an ionized gas down to the micrometer scale (with molecular delivery) and even down to much smaller areas than liquids can reach. Lademann *et al*. demonstrated that plasma could even penetrate along hair down into its follicle, where other treatment approaches have failed thus far.[65]

Very economical devices can be designed, with low device and production costs, low running and maintenance costs, as well as a waste-free application in which only electricity is required. This is an important factor for all contemporary medical devices and treatment approaches.

V. POSSIBLE DRAWBACKS OF CAPS

One limitation of CAPs thus far is their restriction to superficial applications. It is not yet known how deep the plasma can actually penetrate into the tissue. This will strongly depend on the patient's constitution, the tissue type, and of course the plasma source used. Paracrine effects must also be considered.

The first prototypes for the internal use of CAPs (e.g., for endoscopic usage) are currently available, owing to increasing knowledge, changes in design, and the resultant miniaturization processes.[66,67] However, as a result of their brief history, the potential use of CAPs for systemic applications is currently unknown. Therefore, the application can be used either for preventative purposes or as an add-on therapy to a systemic use of an antibiotic.

CAP is a very complex technology and has the same problems that other upcoming technologies have: There is not yet enough knowledge and long-term experience con-

cerning possible negative side effects and safety in the usage of CAPs. In addition, there are few available international guidelines and recommendations regarding safety limits for toxic products/byproducts of plasmas.[58] For these reasons, the plasma community has a great responsibility to precisely explore the safety features of each plasma device for its respective application.

VI. CONCLUSIONS

CAPs have unique features and benefits that are necessary for an upcoming technology to justify its relevant role in medicine. Few technologies have such great variability and adjustability that allow a broad and efficient antibacterial potentiality in addition to nonharming effects on surrounding tissue. Their less probable resistance makes CAPs very interesting tools for the treatment of superficial bacterial infections in overcoming bacterial resistance. The first clinical trials in patients with chronic infected wounds approved these features *in vivo*. Furthermore, it is important to note that no side effects or allergic reactions have been reported to date. However, this is only the beginning. Ongoing and future studies and clinical trials in patients must confirm and strengthen the role of cold plasmas to convince the medical and hygiene communities of their benefits. Only the future will show whether CAPs will successfully exploit their potentiality and if they will manage the steps toward an established method for medical use.

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