SPECIAL REPORT

Improving the Treatment of Acute and Chronic Wounds and the Role of Plasma

The Pathophysiology and Socio-Economic Burden of Chronic Wounds

Traditional Antimicrobial Treatments in Wound Care

Limitations of Wound Disinfection Methods

Plasma Technology: A New Tool in Wound Care

Emerging Trends and Future Outlook for Wound Management

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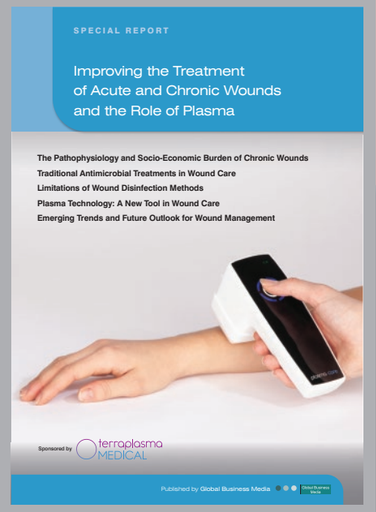

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Foreword

Wound healing is a complex, highly regulated process that is critical in maintaining the structure and function of the skin. The process of wound healing occurs along a continuum, involving numerous molecular pathways that act in concert to repair damaged tissue. Dysfunction at any stage of the healing process can result in delayed or abnormal healing. In these instances, a chronic wound may result, and the tissue is unable to re-establish its structure. Chronic wounds cause significant morbidity including patient discomfort and distress. They also impose a significant economic burden on healthcare systems, as they consume valuable resources in their effective management.

Abnormal wound healing is rarely observed in healthy patients. They most frequently occur as the result of an underlying pathology from a chronic condition. Common comorbidities include diabetes, cardiovascular disease, cancer and malnutrition. Wound infection is a challenge frequently faced in wound care. It is commonly observed in cases of diabetic or venous stasis ulcers. Infection may equally be the underlying cause for the failure of a wound to heal.

Successful wound care involves optimising the environment of a wound to provide a pathogen-free and protective area to promote healing. Numerous treatment options are available to modify both the local and systemic conditions affecting a wound site. While many clinicians still use traditional treatments such as antimicrobial agents, wound dressings and oxygen therapies, there continues to be a constant flow of new technologies available for wound care. This report highlights some of these advances, particularly in plasma treatment devices, in the context of emerging trends in healthcare and wound infection management.

The Report includes an article from terraplasma medical, a leading innovator in cold atmospheric plasma technology, which describes the benefits of plasma-based treatment in wound disinfection and healing.

Dr Sophie Laurenson
Editor

Dr. Sophie Laurenson *is a scientist and social entrepreneur. She obtained a Ph.D. in Oncology (Biophysics / Biochemistry) from the University of Cambridge in 2007 and has worked in industry and academia for 17 years. Currently, she is the Founder and Managing Director of Limeburners Bay International AG, developing medical technology for resource-limited settings.*

IMPROVING THE TREATMENT OF ACUTE AND CHRONIC WOUNDS AND THE ROLE OF PLASMA

The Pathophysiology and Socio-Economic Burden of Chronic Wounds

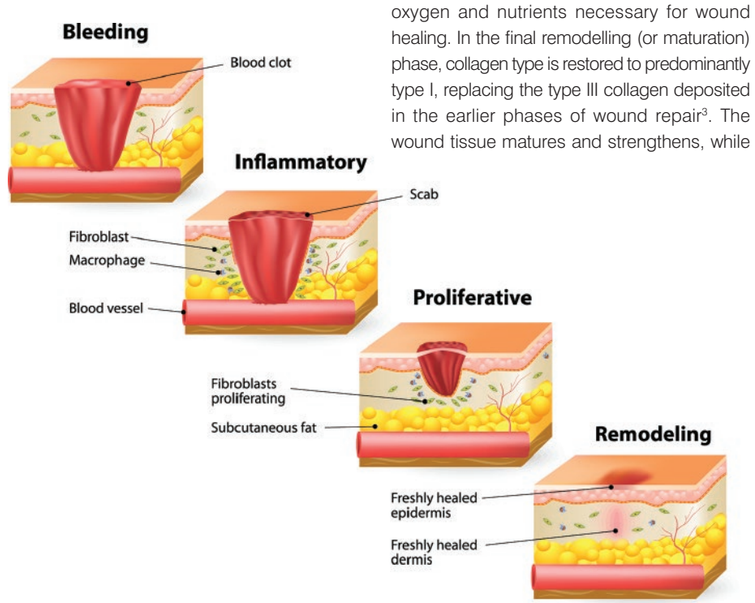
Sophie Laurenson, B.Sc.(Hons), Ph.D. (Cantab)

THE SKIN is the largest organ in the human body and functions as a barrier, protecting the internal organs from the external environment. A superficial skin wound (or cutaneous wound) causes a rupture in this barrier, exposing the body's internal structures to the environment. The process of cutaneous wound healing is complex and highly regulated. Numerous molecular pathways work in coordination to repair the skin's barrier function[1]. Although wound tissue never regains its original strength, the majority of superficial wounds in healthy patients will heal normally. However, when wound healing follows an abnormal process, a chronic wound may result. Chronic wounds can be difficult to manage and represent a significant burden to both patients and healthcare systems.

The Wound Healing Process

The wound healing process can be divided into phases, each encompassing specific processes and pathways: haemostasis, inflammatory, proliferative, migration and remodelling or maturation (Figure 1). The entire wound healing process requires numerous inflammatory cells, growth factors, matrix molecules and nutrients to be delivered to the wound site. Successful execution of each phase is essential to achieving complete wound closure and restoring the normal structure of the skin. Deviations in any phase may be associated with irregular or delayed wound healing.

The inflammatory phase of wound healing immediately follows the establishment of haemostasis. Pathogens and foreign material are cleared from the wound site by immune cells including neutrophils and monocytes / macrophages[2]. These cells phagocytose cellular debris and secrete growth factors and cytokines that promote cell migration and proliferation. During the proliferative phase, fibroblasts promote the deposition of collagen and ground substance onto which new tissue will form. This phase is accompanied by the proliferation of endothelial cells, forming the vasculature that supplies oxygen and nutrients necessary for wound healing. In the final remodelling (or maturation) phase, collagen type is restored to predominantly type I, replacing the type III collagen deposited in the earlier phases of wound repair[3]. The wound tissue matures and strengthens, while



The process of cutaneous wound healing is complex and highly regulated. Numerous molecular pathways work in coordination to repair the skin's barrier function

the vascular network regresses to restore a near-normal skin structure[4].

The wound healing process has a high metabolic demand. In order to support the active proliferation of cells, wound healing requires oxygen and nutrients. Both neutrophils and fibroblasts require oxygen to function efficiently and reductions in oxygen tension are correlated with poor outcomes[5]. Effective collagen deposition is associated with subcutaneous oxygen tension and tissue perfusion levels[6]. Reduced oxygen tension is also associated with higher rates of wound infection[7]. Nutrients are also vital to efficient wound healing, especially protein[8], glucose, vitamins A and C and zinc[9]. The regulation of oxygen and nutrient supply is dependent upon arterial partial pressure of oxygen, which is maintained by vascular processes including cardiac output, vasodilation and capillary function. Vasoconstriction, resulting from hypovolemia, pain or anxiety, environmental factors or chemical agents (particularly α1 agonists, β antagonists or nicotine)[10].

The Socio-Economic Burden of Chronic Wounds

Aberrant wound healing is rarely observed in normal healthy patients. Chronic wounds are those which do not heal within a reasonably normal timeframe (approximately 12-weeks) or have a high rate of recurrence. They are most associated with underlying chronic conditions, such as diabetes, cardiovascular disease, cancer or malnutrition. Chronic wounds result from one or more deviations in the normal physiologic wound healing process, causing it to stall. The underlying mechanism varies, but may include insufficient blood supply, immunosuppression, metabolic diseases, medications or pre-existing local tissue injury. External factors, including moisture, temperature and sustained pressure, also affect the wound healing process.

Chronic diseases are the leading cause of mortality and morbidity worldwide, contributing to poor quality of life and rising healthcare costs. The pathophysiological mechanisms that underpin many common chronic diseases affect the wound healing process. The increasing prevalence of diabetes is one of the leading causes of chronic wounds globally. An estimated 60 million people in Europe are affected by diabetes[11]. Diabetic foot ulcers affect 15% of diabetic patients and are the underlying cause of 84% of lower limb amputations in diabetic patients[12]. Hyperglycaemia modifies protein molecules, altering the permeability of the basement membrane and vasculature[13]. These processes alter the delivery of oxygen and nutrient to wound tissue, inhibiting the healing process. Recent evidence suggests that glycaemic control can positively impact rate of wound healing in diabetic patients[14]. Cardiovascular diseases affecting the arterial and venous systems also contribute to the development of many chronic wounds by restricting blood supply to peripheral limbs.

Chronic wounds can be difficult to manage, especially when the underlying aetiology cannot be addressed. In most instances, chronic wounds are managed at the community level, imposing a significant burden on healthcare resources. An estimated that 4% of total healthcare costs are due to wound care[15]. This percentage is increasing, especially due to chronic wounds resulting from diabetic, venous and pressure ulcers[16]. A substantial number of inpatient hospitalisations result from poor management of chronic wounds, often requiring extended stays. Wound complications are associated with high readmission rates, longer and more intensive treatment and specialist or surgical interventions.

Infections in Chronic Wounds

The most common challenge to wound healing is infection with one or more pathogens. Although the presence of bacteria in wound tissue is normal, excessive bacterial proliferation can impact any phase of the wound healing process. Bacteria may influence both platelets and components of the complement system, preventing the establishment of haemostasis and promoting thrombocytopenia[17]. Pathogen presence also stimulates inflammation and alters the function of key immune cell regulators. These effects are observed when levels of bacteria exceed 105 colony-forming units per gram of tissue, for most species[18]. However, a quantitative inoculum threshold for diagnosis of an infected wound is still widely debated. Furthermore, the presence of specific bacteria such as β-haemolytic streptococci, Staphylococcus aureus and Pseudomonas species, may indicate clinically relevant infection at any inoculum level[19]. The accurate clinical diagnosis of wound infection is important. It is necessary to distinguish between an incidental positive culture and a genuine pathogenic infection[20]. Studies of diabetic foot ulcers have shown that a wound may have more than one pathogenic microorganism present and the presence of more than four types was associated with poor outcomes[19]. Accurate identification enables targeted antimicrobial therapy, improving outcomes and reducing costs[21].

Non-healing chronic wounds impact on patient morbidity and quality of life and increase mortality risk. The true extent of this cost is not widely recognised due to the lack of evidence available at the local level. Consequently, the burden of chronic wounds and the importance of effective wound care may be underestimated.

IMPROVING THE TREATMENT OF ACUTE AND CHRONIC WOUNDS AND THE ROLE OF PLASMA

Traditional Antimicrobial Treatments in Wound Care

Sophie Laurenson, B.Sc.(Hons), Ph.D. (Cantab)

Limitations of antimicrobial treatments include patient discomfort, potential resistance and toxicity

Introduction

Wound care has become increasingly important, given the increasing prevalence of chronic wounds and their associated morbidity. One of the greatest challenges in effective wound care is the prevention and treatment of complications arising from wound infections. There are many preventative and curative procedures available for wound infection management[22]. One of the most established methods is the application of antimicrobial agents to the wound tissue. Options include metallic compounds, iodine-containing compounds and more modern antibiotics applied topically or systemically. However, there are limitations associated with the use of each treatment. Some treatments may be difficult to adhere to, increasing the potential for antimicrobial resistance to develop. Furthermore, the efficacy of some antimicrobial treatments has been questioned in recent years.

Silver Compounds

Silver has been used as an antimicrobial agent in wound care for millennia and remains an ingredient in many wound care treatments today[23]. This is due to the numerous chemical compounds that silver may form, as well as its broad spectrum of antimicrobial activity and limited toxicity. There are also low levels of antimicrobial resistance towards silver-based treatments, making them a popular option for treating resistant pathogens. However, the non-specific nature of silver can be a drawback, as therapy cannot be targeted towards specific pathogenic species. Furthermore, recent evidence has revealed that the popular silver treatment, silver sulfadiazine, has little effect on wound healing outcomes[24]. The relatively short effective half-life of silver compounds is an additional important limitation. Recent advances have included developing specialised wound dressings containing sliver compounds. Nanocrystalline silver dressings have been developed to facilitate sustained release of silver nitrate, maintaining efficacy over longer treatment periods[25].

Iodine-Containing Compounds

Iodine-containing compounds also have a long history of use in wound care. Cadexomer iodine, an iodine compound embedded in starch, has

ANTIBIOTIC CRÈME BEING APPLIED TO A WOUND

One of the greatest challenges in effective wound care is the prevention and treatment of complications arising from wound infections

been promoted as a cost-effective adjuvant for wound healing[26]. However, there are concerns over the toxicity of iodine-containing compounds, especially when used on large areas of tissue. Iodine treatment is also contraindicated in patients suffering with thyroid disorders.

Topical Antibiotics

Numerous topical formulations of antibiotics have been developed for wound healing applications. Early studies of minor injuries in children showed reduced rates of infection resulting from administration of topical antibiotic ointments[27]. These remain popular treatment strategies despite emerging evidence that routine administration provides little benefit to most patients. To maintain moisture within the wound tissue, topic ointments must be applied frequently. Current evidence suggests that they are most effective in clinically-infected wounds exhibiting pain, tenderness, warmth, purulent drainage, erythema, or induration[28]. Furthermore, application of topical ointments often causes patient discomfort and may result in contact dermatitis[29]. Widespread use of antimicrobial ointments may also promote antimicrobial resistance, an issue of increasing global concern. Overall, it is recommended that the use of topical antibiotics should be reserved for clinically infected wounds and specific dermatologic conditions, rather than for general prophylaxis. The administration of systemic antibiotics is also a treatment option, although the evidence supporting this approach for management of venous leg ulcers is limited.

Antimicrobial Dressings

The healing of a superficial wound requires many factors to work in coordination. Wound dressings have evolved considerably to address possible barriers to wound healing, ranging from infection to hypoxia. Wound dressings can help modify the wound environment and optimise healing conditions. This includes providing the appropriate levels of moisture and pressure, as well as preventing microbial access to site. This helps to reduce the likelihood of colonization and subsequent infection. Several recent developments in wound dressings have integrated antimicrobial compounds into the dressing material[29]. These products combine traditional materials such as foams and hydrogels with antimicrobial compounds. Examples include products incorporating silver, betaine, chitin, or polyhexamethylene biguanide.

Many pathogens have evolved sophisticated mechanisms to evade destruction by antibiotics and other antimicrobial agents. The formation of biofilms is particularly problematic in wound care as they create a physical barrier to wound healing and prolong the inflammatory phase. in vitro studies and patient data have demonstrated that antimicrobial wound dressings can have good outcomes for chronic pressure or venous ulcers that may be colonised by biofilms[30].

The development of these products has been in response to some of the limitations of topical antibiotic ointments. In particular, topical antimicrobial ointments must be repeatedly applied to the wound tissue to maintain an effective concentration and moisture. Incorporating antimicrobial agents into the wound dressing helps to address these limitations. However, these materials may not be appropriate for broad-spectrum application to healing wounds. The issues related to antimicrobial resistance and prophylactic efficacy apply equally to advanced wound dressings.

Conclusion

Antimicrobial and antiseptic treatments have a long history of use in wound management. The remain a common approach, although several limitations are now recognised. At present, the evidence supporting the use of antimicrobial agents in treating chronic wounds is mixed. This is partly due to the lack of quality research and mixed methodology used in studies, making the different treatment options difficult to compare. There is no evidence to support the routine use of systemic antibiotics to promote healing in venous leg ulcers, although there is some evidence to support the use of cadexomer iodine. Further research is needed to draw definitive conclusions on the effectiveness of systemic antibiotics and topical preparations in healing chronic wounds such as venous leg ulceration[31]. Given the increasing problem of bacterial resistance to antibiotics, current guidelines recommend that antibacterial treatments should only be used in cases of confirmed clinical infection.

Topical antimicrobial ointments must be repeatedly applied to the wound tissue to maintain an effective concentration and moisture

Limitations of Wound Disinfection Methods

Sophie Laurenson, B.Sc.(Hons), Ph.D. (Cantab)

Existing wound infection treatments including oxygen, light and plasma-based therapies have limitations for use in outpatient settings

Introduction

Wound care is a multidisciplinary activity. There is a plethora of treatment options available, depending on the aetiology and characteristics of the chronic wound. In addition to traditional antimicrobial treatments, wound infection can be prevented and treated using a range of oxygen, light and plasma-based therapies. These platforms leverage different modalities to target pathogenic cells for elimination. A key requirement is to ensure specificity towards the pathogenic species and to limit the risk to host tissues. Each of the methods described below has benefits and potential risks or limitations. Balancing these factors is important in ensuring efficacious and safe wound care strategies.

Oxygen Therapies

Oxygen-based therapies have been used for wound care management for several decades. It is a useful treatment modality for acute, chronic or surgical wounds. Oxygen is required by numerous molecular pathways in the wound healing process[32]. It is required for increasing energy production to fuel cell proliferation, collagen synthesis, reepithelialisation and pathogen defence mechanisms. Oxygen and its derivative, hydrogen peroxide (H_2O_2), both stimulate the production of reactive oxygen species (ROS) such as superoxide[33]. ROS are toxic to bacterial pathogens and stimulate angiogenesis in wound tissue[34]. Reduced oxygen levels are characteristic of chronic wounds. Insufficient oxygenation of wound tissue is associated with delayed wound healing and poor outcomes for a variety of chronic wound conditions.

Several different supplemental oxygen therapies have been developed to aid wound healing. Oxygen dressings and topical oxygen therapy (TWO_2) apply localised delivery of oxygen directly to the wound by external administration. In TWO_2, a portable oxygen delivery instrument applies a continuous flow of transdermal non-pressured oxygen directly to the wound site.

Other therapies leverage systemic oxygen administration through supplemental oxygen inspiration during and after surgery. Hyperbaric oxygen therapy (HBOT) is an alternative system to administer systemic supplemental oxygen.

Cold atmospheric plasma (CAP) is a novel mechanism of eliminating pathogenic microorganisms and has potential applications in chronic wound management

Patients inhale 100% oxygen at a pressure greater than one atmosphere (ATM) within a confined environment. These interventions deliver a high percentage of oxygen to the wound site via the systemic circulatory system. However, they are only effective when there is sufficient blood supply to the wound site. Many chronic wounds result from vascular disorders in which there is reduced or aberrant blood supply to the affected tissues.

A recent systematic review recently compared the efficacy of a range of oxygen therapies for outcomes in wound healing[35]. Supplemental inspired oxygen therapy was found to be more likely to have a positive outcome during specific types of surgical interventions. This is contrast to its widespread application as a mechanism for reducing surgical site infections during routine surgical procedures. However, the lack of clinical data made it difficult to compare within and between the different oxygen-based therapies. Further randomized clinical studies are required, especially on patient-friendly oxygen dressings and topical wound oxygen therapies.

Light-Based Therapies

Light-based technology describes a collection of light-emitting devices with potential applications in wound care. Low-level laser therapy and photodynamic therapy both have wide applications in wound care.

Light wavelengths in the ultraviolet (UV) part of the spectrum may also be used in would management[36]. UVC (wavelengths between 200–280 nm) has strong antimicrobial properties. It can be directly applied to kill pathogens in infected wounds without extensive damage to human tissue[37]. This is a common treatment in clinically infected acute wounds. UVB (wavelengths between 280–315 nm) has been found to stimulate wound healing and has been used to stimulate the immune system via extracorporeal exposure of blood. UVA (wavelengths between 315–400 nm) is known to affect cell signaling pathways but is not yet used in wound care.

One of the limitations of UV therapy, is the inherent low penetration of UV light into tissue. To extend penetration depths, optical technologies may be employed to manipulate the parameters of the UV light. New UV light sources, including lasers, light-emitting diodes (LEDs), and microwave-generated UV plasma are becoming accessible for biomedical applications[36].

Both UVC and UVB are carcinogenic and have the potential to damage host cell DNA. The potential benefits of pathogen elimination by UV administration must be carefully balanced against this risk. The carcinogenic effects of chronic exposure to UV should be considered when planning treatment regimes. Further studies investigating the cellular and molecular consequences of prolonged UV exposure, will aid in designing management strategies to optimize would healing. The effectiveness of UV to influence biological changes is dependent on the chosen irradiation parameters. It is important to select the optimum wavelength and exposure levels that will produce the desired benefits at the lowest irradiation level.

Cold Atmospheric Plasma (CAP) Technology

Cold atmospheric plasma (CAP) is a novel mechanism of eliminating pathogenic microorganisms and has potential applications in chronic wound management[38]. CAPs are partially ionised gases, which interact with the surrounding air and function at low temperatures. They are comprised of a reactive mixture of electrons, ions, excited atoms and molecules, reactive oxygen and nitrogen species. CAPs have been shown to inactivate bacteria and fungi on several substrates and in biofilms, as well as spores and viruses[39,40]. To date, no adverse reactions have been observed in mammalian tissues and no antimicrobial resistance to CAP treatments have been reported.

The utility of CAP therapies in clinical practice are currently being investigated and five CAP devices have recently received regulatory approval in Europe. These systems are predominantly stand-alone, stationary instruments that are designed to be used in a clinical setting. They are relatively bulky and require a reasonable skill level to operate. These features restrict their use in decentralized or home-based care settings.

Conclusion

The development of oxygen, light and plasma-based therapies has increased the treatment options available for non-invasive wound disinfection. However, each of these technologies has inherent risks. Furthermore, their development has centred around the use of these platforms in clinical settings. This restricts their use in decentralised or home-based care settings.

Plasma Technology: A New Tool in Wound Care

terraplasma medical GmbH

Cold atmospheric plasma (CAP) technology is a novel, effective and safe treatment option for chronic wounds.

Introduction

Wound infection has been a major threat to human health throughout history. This changed drastically following the stepwise development of hospital hygiene and the discovery of antibiotics in the 19th and 20th centuries. However, the rise of multi-drug resistant organisms (MDROs) in recent years has revived the threat. Thus, infection continues to be a serious complication in the management of acute and chronic wounds and has a serious impact on health and quality-of-life for patients. Moreover, wound infection also poses a significant economic challenge for healthcare providers.

A novel approach to address this issue is the therapeutic use of plasma technology to inactivate wound pathogens, including MDROs. This technology also supports wound healing in patients, whose cellular survival mechanisms are stimulated by the plasma species.

Cold Atmospheric Plasma

In physics, plasma is the term for an ionized gas and is termed the 4th aggregate state. Gases and gas mixtures are converted into plasma by the application of energy. Cold atmospheric plasma (CAP) is a specific type of plasma. It refers to a gas that is turned into a partially ionized gas at room temperature and atmospheric pressure, so that only one in 10^9 particles is ionized. CAPs generate free electrons, atoms, ions and reactive species from the gas components. They also release radiation in the form of UV- and visible light, heat and an electromagnetic field (Figure 4).

CAP devices that are developed for biomedical applications must fulfil a list of requirements to ensure safety and regulatory compliance. To avoid burning or protein denaturation, the produced plasma must not exceed temperatures of 40°C. Furthermore, the UV emission must fall below threshold limits set by the International

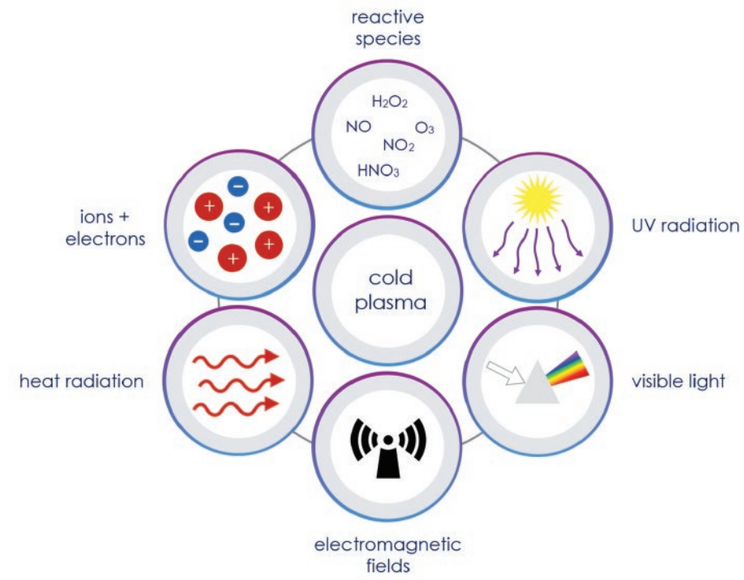
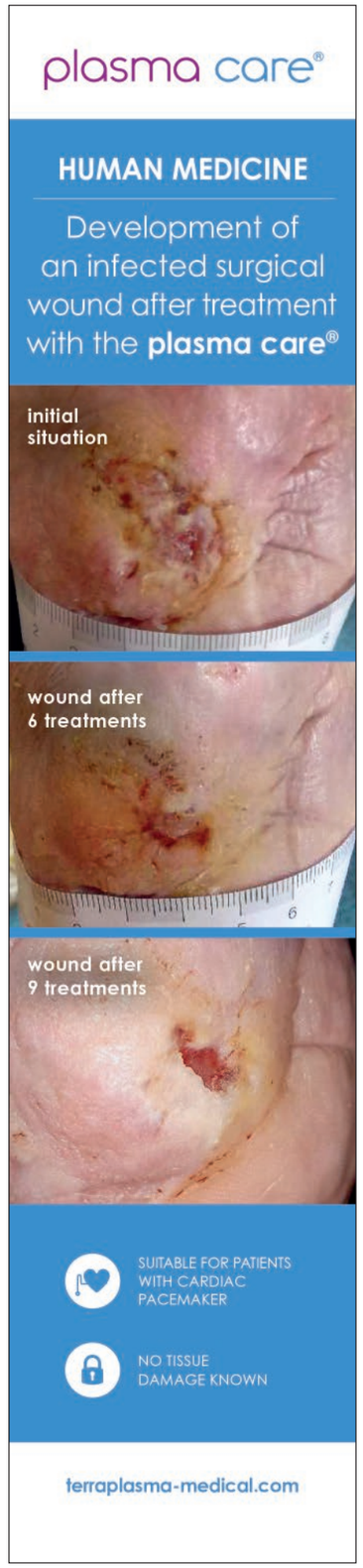


FIGURE 4: THE PLASMA COCKTAIL IS A COMPLEX MIXTURE OF PHYSICALLY AND CHEMICALLY ACTIVE COMPONENTS.



Infection continues to be a serious complication in the management of acute and chronic wounds and has a serious impact on health and quality-of-life for patients

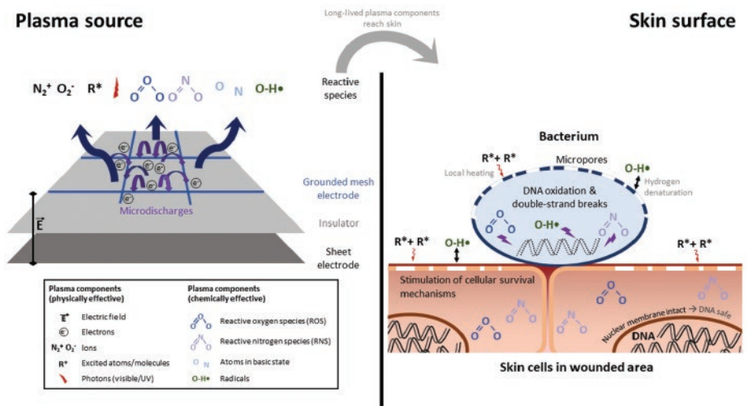


FIGURE 5: THE MECHANISM OF ACTION OF AN INDIRECT SURFACE MICRO-DISCHARGE PLASMA SOURCE [BASED ON SHIMIZU *ET AL.* 2017, LEDUC ET AL. 2009 AND DB GRAVES 2014].

Commission on Non-Ionizing Radiation Protection (ICNIRP). Finally, the plasma device must produce therapeutic components including reactive oxygen (ROS) and nitrogen species (RNS) such as superoxide, hydroxyl radicals and ozone, similar to those produced by the human immune system. Existing threshold limits for these species are specified by the World Health Organization (WHO), Occupational Safety and Health Administration (OSHA) or National Institute for Occupational Safety and Health (NIOSH) and must not be exceeded.

Mechanism of Action

CAP functions by transiently generating nanoscale pores (diameter <5 nm) in the cell membrane. This effect is produced by the plasma components[41]. The highly reactive plasma species enter the cells via these pores and react with intracellular structures such as proteins, lipids and free DNA in prokaryotic cells. In combination, these processes inactive and kill microbial pathogens. To date, no natural evolution of resistance towards CAP treatment has been observed in bacteria.

In human eukaryotic cells, cellular DNA is not affected by the plasma species. Cytobiological repair mechanisms provide protection and the integrity of the nuclear membrane is not affected within a short therapeutic window of several minutes. In addition to fighting infections, CAP treatment can stimulate improved wound healing in some patients, presumably due to a hormetic response towards oxidative stress and the resulting activation of survival mechanisms[42].

Five medical devices that employ CAP have obtained regulatory approval (CE-mark) since 2013. The most recent development is a portable, battery-operated device, the plasma care® (see Figure 6 (A)). The plasma care® is approved for wound treatment in clinical settings as well as in homecare patients. The plasma care® has the size and weight of a large telephone handset and is easy to operate with a single touch button. The plasma care® employs Surface Micro-Discharge (SMD) technology to generate CAP[43]. This

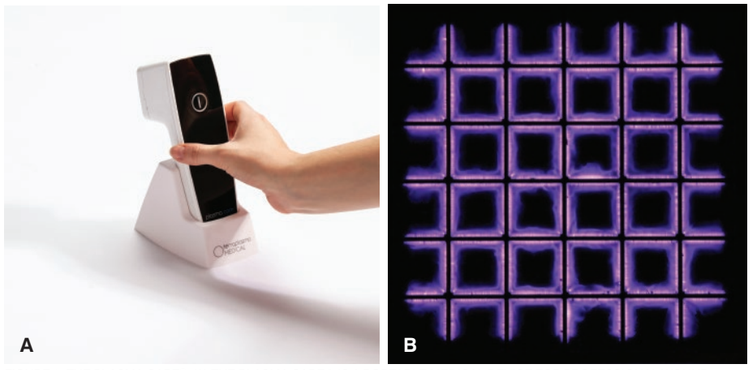


FIGURE 6: THE PLASMA CARE®. (A) THE PLASMA CARE® IS A PORTABLE MEDICAL DEVICE FOR PROFESSIONAL WOUND CARE. (B) ACTIVATED PLASMA SOURCE UNIT IN THE DARK. THE CHARACTERISTIC PURPLE GLOW OF THE PLASMA IS VISIBLE.

process uses a high voltage electrode, a dielectric and a grounded mesh grid electrode (Figure 5, left side). Upon application of a 3.5 kV voltage of 4 kHz frequency, the SMD plasma source produces thousands of micro-discharges superficially on the mesh grid electrode (purple glow in Figure 6 (B); depicted as purple arrows in Figure 5, left side). These micro-discharges interact with the surrounding air, producing a cascade of more than 600 chemical reactions that result in therapeutically active ROS formation. These ROS are transported to the wound surface by diffusion.

Efficacy and Safety *in vitro*

In extensive pre-clinical tests, the plasma care® was proved efficacious against numerous bacterial strains from risk groups I and II including *S. aureus, E. faecalis, P. aeruginosa, E. coli*, MRSA and VRE. There was no significant difference in their sensitivity towards CAP (unpublished data). On agar, 99.999 % of the tested bacteria and of the yeast *C. albicans* were inactivated within 60 seconds. Moreover, the plasma care® was effective upon application to biofilms of *E. faecalis* (99.9% reduction within 1 minute)[44]. Under more life-like conditions in *ex vivo* models of pig and human skin, 69 to 83 % of bacteria were killed within the same time period (unpublished data). However, the plasma treatment is not a targeted therapy. Healthy human tissue also comes into contact with the CAP. Therefore, potential damage to human tissue including primary human fibroblasts and keratinocytes or in human skin was investigated. Plasma treatments of up to 3 minutes (longest permissible treatment period for a single wound area within 24 hours) had no impact on vitality, viability or migration behavior of primary human fibroblasts and keratinocytes *in vitro* (unpublished data). Furthermore, no histological or pro-apoptotic changes were observed in "normal" or "sensitive" skin from healthy donor biopsies. Mutagenicity studies (HGPRT test using V79 cells) provided no evidence of any genotoxic potential of the CAP from the plasma care®. For this purpose, treatment periods of up to 5 minutes were examined (longer treatment periods are not possible due to the limitations in cell tests) (unpublished data). Finally, the UV-irradiation, ozone and nitrogen dioxide levels produced by the plasma care® were tested with respect to health and occupational safety. Based on their very low intensity and concentrations, respectively, neither UV-irradiation nor ozone or nitrogen dioxide levels produced by the plasma care® represent a health or occupational safety hazard (unpublished data).

Thus, the results from the extensive pre-clinical studies of the plasma care® show that this medical device is effective and safe. It is expected that similar advantages for the wound treatment of patients can be obtained with the newly developed plasma care®, as have been described using other cold plasma therapies. To generate clinical data in support of this assumption, a randomized placebo-controlled clinical trial for the plasma care® was started following CE-mark approval in June 2019 (ISRCTN98384076)[45]. The trial is expected to last for approximately twelve months and is conducted at four trial sites in Germany.

Clinical Experience

In addition to the post-market clinical follow-up trial, the plasma care® is being tested in several settings including clinics, private practices and homecare services. Initial results appear encouraging, with examples from veterinary and human medicine shown below.

The first case study (see Figure 7) shows the left foreleg of an 8-year old mare with an infected post-operative wound that failed to heal in three months. Upon initiation of cold plasma therapy, the infection with partially resistant *E. coli* was controlled and wound healing re-initiated. After 14 plasma treatments for 2 minutes per 13 cm² area in 19 days the wound was re-epithelialized. The mare was released after four weeks with full wound closure (unpublished data).

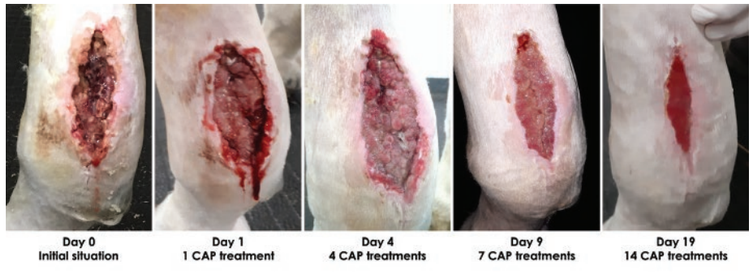


FIGURE 7: WOUND PROGRESSION IN AN 8-YEAR OLD MARE WITH A POST-OPERATIVE WOUND INFECTION. THE MARE WAS TREATED WITH COLD PLASMA 14 TIMES IN THE COURSE OF THREE WEEKS (IMAGES COURTESY OF PFERDEKLINIK AM KIRCHBERG GMBH).

The results from the extensive pre-clinical studies of the plasma care® show that this medical device is effective and safe

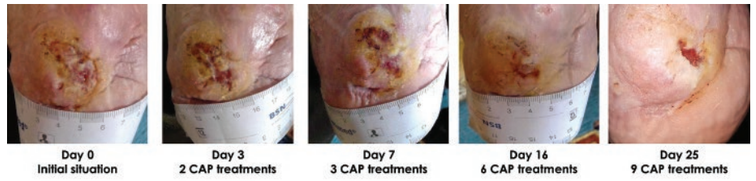


FIGURE 8: WOUND PROGRESSION IN A LEFT LOWER LIMB AMPUTEE WITH A NON-HEALING WOUND. THE PATIENT ALMOST ACHIEVED FULL WOUND CLOSURE AFTER NINE TREATMENTS (IMAGES COURTESY OF ELLIPSA MEDICAL SERVICES GMBH).

The second case study (see Figure 8) shows a non-healing wound in a patient with a lower limb amputation, who was cared for by Ellipsa medical services GmbH since January 2018. Plasma treatment was commenced on August 5th and performed two times per week for four weeks. The patient responded very well to the treatment and almost achieved full wound closure after nine plasma treatments for 1 minute per 13 cm^2 area (unpublished data).

The results that have been achieved with the plasma care® so far encourage terraplasma medical GmbH to expand the available clinical data. The Munich-based Start-Up aims to gain and provide further insights into the benefits and potential limitations of cold plasma technology. This will enable healthcare providers to offer their patients a scientifically sound and innovative therapy option.

The results that have been achieved with the plasma care® so far encourage terraplasma medical GmbH to expand the available clinical data

IMPROVING THE TREATMENT OF ACUTE AND CHRONIC WOUNDS AND THE ROLE OF PLASMA

Emerging Trends and Future Outlook for Wound Management

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The prevalence of chronic conditions and associated wounds can be addressed by enabling decentralised wound care

Chronic Conditions and Wounds

Chronic diseases impose an immense societal and financial burden on both high-income countries and emerging markets. Prevention and effective management of chronic diseases can provide patients with improved quality of life, lessen lost productivity from disability and reduce unnecessary medical costs. Successful management of chronic wounds leverages a combination of therapeutic approaches. This is particularly true for chronic wounds that result from chronic conditions, where management of the underlying pathology should ensure improved outcomes for wound care.

The factors contributing to the increased burden of chronic diseases include increased life-expectancy combined with risk factors imposed by lifestyle and environmental exposures. Exposure to tobacco smoke and increasing levels of obesity contribute to early death and disability, measured by disability-adjusted life years (DALYs). In 1990, the leading risk factors for mortality and morbidity were short gestation for birth weight, low birth weight for gestation and child wasting. In 2017, the leading risk factors were high blood pressure (hypertension), tobacco smoking and elevated blood sugar levels. Between 1990 and 2017, the total global burden of disability increased by 52%. An estimated 80% of disability in 2017 was caused by non-communicable diseases[46].

Decentralised and Home-Based Care

The increase in chronic wound prevalence is directly related to this increase in chronic disease burden. As healthcare systems continue to experience increased demand for wound care services, there is an emerging trend towards providing decentralised and home-based care. The majority of wound care is already managed at the community-level. Recent recommendations from The Vascular Society of Great Britain and Ireland included extensive implementation of diabetic ulcer treatment in outpatient settings[47]. Their recommendations include the establishment of “one-stop clinics” providing comprehensive services at the time of initial clinical assessment. This provides convenience for patients and reduces the demand for follow-up appointments, ensuring efficiency. It is thought to be particularly important for complex cases requiring a multidisciplinary approach to treatment. Providing a range of services in a single location, close to the patient, has the potential to reduce delays in the pathway from diagnosis to treatment, improving patient care.

This approach to improving patient care and reducing costs by decentralisation extends to home-based care. Implementation of technologies to monitor and treat wounds within the home will drive innovations in this field. The design requirements of these devices are onerous. The systems must be sufficiently simple to operate safely in the absence of a trained healthcare professional yet still deliver therapeutic benefit. Training, maintenance and calibration all add to the total cost of ownership associated with medical equipment. Development of simplified, safe devices to aid in wound healing will help to reduce the financial burden of wound management in clinical settings in addition to facilitating home-based care.

Future Outlook

The increasing threat of antimicrobial resistance has highlighted the need to investigate alternative strategies to prevent and treat wound infections. Recent advances in wound dressing technologies involving both dressing materials and antimicrobial agents is likely to continue. Major advances in tissue engineering and stem cell research have promoted the development of

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artificial human skin as an alternative to dressings for the prevention of microbial colonization.

There remains a gap in our understanding of the processes and dynamics involved in wound healing. Further research should reveal the molecular and cellular aetiologies of chronic wounds that occur as a result of underlying pathologies. This would aid in developing interventions to prevent chronic wounds from forming. in vivo cell-based therapies treating ischemic events and correcting diabetic vascular complications could be useful to prevent ulcer formation in diabetic patients[47]. Regulatory approved and clinically efficacious treatments include PDGF-BB[48], fibroblasts embedded in absorbable meshes[49] and fibroblasts and keratinocytes enmeshed in type 1 collagen[50]. Treatments targeting NOS activation and epithelial cell migration may also have a significant impact on wound healing, especially in combination with existing therapies. Delivery systems that control the spatial and temporal profile of reagent release would greatly enhance wound management strategies.

In recent years, emphasis has shifted towards providing value-based, patient-centered care. Prevention and monitoring are the cornerstones of this care paradigm. There have been calls to develop wound management monitoring systems, to improve outcomes and reduce costs. Wound dressings containing embedded sensors to monitor key environmental and biomarkers are currently in development[51]. These advances could enable remote monitoring of chronic wounds, facilitating the early detection of infections or excessive inflammation and timely interventions. When combined with home-based wound treatment devices, these innovations have the potential to reduce the resources that health systems invest in community-level wound management, reduce hospital admissions and associated morbidity.

IMPROVING THE TREATMENT OF ACUTE AND CHRONIC WOUNDS AND THE ROLE OF PLASMA

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