SKIN

IN-DEPTH REVIEW

Nonthermal Atmospheric Pressure Plasma Technology in Dermatology

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ABSTRACT

Nonthermal atmospheric plasma (NTAP), also known as cold atmospheric plasma (CAP), is an emerging tool with important effects on biological systems. NTAP harnesses physical plasma, generating a low energy ion environment in which reactive oxygen and nitrogen species are formed. These ionic species can modify proteins and cell membranes in a non-invasive manner. The use of NTAP therapy, a technology once used for medical sterilization, is rapidly expanding, particularly in the field of dermatology. In addition to potent anti-microbial properties, NTAP has demonstrated promise in skin regeneration and cancer-related indications. NTAP affords a unique advantage to pharmacological therapies in that there is no risk of drug-drug interactions. A preliminary safety profile for NTAP has been established, with no adverse effects such as pain, inflammation, blistering, bruising, and pruritus noted to date. In this review, we discuss the clinical application of NTAP in the treatment of onychomycosis, warts, actinic keratosis, and wound healing.

INTRODUCTION

In the past 20 years, significant advancements have been made in the understanding of physical plasma. The advent of nonthermal atmospheric plasma (NTAP) devices has spurred the advancement of applications of physical plasma in medicine, especially in the field of dermatology. Plasma is known to cause cell membrane permeabilization, regulate skin regeneration, and offer both antibacterial and antiviral properties.[1-3] In this review, we discuss recent applications in the treatment of skin conditions.

REVIEW

Nonthermal Atmospheric Plasma

The concept of “plasma” in the world of physics refers to an ionized gas. Physical plasma exists in a higher energy state than normal gas, which allows for electrons and ions to move independently of one another. Plasma can be further subdivided into “hot” and “cold” plasma. Hot plasma occurs when electrons and ions are in energy equilibrium with one another. While applications for hot plasma exist in the medical field, they are mostly limited to surgical tools. NTAP, which

is also known as cold atmospheric plasma (CAP) or non-equilibrium plasma, refers to plasma that is not in thermodynamic equilibrium, as only the electrons are at a very high temperature, while the ions are at lower temperature. As electrons are quite small, it is the ion component of plasma that makes up the significant portion of its mass. When electrons are thermalized, their velocity distribution is very different from that of the ion velocity distribution, which leads to modification in proteins and cell membranes. Cold plasma is room temperature and therefore does not cause thermal injury to the skin or skin functions, indicating a very favorable safety profile for its application in treating cutaneous pathologies.[4,5]

Plasma can be created in a vacuum, or under atmospheric pressure conditions. The majority of NTAP is generated in air, helium, or argon mixed with other reactive gases.[6] This creates a low energy ion environment in which reactive oxygen and nitrogen species (ROS and RNS, respectively) are formed, along with other plasma components such as UV photons and hydroxyl radicals.[7] Plasma affects cells in a dosage-dependent manner by disrupting cell-cell adhesions and cell detachment from substrates.[1] The short-term exposure temporarily causes cell membrane permeabilization, which inhibits cell migration. Longer exposure times and higher intensity plasma induces apoptosis or necrosis by ROS and RNS. While NTAP has complex effects on tissues, the ROS and RNS generated contribute to an antiviral and antibacterial milieu,[2] allowing plasma to alter tissues at the cellular level, without causing additional damage.[7]

Several additional cellular and molecular mechanisms that modulate the biological effects of NTAP therapy have been elucidated. The Wingless-Int (Wnt)/β-catenin signaling pathway has been reported as the major regulator of skin regeneration following NTAP treatment.[8] β-Catenin is a transcriptional factor that positively regulates Wnt signaling, playing an important role in stem cell renewal, cell proliferation, and cell survival.[9] Moreover, *in vitro* application of NTAP therapy to keratinocytes in culture stimulated cell growth and proliferation, as evidenced by increases in the proportion of treated cells in S and G2 phase.[8] Additionally, NTAP treatment disrupted E-cadherin mediated cell-to-cell interactions, allowing for increased nuclear localization of β-catenin. Increased nuclear presence of β-catenin increased the transcription of c-Myc and cyclin D1, further supporting the increases in cell growth, proliferation, and overall “cell stemness” observed. These findings were recapitulated *in vivo*, supporting NTAP mediated epidermal expansion and increases in activated β-catenin in the absence of DNA damage. NTAP induction of cell stemness is an exciting finding that demonstrates great promise for dermatological applications.

Interestingly, despite demonstrating the capacity to promote cell growth and proliferation, NTAP has demonstrated efficacy in the treatment of cutaneous malignancies, such as *in vitro* melanoma and squamous cell carcinoma. Longer NTAP exposures disrupt calcium homeostasis in cancer cells, leading to increased intracellular calcium levels.[10] Calcium is a critical secondary messenger responsible for regulating tumor survival, growth, and invasion. Additionally, calcium plays a critical role in triggering mitochondrial cytochrome c release and subsequent apoptosis in cells experiencing extreme stress. Prolonged NTAP exposure induced apoptotic behavior in melanoma cells in the presence of increased intracellular calcium. Evidence suggests

ROS and RNS mediated disruption in cellular calcium handling may play a critical role in NTAP-mediated anti-cancer activity. Further evidence of NTAP activity will be provided in this review.

Nonthermal Plasma Technology

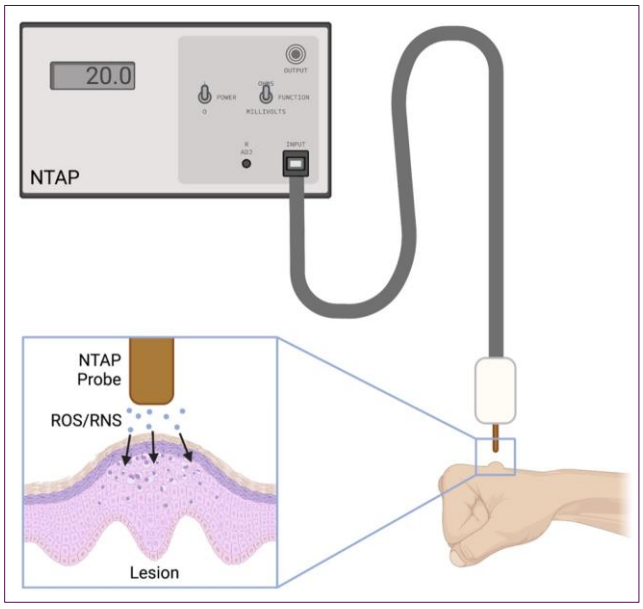
Biomedical application of physical plasma at lower temperature has been regarded as one of the most significant opportunities for advancement in plasma science. NTAP allows for the direct delivery of cold plasma to the skin's surface without the burden of a vacuum chamber or the need to burn tissue.[11] NTAP has a broad range of applications from disinfection of living tissues, to blood coagulation, to the induction of apoptosis in malignant tissues.[6,12-15] The first small scale clinical studies have shown plasma treatments to be well tolerated, painless and without side effects.[4,16-21] In a risk assessment of UV radiation and temperature, it was shown that the UV radiation generated by plasma is an order of magnitude lower than the minimal erythema dose (MED) necessary to produce sunburn on the skin *in vivo*. Further, thermal damage of tissues by plasma can be eliminated due to the nature of cold plasma. Plasma is non-toxic and chemical-free, and devices may be used for multiple years. Thus, NTAP devices results in a significant reduction of effluents, which is beneficial from both an economic and environmental perspective.[22]

Nonthermal Atmospheric Plasma Devices

Three types of NTAP devices exist: the floating electrode-dielectric barrier device (FE-DBD), which supplies direct plasma; atmospheric pressure plasma jet (APPJ), which indirectly supplies plasma; and the surface microdischarge device, which is a hybrid of FE-DBD and APPJ. The individual plasma devices and their differences are reviewed extensively elsewhere.[23] Most of the literature regarding NTAP centers on APPJ, which has been studied extensively with ulcer and wound healing. For this review, we focus on FE-DBD, which is increasingly being used as the NTAP system of choice in dermatology.

FE-DBD creates plasma using a pulse generator. For a typical FE-DBD used in dermatology, this pulse generator supplies a 20-kV pulse of 20-ns pulse width at 200 Hz to a 5-mm diameter quartz-covered copper electrode of 10-cm length and 1-mm quartz thickness [Figure 1].[13] These nanosecond pulse parameters offer a high level of plasma uniformity which allows for the avoidance of tissue damage. However, this is but one example of FE-DBD. Lipner's study of onychomycosis used a machine with a different electrode shape and size, as well as different settings.[24] For in vitro and animal studies, there are many variations of electrode design and pulse generator settings.

Figure 1. Floating electrode-dielectric barrier device



Plasma devices use a variety of feed gases, such as atmospheric air, argon, and helium; however, NTAP generates plasma using air or helium. During the generation of plasma, there is no electric contact with the tissue being treated. The flow of the gas is slow, thus there is no mechanical effect on tissue.

Applications in Dermatology

Treatment with NTAP has been shown to be well tolerated and without adverse effects on the skin, even on skin surfaces that are denuded, eroded, or ulcerated. Given its outstanding safety profile and painlessness of application, NTAP technology has been employed in a variety of dermatologic clinical studies. These studies have investigated the treatment of onychomycosis, actinic keratosis, warts, and wound healing.[25]

Onychomycosis

NTAP has demonstrated efficacy *in vitro* and *in vivo* in the treatment of onychomycosis.[13,24,25] Onychomycosis is a fungal infection of the nails, commonly caused by dermatophytes of the Trichophyton genus.[26] Treatment of onychomycosis typically involves the use of systemic anti-fungal agents, such as terbinafine , and/or topical antimicrobials. Oral antifungal agents can promote significant drug-drug interactions and undesirable side effects, whereas topical treatments have low complete cure rates.[27,28] Additionally, these agents require long-term daily administration. As such, there is an unmet need for a quick, non-invasive, and efficacious treatment for onychomycosis.

In vitro studies assessing dermatophyte inactivation by NTAP demonstrated significant sensitivity to plasma therapy, providing rationale for clinical studies. A study by Xiong et al. examined the efficacy of NTAP in treating model nails coated with either *Escherichia coli* (*E. coli*) bacteria or *Trichophyton rubrum* (*T. rubrum*) fungus.[13] Three different NTAP devices were employed: helium plasma jet, surface micro discharge plasma, and FE-DBD. All three NTAP devices significantly reduced bacterial and fungal load on the nail surface; however, the FE-BDD significantly outperformed all NTAP devices. In a pilot study of 13 patients with toenail onychomycosis, there was a 53% clinical and 15% mycological cure rate with no side effects when using NTAP.[24] Clinical cure was defined as a 3-5 mm increase in clear nail six-months following treatment. Notably, 61.5% of patients were satisfied with their treatment; 76.9% of patients expressed willingness to pay for NTAP treatment, and 100% of patients felt that treatment was tolerable. These data establish a clinical benefit of NTAP in treating onychomycosis and support the need for larger clinical studies.

Actinic Keratosis

Actinic keratoses (AKs) are precursor lesions to squamous cell carcinoma (SCC) that are commonly associated with chronic sun exposure. AKs are typically treated with blue light therapy or topical compositions containing 5-fluorouracil, depending on lesion location and thickness. Conventional treatments have drawbacks, such as treatment site pain and inflammation; incomplete resolution of the lesion, and frequent recurrence of the lesion.[13] *In vitro* studies have demonstrated a pro-apoptotic benefit of NTAP in SCC cells derived from patient tumors. Additionally, NTAP therapy promotes macrophage migration and activation *in vitro*, suggestive of a potential immune-mediated attack of pre-cancerous

cells in target lesions.[12,13,29] These data have inspired clinical studies involving the use of NTAP in treating patients with AKs.

A clinical study involving the use of electrical plasma, a form of NTAP, demonstrated efficacy in the treatment of AKs.[12,30] Five patients were enrolled in a pilot study, comprising 17 lesions. Patients underwent a single NTAP treatment to each lesion with one-month follow-up. Pre- and one-month-post-treatment images were compared. Treatment outcomes were stratified into one of three categories: fully resolved, significant improvement (>50% regression), or no improvement (<50% regression). Of the 17 lesions studied, nine lesions (53%) completely resolved; five lesions demonstrated significant improvement (29%), and three lesions were unchanged (18%). Overall, 70% of lesions demonstrated a therapeutic benefit without discomfort during or after treatment. These data suggest a role for NTAP in the treatment of AKs and th need for follow-up studies in larger patient populations.

A clinical study using APPJ also demonstrated efficacy for NTAP in the treatment of AKs.[31] This proof-of-concept study enrolled seven patients with a total of 115 lesions treated over eight treatment areas. Patients underwent a total of seven treatments applied twice weekly for two minutes each. Olsen grading pre- and post-treatment were compared. Clinical downgrading as per Olsen was found in all treated areas, indicating a decline in AK characteristics such as erythema, scaling, crusts, and thickness. The total lesion number decreased in 75% of the treated areas. No adverse events were observed. These data suggest that NTAP may represent a novel, safe treatment that lacks side-effects, though prospective clinical trials with long-term follow-up are needed.

A similarly APPJ-based study demonstrated the effectiveness of NTAP in treating multiple AKs, as measured using high-frequency ultrasound (HFUS).[32] Twelve patients with multiple AKs of the face and scalp who were either intolerant or resistant to conventional field-directed treatments underwent twice weekly sessions of NTAP for two minutes. Treatment was performed until lesion resolution or a maximum of seven weeks of treatment was reached. Performance indexes were determined using three-dimensional digital pictures at baseline and three months post-treatment. All clinical variables, including number of lesions, cumulative area of AKs, and the actinic keratosis area and severity index, showed a significant reduction following NTAP. HFUS revealed that total, epidermal, and dermal thickness of target AKs did not change with treatment. Instead, NTAP significantly increased dermal density of target AKs and surrounding sun damaged skin, and significantly decreased the thickness of the subepidermal low-echogenic band in perilesional skin. This band represents an ultrasound sign of sun damage. This was the first clinical study using evaluation other than observation alone to demonstrate the efficacy of NTAP.

NTAP has proven to be as effective as diclofenac 3% gel, a conventional therapy used in the treatment of AKs, in a prospective randomized clinical trial.[33] Sixty participants were enrolled in the study. Two anatomically treatment areas were chosen in each patient and were randomized in a 1:1 ratio to receive either NTAP twice weekly for three minutes or diclofenac 3% gel twice daily for three months. Blinded assessment of number of lesions, surface area affected, Olsen grading, local skin response score, and cosmetic outcome were compared at baseline, once per month

during treatment, and three-months post-treatment. At three-month follow-up, both treatment methods had significantly reduced total lesion count (NTAP: 40%, CI 28-53%, diclofenac: 40%, 95% CI 27 – 52%) and affected surface area (NTAP: 49%, 95% CI 37 – 61%, diclofenac: 36%, 95% CI 19 – 53%). NTAP showed significantly better effectiveness in reducing lesion count (NTAP: 33%, 95% CI 20 – 45%, diclofenac: 21%, 95% CI 9 – 33%) and AK-affected area (NTAP: 41%, 95% CI 28 – 54%, diclofenac: 27%, 95% CI 13 – 42%) by the end of treatment. This study demonstrated that plasma is as good as a well-established modality for the treatment of AKs.

Warts

Warts, also known as verruca vulgaris, are benign skin growths that are caused by the human papillomavirus (HPV). Although harmless, these lesions can spread and cause symptoms, such as inflammation, pruritus, dermatitis, and scarring. Standard of care (SOC) treatment of warts involves cryotherapy, which may be poorly tolerated, especially in children. As such, there is a need for an efficacious and painless intervention for the treatment of warts.

Studies have shown that NTAP leads to an influx of intracellular calcium, thereby inhibiting viral replication and promoting resolution of the wart.[34] A 2020 case series involving five pediatric patients demonstrated that NTAP is both safe and effective in the treatment of warts.[34] All patients treated with NTAP in the case series achieved complete clearance of their warts. Patients found the treatment to be painless and equally efficacious, suggesting a potential advantage over cryotherapy in children. There were no adverse events reported, including blistering, scarring, significant pigmentary alteration, persistent nail changes, or pain. Although promising, the clinical benefit and safety profile of NTAP has yet not been extensively studied in pediatric patients with warts to date. Future studies comparing the efficacy of NTAP to cryotherapy in larger patient populations are required.

Wound Healing

Wound healing is a natural process that is achieved by four-steps: hemostasis, inflammation, proliferation, and remodeling.[35] Impaired wound healing results from a disruption in the normal sequence, typically due to poor circulation, weakened immune system, diabetes, and/or the persistence of microbes. The anti-microbial properties of NTAP have instigated both pre-clinical and clinical studies in wound healing utilizing the APPJ device. NTAP has demonstrated *in vitro* and *in vivo* efficacy in pre-clinical models of wound healing.[36] NTAP promotes the release of transforming growth factor (TGF)-β1 cytokine from cells *in vitro*. TGF-β1 is a pro-inflammatory cytokine that promotes myofibroblast activation, which promotes extracellular matrix production and wound contraction.[37] In a mouse model of diabetic ulceration, NTAP treatment promoted cell proliferation, neovascularization, and regeneration of the epidermal layer, thus accelerating wound healing.[36] NTAP has demonstrated efficacy in over 20 pre-clinical studies, which are reviewed extensively elsewhere.[38]

A 2020 study investigated the efficacy of NTAP in the treatment of diabetic foot ulcers.[36] A total of 44 patients received standard care with or without NTAP (N=22/group). Treatment was applied three times per week for three weeks total. The NTAP device used a noncontact probe and generated low temperature plasma using

helium gas. Primary outcomes were wound size, number of cases reaching a wound size of less than 0.5 cm, and bacterial load reduction from baseline. Results demonstrated a statistically significant reduction in wound size; a reduction in the fraction of wounds greater that 0.5 cm in size, and a reduction in bacterial load immediately preceding NTAP treatment. It should be noted, however, that bacterial load only remained suppressed for ten hours. These data demonstrate a transient anti-microbial effect following NTAP treatment and suggest the need for studies with more frequent application, particularly in an inpatient setting where repeat treatment exposure is feasible. A subsequent study assessed the efficacy of NTAP in patients following once weekly treatment for eight weeks total.[39] A total of 50 patients with diabetic pressure ulcers were split into two groups, consisting of SOC versus SOC plus NTAP treatment. Like the previous study, the NTAP device utilized a noncontact probe and generated low temperature plasma using argon gas. Patients that received NTAP in addition to SOC treatment had significantly improved Pressure Ulcer Scale for Healing (PUSH) scores and a reduction in exudate following one week of treatment. Wound size and PUSH scores were significantly improved following the eight-week treatment course. These data, taken together, suggest the wound healing efficacy of NTAP may not be restricted to anti-microbial activity. Comparably, a 2020 study employed NTAP once or twice weekly in patients with therapy-refractory chronic wounds.[23] NTAP significantly reduced wound size and bacterial load following 12-week treatment. The results indicate that once weekly therapy is equally effective in comparison to twice or thrice weekly NTAP. Additionally, these data suggest chronic serial NTAP administration can lead to sustained anti-microbial effects.

CONCLUSION

The use of NTAP therapy is rapidly expanding, particularly in the field of dermatology. A technology once used for medical sterilization has proven therapeutic in a wide variety of dermatological conditions.[14] NTAP has been extensively studied in wound healing applications in large patient populations and has proven efficacious, especially when co-administered with SOC therapy. The use of NTAP in onychomycosis, warts, and AKs has been restricted to pilot studies. Data from these studies are promising and should encourage future studies in larger patient populations. NTAP has proven beneficial in reducing microbe count, promoting cell migration, healing, and in the destruction of aberrantly active cell populations. As such, we suggest NTAP therapy deserves special consideration as an emerging pain-free and safe therapy in the treatment of dermatological conditions.

Conflict of Interest Disclosures: None

Funding: None

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