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Harnessing Plasma Technology in Cream Formulations for Medical Applications as a Nitric Oxide Donor: Proof-of-Concept

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ABSTRACT: Plasma's role in healthcare has been steadily gaining recognition, particularly for its capacity to produce reactive species that foster wound healing, combat microbial infections, and augment drug delivery. Despite its promise, implementation of plasma technologies is often impeded by logistical constraints, accessibility issues, and challenges integrating with established medical treatments. In this paper, we describe an innovative solution to deliver the benefits of plasma in healthcare: plasma-activated cream (PAC). PAC offers a versatile lipid-based platform for medical applications that transcends the traditional boundaries of plasma application by its flexible integration into a variety of treatment forms: as a cream base for transdermal applications, oil base for injectables, or incorporation with other biologics and lipid-soluble compounds. In this study, we reveal the novel method of creating PAC by infusing a lipophilic base with plasma-activated species, specifically focusing on nitric oxide (NO) and its related compounds (NOx). By measuring NOx concentrations before and after plasma treatment, we successfully validated the use of gliding-arc plasma to synthesize PAC. The NOx concentration rose from a baseline of 0 mg/L to an average of 2.0 mg/L post-treatment, indicative of successful infusion of plasma-activated species into PAC. This preliminary experiment unveils a novel pathway for incorporating plasma's beneficial effects into a lipid-based cream and shows the potential for PAC to act as NO storage. PAC not only brings forth new possibilities in wound-healing and antimicrobial treatments but also lays the groundwork for further exploration of plasma's role in enhancing drug delivery and NO storage.

KEY WORDS: nitric oxide, nitric oxide storage, enhanced drug absorption, drug delivery, drug potentiation, wound healing

I. INTRODUCTION

Plasma, often termed the fourth state of matter, has long been acknowledged for its potential in various scientific fields, including healthcare. The interest in plasma technology in healthcare has been largely driven by its ability to generate reactive oxygen and nitrogen species (RONS), which have been found to promote wound healing, reduce microbial infections, selectively kill some types of cancer cells, and potentially enhance drug delivery.[1–21] These effects are not limited to the direct discharge of plasma at target sites, but have been seen in the application of plasma-activated mediums to those sites:

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specifically water based solutions or simply plasma activated water.[13,22–25] However the integration of plasma technology into creams and oils—common vehicles for nonpolar drug delivery—is relatively unexplored.

Creams are routinely used for topical application of drugs, offering advantages such as sustained release, enhanced absorption, and localized effect. Oils are also used both topically and in injections. In terms of plasma, it is well known that small gaseous molecules are significantly more soluble in lipids than in water. Indeed, one of the most potent RONs generated by plasma discharges using air is nitric oxide (NO). NO production by plasma has been extensively studied,[1–19,24,25] and NO is known to be up to 1000 times more soluble in lipids than in water.[26–28] NO is also highly reactive, and it is known to react with lipids to form L-NO2.[26–28] L-NO2 readily dissociates to release NO in the presence of water ($k = 9.67 \times 10^{-6} s^{-1} M^{-1}$, pH 10.4);[26–28] however, without water, this dissociation is abrogated.[26–28] It is hypothesized that if the NO produced by plasma can enter a water-free lipid/cream, and if this is then stored isolated from water, the new plasma activated cream can function as a form of NO storage, since the NO will only dissociate in presence of water (such as when applied to the target site on the human body).

The enhanced ability of lipids to hold and potentially store NO produced from plasma, combined with the therapeutic benefits of a cream, has led to the development of plasma-activated cream (PAC) described in this paper. This development of PAC aims to harness the benefits of plasma technology in lipid solutions to both provide the medical benefits of plasma, as well as synergize with easily incorporable therapeutic agents.[4,7,13,15,16]

II. MATERIALS AND METHODS

A. Generation of PAC

To generate PAC, we used a device (pending patent application filed by Drexel University, Philadelphia, PA) with sample schematic seen in Fig. 1.

Briefly, a gliding arc plasmatron, which is supplied with a gas (either air or an alternative gas, such as N2), through a system of electrodes emits ionized gas with a temperature range of 20–300°C. The species produced in the gliding arc plasmatron (air enriched with RONS active species) is passed through the liquid cream/gel/ointment in the loading zone. For the experiments described in this paper, hemp oil and coconut oil were used. The specific operating parameters used in the experiments were 8.0 kV, 0.39 A, with gas flow rates of 1.2 scfm air, and 1.0 scfm nitrogen.

B. Detection of Plasma Activation

To detect the presence of plasma activated species, we measured NOx concentration before plasma treatment and then after using NOx strips (QUANTOFIX Nitrate/Nitrite semi-quantitative test strips (Macherey-Nagel, Germany). We expected 0 mg/L NOx concentrations in oil initially since they are not present in the oil sample. To measure

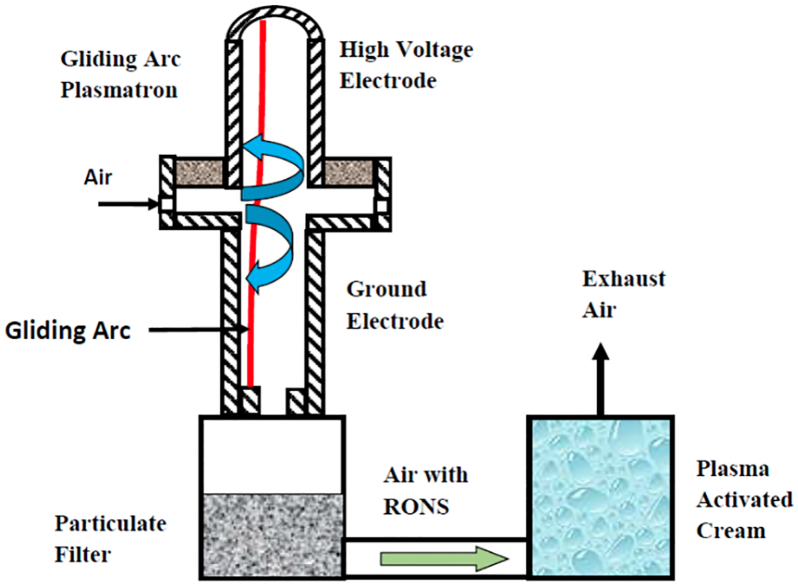

FIG. 1: Plasma system for production of plasma activated cream enriched with reactive oxygen/nitrogen species.

NOx in oil, a 90-mL sample of our oil was mixed with 10 mL distilled water, and then placed in a centrifuge at 3000 RCF for 15 minutes to separate into distinct aqueous and non-aqueous phases. Finally, the NOx concentration was measured in the aqueous phase using NOx strips.

III. RESULTS AND DISCUSSION

Before plasma treatment of both the hemp oil and coconut oil, we measured an average initial NOx concentration of 0.0 mg/mL using the NOx strips, proving that pure hemp and coconut oils do not have any measurable NOx in them. After plasma treatment, we found an average concentration (4 replicates per sample) of 2.25 mg/L (SD = 0.1155, 95% CI [2.14, 2.36]) in the hemp oil and average of 1.8 mg/L (SD = 0.0816, 95% CI [1.72, 1.88]) in the coconut oil. These results demonstrate the use of gliding arc plasma to create PAC that contains NOx and the study unveils a novel method of creating PAC by infusing two different oils with plasma-activated species. The infusion of PAC was achieved by emitting ionized gas from a gliding arc plasmatron into the cream, as seen in Fig. 2. The resultant PAC successfully demonstrated the presence of plasma-activated species, specifically NOx, through the increase in NOx concentration from zero to an average of 2.0 mg/L, indicative of successful plasma infusion.

The novelty of PAC lies in its potential to function as a form of NO storage. This is attributed to the fact that the NOx species react with the lipids to form LNO2, which dissociates to release NO only upon exposure to water.[26–28] As such, storing the PAC in a

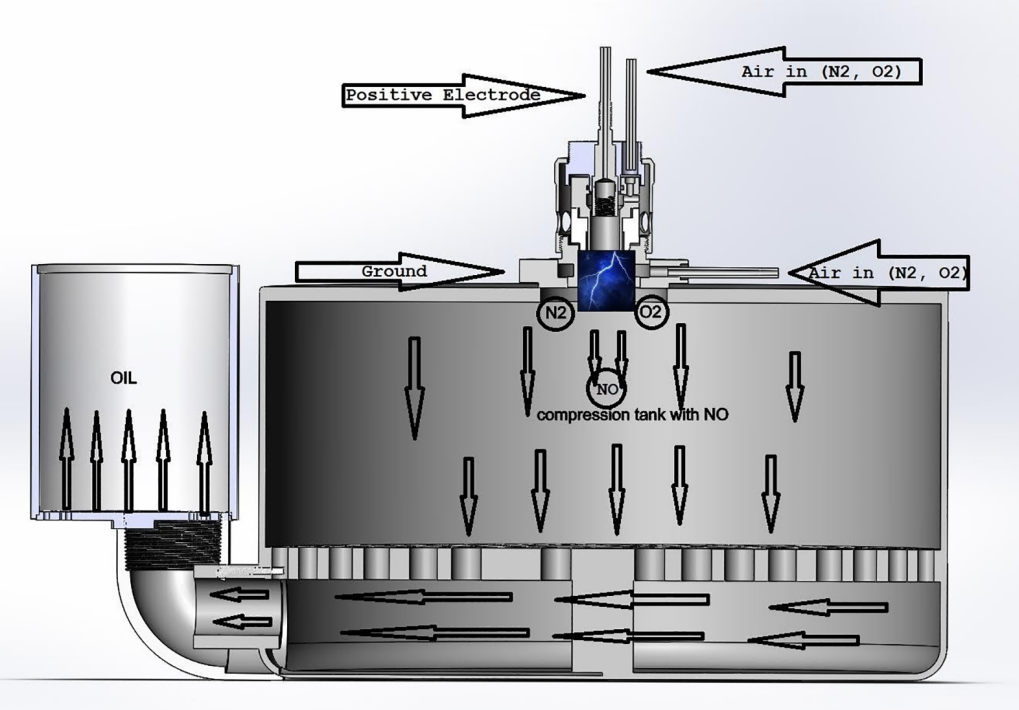

FIG. 2: Air is fed into the gliding arc plasmatron. Voltage is applied, generating free electrons, which react with the nitrogen and oxygen molecules in air producing NO rich plasma. The reactive species generated, including NO, are immediately compressed and conducted into our oil. NO is then free to react with lipids in oil, leading to NO attack of double bonds and nitrosated lipid generation. Excess gas is vented out, and isolated, anhydrous, nitrosated lipid is the end product.

water-free environment may allow it to act as a reservoir of NO until it is applied to the target site on the body, which is typically water-rich, leading to the release of NO at the location. This potential NO storing effect demands further research, and further experiments will need to characterize the presence of LNO2 in PAC.

In addition to potential storage of NO, there are multiple reasons why plasma activated cream has the potential to provide synergy. First, plasma has been shown to alter cellular permeability leading to enhanced drug absorption.[7,13,15] Also, with the nature of creams leading to sustained slow release, PAC could potentially offer a more stable and longer-lasting formulation relative to other plasma activated water based mediums, prolonging plasma's therapeutic effects. One such potential synergy could be the use of PAC as the base for a healing, regenerative, or antibacterial cream that incorporates other drugs since PAC would potentiate the effects of the therapeutics by improving drug uptake, while also providing directly synergistic therapeutics. To illustrate the potential synergy we will discuss some examples.

In diabetic wounds blood flow is compromised, but plasma has been shown to both increase drug uptake and improve angiogenesis.[4,7–15] Incorporating medicines into a PAC base would have the potential to allow both better absorption of drugs, and increased access of those drugs to tissue through the increased angiogenesis from the effect of the plasma activated species (RONs) in the PAC. In the case of antibacterial cream, the sterilizing potential of the RONs species produced by plasma could complement antimicrobial drugs and broaden the treatment spectrum of this cream.

These are just two examples, but they illustrate the unique point of PAC as more than a direct therapeutic, but as a synergistic base that could both add to and potentiate a variety of medical treatments. Additionally, the reactive species of plasma (RONs) are known to dissolve better in lipids than in water. Our research on the development of PAC, using a novel method to infuse active plasma species into a hydrophobic base, brings us a step closer to unlocking the full potential of plasma technology in healthcare.

At its core, PAC is a plasma-treated lipid, and it could be applied locally (topical, injection, etc.), to any areas where it could provide therapeutic benefit, whether alone or in combination with another drug. Our successful proof of concept experiment on the development of PAC paves the way for our future research efforts. These will include characterizing the specific compounds generated through the reaction of plasma with lipids, clinical efficacy trials, and evaluation of synergistic effects of PAC with a variety of therapeutic agents.

REFERENCES

1. Keidar M, Walk R, Shashurin A, Srinivasan P, Sandler A, Dasgupta S, Ravi R, Guerrero-Preston R, Trink B. Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. Br J Cancer. 2011;105(9):1295–301.
2. Fridman G, Shereshevsky A, Jost MM, Brooks AD, Fridman A, Gutsol A, Vasilets V, Friedman G. Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. Plasma Chem Plasma Process. 2007;27(2):163–76.
3. Bruno G, Van Boxem W, Giraud M, Nikiforov A, Lu X, Leys C, Benedikt J. On the liquid chemistry of the reactive nitrogen species peroxynitrite and nitrogen dioxide generated by physical plasmas. Biomolecules. 2020;10(12):1687.
4. Wenske S, Haupt A, Knake N, Reuter S, Reineke K, Ehlbeck J, Weltmann K-D, Stope MB, von Woedtke T, Partecke LI. Nonenzymatic post-translational modifications in peptides by cold plasma-derived reactive oxygen and nitrogen species. Biointerphases. 2020;15(6):061008.
5. Brun P, Bernabè G, Marchiori C, Scarpa M, Zuin M, Cavazzana R, Zaniol B, Martines E. Antibacterial efficacy and mechanisms of action of low power atmospheric pressure cold plasma: Membrane permeability, biofilm penetration and antimicrobial sensitization. J Appl Microbiol. 2018;125(2):398–408.
6. Dong X, Chen M, Wang W, Fan X, Xu H, Xu Y, Luo X. A novel approach to regulate cell membrane permeability for ATP and NADH formation in Saccharomyces cerevisiae induced by air cold plasma. Plasma Sci Technol. 2017;19(2):024001.
7. Lee S, Choi J, Kim J, Jang Y, Lim TH. Atmospheric pressure plasma irradiation facilitates transdermal permeability of aniline blue on porcine skin and the cellular permeability of keratinocytes with the production of nitric oxide. Appl Sci. 2021;11(5):2390.
8. Haralambiev L, Nitsch A, Jacoby JM, Strassenburg S, Hahnel M, Mustea A, Ekkernkamp A, Stope

MB, Kramer A. Cold atmospheric plasma treatment of chondrosarcoma cells affects proliferation and cell membrane permeability. Int J Mol Sci. 2020;21(7):2291.
9. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24(10):R453–R462.
10. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, Bhasker V, Gordillo GM, Sen CK, Roy S. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One. 2010;5(3):e9539.
11. Peiris-Pagès M, Sotgia F, Lisanti MP. Chemotherapy induces the cancer-associated fibroblast phenotype, activating paracrine Hedgehog–GLI signalling in breast cancer cells. Oncotarget. 2018;9(43):26737.
12. Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, Wahl GM, Izpisúa Belmonte JC. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. Nature. 2009;460(7259):1140–4.
13. Fridman A, Friedman G. Plasma medicine. Hoboken, NJ: Wiley; 2013.
14. Kalghatgi S, Kelly CM, Cerchar E, Torabi B, Alekseev O, Fridman A, Friedman G, Azizkhan-Clifford J. Applications of non thermal atmospheric pressure plasma in medicine. In: Güçeri S, Fridman A, Gibson K, Haas C, editors. Plasma assisted decontamination of biological and chemical agents. Dordrecht, Netherlands: Springer; 2008. p. 173–81.
15. Dobrynin D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of direct plasma interaction with living tissue. New J Phys. 2009;11(11):115020.
16. Jacofsky MC, Pastis NJ, Keymel KR, Privett JH, Branam J, Hinds WC, Eiceman GA. Spatially resolved optical emission spectroscopy of a helium plasma jet and its effects on wound healing rate in a diabetic murine model. Plasma Med. 2014;4(1-4).
17. Wu AS, Kalghatgi S, Dobrynin D, Sensenig R, Cerchar E, Podolsky E, Dulaimi E, Paff M, Wasko K, Arjunan KP, Garcia K, Fridman G, Balasubramanian M, Ownbey R, Barbee KA, Fridman A, Brooks AD. Porcine intact and wounded skin responses to atmospheric nonthermal plasma. J Surg Res. 2013;179(1):e1–e12.
18. Kalghatgi S, Friedman G, Fridman A, Clyne AM. Endothelial cell proliferation is enhanced by low dose non-thermal plasma through fibroblast growth factor-2 release. Ann Biomed Eng. 2010;38:748–57.
19. Arjunan KP, Sharma VK, Ptasinska S, Clyne AM. Non-thermal dielectric barrier discharge plasma induces angiogenesis through reactive oxygen species. J R Soc Interface. 2012;9(66):147–57.
20. Priya Arjunan K, Morss Clyne A. Hydroxyl radical and hydrogen peroxide are primarily responsible for dielectric barrier discharge plasma-induced angiogenesis. Plasma Process Polym. 2011;8(12):1154–64.
21. Haertel B, Wende K, von Woedtke T, Weltmann K-D. Non-thermal atmospheric-pressure plasma possible application in wound healing. Biomol Ther. 2014;22(6):477.
22. Tanaka H, Mizuno M, Ishikawa K, Nakamura K, Kajiyama H, Kano H, Kikkawa F, Hori M. Cell survival and proliferation signaling pathways are downregulated by plasma-activated medium in glioblastoma brain tumor cells. Plasma Med. 2012;2(4):207–20.
23. Tanaka H, Mizuno M, Ishikawa K, Toyokuni S, Kajiyama H, Kikkawa F, Hori M. Plasma-activated medium selectively kills glioblastoma brain tumor cells by down-regulating a survival signaling molecule, Akt Kinase. Plasma Med. 2011;1(3-4):265–77.
24. Girard PM, Arbabian A, Fleury M, Bauville G, Puech V, Dutreix M, Sousa JS. Synergistic effect of H_2O_2 and NO_2 in cell death induced by cold atmospheric He plasma. Sci Rep. 2016;6(1).
25. Zhou R, Zhou R, Wang P, Xian Y, Mai-Prochnow A, Lu X, Cullen PJ, Becker KH, Ostrikov K, Bazaka K. Plasma-activated water: Generation, origin of reactive species and biological applications. J Phys D Appl Phys. 2020;53(30):303001.
26. Baker PR, Schopfer FJ, O'Donnell VB, Freeman BA. Convergence of nitric oxide and lipid signaling: Anti-inflammatory nitro-fatty acids. Free Radic Biol Med. 2009;46(8):989–1003.
27. Schopfer FJ, Baker PR, Giles G, Chumley P, Batthyany C, Crawford J, Patel RP, Hogg N, Branchaud

BP, Lancaster JR Jr, Freeman BA. Fatty acid transduction of nitric oxide signaling: Nitrolinoleic acid is a hydrophobically-stabilized nitric oxide donor. J Biol Chem. 2005.
28. Lima ES, Bonini MG, Augusto O, Barbeiro HV, Souza HP, Abdalla DSP. Nitrated lipids decompose to nitric oxide and lipid radicals and cause vasorelaxation. Free Radic Biol Med. 2005;39(4):532–9.