

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/348372649>

Tolerability of Six Months Indirect Cold (Physical) Plasma Treatment of the Scalp for Hair Loss

Article in *Journal of Drugs in Dermatology* · December 2020

DOI: 10.36849/JDD.2020.5186

CITATIONS

7

READS

581

6 authors, including:



Shilpa Malik

Touro University College of Osteopathic Medicine

7 PUBLICATIONS 18 CITATIONS

SEE PROFILE



Jasmit Walia

2 PUBLICATIONS 7 CITATIONS

SEE PROFILE



Greg Fridman

AAPlasma LLC

174 PUBLICATIONS 9,383 CITATIONS

SEE PROFILE



Peter Friedman

The Skin Center Dermatology Group

55 PUBLICATIONS 1,699 CITATIONS

SEE PROFILE

Tolerability of Six Months Indirect Cold (Physical) Plasma Treatment of the Scalp for Hair Loss

Afshan Khan,^{a*} Shilpa Malik,^{a*} Jasmit Walia,^a Gregory Fridman PhD,^b Alexander Fridman PhD,^c
Peter C. Friedman MD PhD^a

^aThe Skin Center Dermatology Group, New City, NY

^bAA Plasma, Philadelphia, PA

^cC&J Nyheim Plasma Institute, Drexel University, Camden, NJ

*The first and second authors contributed equally to this work.

ABSTRACT

Androgenetic alopecia (AGA) is a chronic form of hair loss. Cold atmospheric (physical) plasma (CAP) is partly ionized gas with various widely researched effects on living tissues. CAP is an emerging treatment modality in dermatology with uses for chronic leg ulcer, actinic keratosis, warts, and other applications. Its previously demonstrated ability to induce stem cell differentiation in various cell types makes CAP a possible treatment option for AGA. Directly creating CAP on the scalp surface has drawbacks, but indirect CAP treatment—when a CAP-treated liquid is used as topical therapy—offers an alternative. In a clinical pilot study, we treated 14 patients with AGA using the indirect CAP method for three months (4 patients) and six months (10 patients). The indirect CAP treatment was well tolerated and while the primary goal of the study was not to assess efficacy, most patients reported improvement, and the investigator's assessment also showed improvement in most patients. Our findings create the foundation for longer, extensive trials to systematically assess the efficacy of indirect CAP treatment for AGA.

ClinicalTrials.gov: NCT04379752

J Drugs Dermatol. 2020;19(12):1177-1180. doi:10.36849/JDD.2020.5186

INTRODUCTION

Androgenetic alopecia (AGA) is a chronic form of hair loss characterized by the miniaturization of hair follicles. There are different prescription and over-the-counter treatments for AGA, which have various advantages and drawbacks, but the search for the ideal AGA treatment continues.¹ Cold atmospheric pressure (physical) plasma (CAP), a partially ionized gas, is an emerging treatment modality with expanding use in dermatology.² There is a vast body of literature exploring the effects of CAP in cell cultures and animal models. It is shown to have a deleterious effect on bacteria and fungi.³ It also selectively causes cell death in malignant cell lines in vitro.⁴ Other experiments indicate the ability of CAP to induce stem cell differentiation.⁵ This important effect has been described in various cell types, including adipose derived stem cells,⁶ and neural stem cells.⁷ Clinical dermatological use of CAP originated in the treatment of chronic ulcers, based on its antiseptic effects.⁸ Small pilot studies showed efficacy of CAP treating actinic keratosis and warts.^{9,10,11}

Minoxidil, one of the most established treatment modalities for AGA, may induce hair follicle stem cell differentiation and hair growth via triggering cellular calcium influx.¹² As CAP has also been shown to induce cellular calcium influx in vitro,¹³ one can

hypothesize that it may be able to improve AGA by inducing hair follicle stem cell differentiation based on its similar effect on other stem cell populations, and specifically via triggering cellular calcium influx. We are not aware of any prior reports describing the use of CAP to induce hair growth in humans, but a recent rat model based study showed that CAP treatment using a nitrogen-CAP jet-type device increased hair diameter.¹⁴ If proven to be safe and effective, CAP may offer an alternative for the treatment of AGA.

CAP is used to treat living tissues in two main ways. Direct CAP treatment is when the partly ionized gas is created directly on the treated surface or delivered to the surface immediately by a gas jet stream. Indirect CAP treatment is a process when a liquid is exposed to CAP and the created mixture is delivered to a cell culture or living tissues. Plasma is a complex ionized gas, which contains various reactive species and charged particles. Although applying direct CAP to the surface of human skin was shown to be safe and tolerable in different settings,^{15,16} the depth of penetration of direct CAP treatment is limited,¹⁷ and may not be sufficient to reach the hair follicles, especially on thick human scalp skin. Currently available methods of delivering direct CAP treatment are also time-consuming and impractical

if the treated area is large. Furthermore, CAP devices typically emit ultraviolet light and ozone, thus long-term and regular exposure to a direct CAP device raises safety concerns. Indirect CAP treatment, however, not only improves the depth of penetration significantly and is easy to apply to a large surface, such as the entire scalp, but in an indirect treatment setting, the CAP device is not present at the treated area, eliminating safety concerns arising from UV and ozone production. To our knowledge, there has been no prior study assessing the tolerability of long-term indirect CAP treatment of the human scalp, a prerequisite to treating chronic hair loss, such as AGA.

MATERIALS AND METHODS

Patients

To explore the tolerability and assess patient opinion of indirect CAP treatment, we initiated a pilot study for patients with AGA. We searched the electronic medical record system of a single specialty dermatology practice (The Skin Center Dermatology Group, New City, NY) for patients with androgenetic alopecia. The identified patients were called and offered participation in screening for a clinical trial using a topical at-home treatment for hair loss. The protocol was approved by the Western Institutional Review Board (4/9/2019; 20130084). Those interested were screened and patients meeting study criteria were enrolled in the trial. Inclusion and exclusion criteria are listed in Table 1.

Treatment Protocol and Evaluation

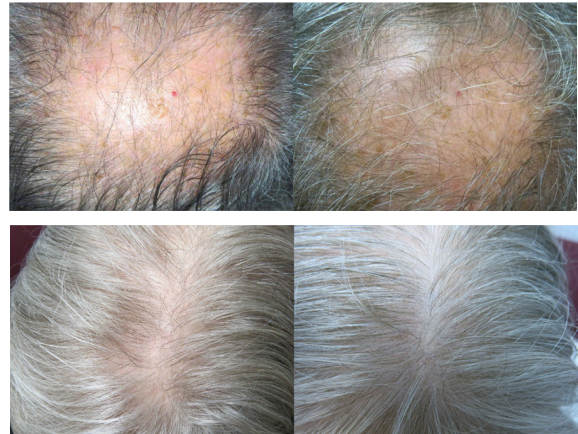
The study treatment consisted of twice daily application of 1 ml plasma-treated liquid compound to the scalp. Patients were given 14 ml amounts of the liquid compound once a week in bottles equipped with droplet applicators. The patients were seen weekly for visual scalp inspection to ensure the early detection of adverse effects and they were asked about any adverse reactions. At the end of the trial, participants were asked to complete a written questionnaire regarding the treatment. Before and after photographs were taken.

Preparation of Plasma-Treated Liquid Compound

The base of the liquid compound was a mixture of 20% isopropyl alcohol and 80% water. The base mixture was treated with plasma generating device to create a stock solution that was further diluted before distribution to patients. To ensure uniformity of the treatment, we used hydrogen peroxide concentration as a surrogate marker to measure the effectiveness of plasma delivery to the base mixture. Stock solutions were generated to reach a hydrogen peroxide concentration of 1000 mg/L (measured using Quantofix Peroxid 1000 test strips by Macherey-Nagel, Duren, Germany).

During the first 6 weeks of the study, the compound was created by treating 20 ml of the base mixture with a commercially available helium plasma jet device (Piezobrush, manufactured by Relyon Plasma GmbH (formerly Reinhausen Plasma GmbH),

FIGURE 1. Clinical photos of a male (top panel) and a female (bottom panel) patient taken before and after completion of 6 months CAP treatment.



Regensburg, Germany) for 50 minutes. This device creates CAP using an electrode inside a hand piece. The plasma is carried out through an opening by a continuous helium stream and gets mixed into the medium placed under the hand piece. During the remainder of the study, CAP was created by placing a quartz-covered metal electrode 1 mm above the surface of 5 ml of the medium and connecting the electrode to a power supply generating 10 microsecond, 20 kHz pulsed discharge as described previously¹⁸ for 30 minutes. The generated CAP diffused into the medium. In both cases, the treated stock mediums were diluted 1:3 before distribution.

RESULTS

16 patients (7 females and 9 males aged 31–76; average age, 54.3 years) were enrolled and completed the informed consent process. Ten patients (7 males and 3 females) completed 6 months of treatment; four patients (2 males and 2 females) completed 3 months of treatment; two females dropped out due to inability to keep the weekly appointments. Only one patient reported transient scalp irritation sensation at one of the weekly visits, which resolved spontaneously without dropping out of

TABLE 1.

Enrollment Criteria
Inclusion Criteria
Age above 18
Diagnosis of androgenetic alopecia
Ability to apply trial treatment compound twice a day for 3 months
Ability to present for weekly evaluations.
Exclusion Criteria
Any type of hair loss treatment in the preceding 3 months
Any type of treatment for any scalp condition in the preceding 3 months
Any active scalp conditions

TABLE 2.**Responses to Questions at End of the Trial from 4 Patients Completing Only 3 Months of Treatment**

Have you experienced any side effects?	Yes: 1*	No: 3
Did you find the application procedure practical:	Yes: 4	No: 0
Did you find the application procedure easy to perform?	Yes: 4	No: 0
Have you seen any improvement with the treatment?	Yes: 2	No: 2
Would you like to continue the study treatment?	Yes: 4	No: 0

*Mild, occasional itch during the first 3 weeks only

TABLE 3.**Responses to Questions at End of Trial from 10 patients Completing 6 Months of the Study**

Have you experienced any side effects?	Yes: 1*	No: 9
Did you find the application procedure practical:	Yes: 9	No: 1
Did you find the application procedure easy to perform?	Yes: 10	No: 0
Have you seen any improvement with the treatment?	Yes: 9	No: 1
Would you like to continue the study treatment?	Yes: 8	No: 2

*Transient burning sensation that resolved spontaneously without dropping out of the trial.

TABLE 4.**Averages of Patient Ratings* of Different Aspects of Improvement after 3 Months Treatment (14 patients) and 6 Months Treatment (10 patients)**

	3 Months	6 Months
Decrease in hair shedding:	3.7	5.6
Increase in fine ('baby') hair growth:	4.5	3.9
Increase in normal hair growth:	5.3	5.1
Thickness of hair (with and strength of individual hairs):	4.5	4.1
Density of hair (fullness of hair):	4.3	4.1
Increased length of uncut hair	2.9	5.2

*Scale: 1 is 'no improvement' - 10 is 'lot of improvement'.

the study and another patient reported occasional mild itch during the first 3 weeks of treatment. No other patients reported any side effects during and at the conclusion of the trial. Most patients found the treatment practical and easy to apply and they were interested in ongoing use of the study treatment. At the weekly follow-ups there was no sign of any irritation, inflammation, or any other scalp skin changes for any of our patients. The majority of patients (12 of 14) responded in our final questionnaire that they saw improvement of AGA after the indirect CAP treatment. The answers to the final questionnaire are summarized in Tables 2-4. Investigator assessment based on photographs taken before the beginning and after the completion of the treatment course concluded that there was

visible improvement on 6 of the 10 patients completing six months of treatment and 2 of the 4 patients completing three months of treatment.

DISCUSSION

We have conducted a small pilot study to assess the tolerability and patient opinion of indirect CAP treatment of the human scalp. We have found that 6 months continuous application of CAP treated liquid compound (created by using two different types of CAP sources) was very tolerable and the patients found the application practical and easy to perform. The primary goal of the study was not to evaluate hair growth, but we asked patients to self-assess at the end of the trial and investigator assessment based on photographs was also done. Although most patients observed positive effects, we did not perform hair count, pull test, or any other objective measurements of possible hair growth. The investigator assessment also demonstrated improvement in the majority of cases, but it was only based on overall impression comparing clinical photographs and no standards were used. To our knowledge, this is the first study examining the tolerability of indirect CAP treatment of the human scalp, paving the way for systematic trials to objectively assess the efficacy of indirect CAP treatment of AGA.

Limitations of this study were that the number of patients was low and there were no control subjects. The low patient number did not allow for grouping based on gender, severity of hair loss, or age. We have not performed any tissue or cellular level analysis of possible damage caused by CAP. Even though the safety of plasma treatments has been investigated and confirmed in various settings,^{19,20,21,22,23,24,16} it would be prudent to verify those results after long term application, such as in our case. There were no objective measurements (for example hair count) used to evaluate improvement, partly because the main goal of this study was to assess the tolerability of CAP treatment and not its efficacy. To reach a conclusion regarding the efficacy of this modality to treat AGA, objective measures will need to be used in extensive trials.

DISCLOSURES

The authors have no conflict of interest to declare.

REFERENCES

- Adil A, Godwin M. The effectiveness of treatments for androgenetic alopecia: A systematic review and meta-analysis. *J Am Acad Dermatol.* 2017;77(1):136-141.e5. doi:10.1016/j.jaad.2017.02.054
- Friedman PC. Cold atmospheric pressure (physical) plasma in dermatology: where are we today? *Int J Dermatol.* 2020;59(10):1171-1184. doi:10.1111/ijd.15110.
- Bourke P, Zuzina D, Han L, Cullen PJ, Gilmore BF. Microbiological interactions with cold plasma. *J Appl Microbiol.* 2017;123(2):308-324. doi:10.1111/jam.13429
- Khalili M, Daniels L, Lin A, et al. Non-thermal plasma-induced immunogenic cell death in cancer. *J Phys D Appl Phys.* 2019;52(42):423001. doi:10.1088/1361-6463/ab31c1
- Li Y, Choi EH, Han I. Regulation of redox homeostasis by nonthermal biocompatible plasma discharge in stem cell differentiation. *Oxid Med Cell Longev.* 2019;2019. doi:10.1155/2019/2318680

6. Griffin MF, Ibrahim A, Seifalian AM, Butler PEM, Kalaskar DM, Ferretti P. Argon plasma modification promotes adipose derived stem cells osteogenic and chondrogenic differentiation on nanocomposite polyurethane scaffolds; implications for skeletal tissue engineering. *Mater Sci Eng C*. 2019;105(Feb):110085. doi:10.1016/j.msec.2019.110085
7. Xiong Z, Zhao S, Mao X, et al. Selective neuronal differentiation of neural stem cells induced by nanosecond microplasma agitation. *Stem Cell Res*. 2014;12(2):387-399. doi:10.1016/j.scr.2013.11.003
8. Brehmer F, Haenssle HA, Daeschlein G, et al. Alleviation of chronic venous leg ulcers with a hand-held dielectric barrier discharge plasma generator (PlasmaDerm® VU-2010): Results of a monocentric, two-armed, open, prospective, randomized and controlled trial (NCT01415622). *J Eur Acad Dermatol Venereol*. 2015;29(1):148-155. doi:10.1111/jdv.12490
9. Friedman PC, Miller V, Fridman G, Lin A, Fridman A. Successful treatment of actinic keratoses using nonthermal atmospheric pressure plasma: A case series. *J Am Acad Dermatol*. 2017;76(2):349-350. doi:10.1016/j.jaad.2016.09.004
10. Friedman PC, Miller V, Fridman G, Fridman A. Use of cold atmospheric pressure plasma to treat warts: a potential therapeutic option. *Clin Exp Dermatol*. 2019;44(4):459-461. doi:10.1111/ced.13790
11. Friedman PC, Fridman G, Fridman A. Using cold plasma to treat warts in children: A case series. *Pediatr Dermatol*. 2020;37(4):706-709. doi:10.1111/pde.14180
12. Goren A, Naccarato T, Situm M, Kovacevic M, Lotti T, McCoy J. Mechanism of action of minoxidil in the treatment of androgenetic alopecia is likely mediated by mitochondrial adenosine triphosphate synthase-induced stem cell differentiation. *J Biol Regul Homeost Agents*. 2017.
13. Dobrynin D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of direct plasma interaction with living tissue. *New J Phys*. 2009;11. doi:10.1088/1367-2630/11/11/115020
14. Babossalam S, Abdollahimajd F, Aghighi M, et al. The effect of nitrogen plasma on the skin and hair follicles: a possible promising future for the treatment of alopecia. *Arch Dermatol Res*. 2019. doi:10.1007/s00403-019-02020-w
15. Isbary G, Heinlin J, Shimizu T, et al. Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: Results of a randomized controlled trial. *Br J Dermatol*. 2012;167(2):404-410. doi:10.1111/j.1365-2133.2012.10923.x
16. Lademann J, Richter H, Alborova A, et al. Risk assessment of the application of a plasma jet in dermatology. *J Biomed Opt*. 2009;14(5):054025. doi:10.1117/1.3247156
17. Szili EJ, Hong SH, Oh JS, Gaur N, Short RD. Tracking the Penetration of Plasma Reactive Species in Tissue Models. *Trends Biotechnol*. 2018;36(6):594-602. doi:10.1016/j.tibtech.2017.07.012
18. Fridman G, Peddinghaus M, Balasubramanian M, et al. Blood Coagulation and Living Tissue Sterilization by Floating-Electrode Dielectric Barrier Discharge in Air. *Plasma Chem Plasma Process*. 2006;26(4):425-442. doi:10.1007/s11090-006-9024-4
19. Ma M, Duan J, Lu X, He G. Genotoxic and mutagenic properties of atmospheric pressure plasma jet on human liver cell line L02. *Phys Plasmas*. 2019;26(2). doi:10.1063/1.5087148
20. Rutkowski R, Daeschlein G, von Woedtke T, Smeets R, Gosau M, Metelmann HR. Long-term Risk Assessment for Medical Application of Cold Atmospheric Pressure Plasma. *Diagnostics*. 2020;10(4):1-12. doi:10.3390/diagnostics10040210
21. Boehm D, Bourke P. Safety implications of plasma-induced effects in living cells - A review of in vitro and in vivo findings. *Biol Chem*. 2018;400(1):3-17. doi:10.1515/hsz-2018-0222
22. Isbary G, Köritzer J, Mitra A, et al. Ex vivo human skin experiments for the evaluation of safety of new cold atmospheric plasma devices. *Clin Plasma Med*. 2013;1(1):36-44. doi:10.1016/j.cpme.2012.10.001
23. Lademann J, Ulrich C, Patzelt A, et al. Risk assessment of the application of tissue-tolerable plasma on human skin. *Clin Plasma Med*. 2013;1(1):5-10. doi:10.1016/j.cpme.2013.01.001
24. Daeschlein G, Scholz S, Ahmed R, et al. Cold plasma is well-tolerated and does not disturb skin barrier or reduce skin moisture. *JDDG - J Ger Soc Dermatol*. 2012;10(7):509-515. doi:10.1111/j.1610-0387.2012.07857.x

AUTHOR CORRESPONDENCE

Peter C. Friedman FAAD

E-mail:..... pbc9@columbia.edu