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Research Article

Cold Atmospheric Plasma Inhibits Lipogenesis and Proliferation of Human Sebocytes and Decreases Sebum Production in Human Facial Skin

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Background. Although several energy devices targeting sebaceous glands have been developed, an effective and safe therapeutic tool for hyperseborrhea is still needed. Nonthermal atmospheric-pressure plasma (NTAPP) induces microscopic tissue reactions in sebaceous glands of rat skin. *Objective*. The aim of the study is to investigate the effects of NTAPP on sebum production in human skin *in vivo* followed by an experimental study of human sebocytes. *Methods*. Fourteen healthy volunteers with oily facial skin underwent three sessions of argon- and nitrogen-NTAPP treatment at a 1-week interval and were followed up for 8 weeks. The casual sebum level, sebum excretion rate, and porphyrin index were evaluated. Histological analysis was performed using skin biopsy specimens taken from two subjects at the baseline and week 2. SZ95 sebocytes were stimulated with testosterone and linoleic acid (T/LA) with or without treatment with NTAPP. BODIPY and Nile red staining were used for qualitative lipids analysis. Proliferation and differentiation markers were also assessed. *Results*. Casual sebum levels and sebum excretion rates in facial skin decreased by 26 and 24%, respectively, at week 4 compared to those of the baseline. Porphyrin index also decreased by 38% at week 2. Histologically, NTAPP-treated human skin showed no obvious thermal injury, but the number of $Ki67^+$ cells in the sebaceous glands decreased at week 2. Argon- and nitrogen-NTAPP attenuated T/LA-induced increases in neutral lipid accumulation, $Ki67^+$ cells, and peroxisome proliferator-activated receptor-ɣ transcription in human sebocytes at energy settings that did not induce apoptosis. *Conclusion*. Argon- and nitrogen-NTAPP can be a safe and effective therapeutic tool for hyperseborrhea-associated diseases such as acne. This trial is registered with NCT04917835.

1. Introduction

Plasma is a partly ionized gas created by supplying energy to various gaseous sources [1, 2]. Among these, nonthermal atmospheric-pressure plasma (NTAPP) is generated at the room temperature range and hence can be applied to human tissues without direct thermal tissue damage [1–8]. Based on its bactericidal properties and its effects on cell proliferation and migration, NTAPP has recently been applied to treat various infections and acute and chronic wounds [9–12]. Several inert gaseous sources, particularly argon and nitrogen, are used to generate NTAPP for medical purposes [13–15]. Recently, we reported that argon- and nitrogen-NTAPP induced tissue reactions in the sebaceous glands in rat skin [16]. Argon-NTAPP induced a vacuolar tissue reaction in the epidermis, which propagated along the follicular epithelium to the entrance of the sebaceous duct and caused thermal modification in sebaceous cells but not in the surrounding dermis. Low-energy nitrogen NTAPP also elicited thermal tissue reactions in the sebaceous glands,

while preserving the surrounding dermal stromal tissue [16]. NTAPP-generated reactive oxygen and nitrogen species have been shown to penetrate the skin not only across the stratum corneum lipid layer but also through the appendageal route such as hair follicles and sebaceous glands [17, 18]. Indeed, nitrogen plasma treatment on the rat skin led to an increase in the diameter of hair follicles on day 15 post-treatment [19]. These findings suggest potential effects of these ionized-gas sources on hair follicles, leading us to hypothesize that NTAPP may also affect the sebaceous glands surrounding hair follicles. However, supporting data are lacking. Excess sebum production can contribute to the development of acne vulgaris. Therefore, we investigated the effect of NTAPP using argon- and nitrogen gas on sebum production in oily human skin. The effects of NTAPP on sebocyte functions, including lipogenesis, proliferation, and differentiation, were also investigated in cultured human sebocytes *in vitro*.

2. Materials and Methods

2.1. Human Study. This study was approved by the Institutional Review Board of the Yonsei University College of Medicine (IRB No. 3-2019-0239) and carried out in accordance with the Declaration of Helsinki and Good Clinical Practice as defined under the Republic of Korea Food and Drug Administration. Written informed consent was obtained from all participants prior to inclusion in the study (ClinicalTrials.gov identifier: NCT04917835).

Fourteen healthy volunteers aged 19–35 years with oily skin, defined as high casual sebum levels (>220 μg/cm [2] on forehead and >180 μg/cm [2] on cheeks) [20, 21] when measured using a Sebumeter® (SM815; Courage + Khazaka Electronic GmbH, Cologne, Germany), were enrolled in and completed the study (Table 1). Volunteers who had been treated with systemic or topical retinoids within the prior 6 months or 4 months, respectively, had acne of grade 3 or higher, had a history of hypertrophic scars or keloids, or were pregnant were excluded. To avoid diurnal variation, sebum levels were measured between 10:00 and 12:00 hours. Participants were instructed not to wear cosmetics for 2 hours before the measurements. All measurements were made by the same trained physician in the same room under conditions of 20–22°C and 20–40% humidity after 30 min of acclimatization.

To examine the amount of porphyrins in the facial skin, we used digital UV photographs with a Mark-Vu® skin analysis imaging system (PSI Plus, Suwon, Republic of Korea). Pictures were obtained from all 5 zones of the face (forehead, nose, chin, left cheek, and right cheek). The resulting images were analyzed for RGB (red, green, and blue) values. The area of sebum spots was determined by calculating the percentage of the area covered by white and red spots. UV-induced red fluorescence in the follicle indicates the presence of porphyrins. Thus, only the red fluorescence spots were measured to determine the amount of porphyrin. The porphyrin index was determined as the ratio of the area occupied by porphyrin to the area of sebum spots and expressed as a percentage. The average porphyrin index was the average of porphyrin index values obtained from three independent images.

Table 1: Subject demographics.

Characteristics	Value ($n = 14$)
Age, years	
Mean ± standard deviation	28.1 ± 5.2
Range	19–35
Sex, n (%)	
Male	7 (50)
Female	7 (50)
Sebumeter values (mg/cm^2)[a]	
Forehead	238.2 ± 58.18
Both cheeks	191.7 ± 51.66
Fitzpatrick's skin type, n (%)	
I	0
II	0
III	14 (100)
IV	0
V	0
Republic of Korean acne grading system, n (%)	
1	12 (86)
2	7 (14)

[a]Data are presented as mean ± standard deviation.

An NTAPP generator (PlaDuo™; Shenb Co., Ltd., Seoul, Republic of Korea) was utilized to generate argon- and nitrogen-NTAPP pulses. 2.45 GHz microwave energy converted the loaded gaseous sources to plasma. All participants underwent three NTAPP treatment sessions at a 1-week interval. Two passes of argon (0.8 J/pulse with a 12 ms of pulse duration)- and nitrogen (0.75 J/pulse with a 7 ms of pulse duration)-NTAPP treatment were sequentially performed on forehead, nose, and both cheeks in each session. The nozzle diameter was 5 mm, and the distance from the nozzle tip to the skin was 10 mm. The treated areas were cooled with icepacks immediately after treatment. All participants were followed up at 2, 4, and 8 weeks after the last session of treatment. The extent of erythema, edema, pain, desquamation, and post-inflammatory hyperpigmentation were scored at each visit by grading on a visual analog scale of 0 to 3 (0: absent; 1: mild; 2: moderate; 3: severe). At the baseline and at week 2, punch biopsies (2 mm) were obtained from the skin of the zygomatic area of two volunteers who completed the study. Skin specimens were sectioned and stained with hematoxylin and eosin. Cell proliferation was assessed using sections of skin specimens stained with a rabbit anti-mouse Ki67$^+$ antibody (1 : 200; Abcam, Cambridge, MA, USA). The stained slides were observed under a BX51 Olympus microscope.

2.2. SZ95 Sebocyte Culture and Treatment. The immortalized human SZ95 sebaceous gland cell line [16] was cultured in Dulbecco's modified Eagle medium/F-12 culture medium supplemented with 2 mM GlutaMAX I, 10 μg/mL gentamicin, 50 ng/mL human epidermal growth factor, 10% fetal bovine serum, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (all purchased from Gibco BRL, Gaithersburg, MD, USA) and maintained at 37°C and 5% CO_2. Sebocytes were seeded into 6-well plates and allowed to reach approximately 90% confluence. To stimulate

lipogenesis, sebocytes were treated with a combination of 2×10^{-8} M testosterone and 10^{-4} M linoleic acid (T/LA) for 48 hours. All compounds were diluted in dimethyl sulfoxide (DMSO) and diluted with the culture medium (the final concentration of DMSO was 0.1%). Argon- or nitrogen-NTAPP were delivered to sebocytes immediately or 24 hours after treatment with T/LA. The 20 or 60 passes of argon-NTAPP at the energy of 0.75 J and pulse duration of 11 ms or 5 or 10 passes of nitrogen-NTAPP at the energy of 0.75 J and pulse duration of 13 ms were administered to each well at a 5 mm distance. All controls were maintained in the culture media during the experiment to ensure equivalent treatment conditions.

2.3. Assessment of Cell Viability. SZ95 sebocytes were seeded into six-well plates at a density of 2×10^5 cells/well and then irradiated with 5, 10, 20, or 60 pulses of argon-NTAPP at the energy of 0.75 J or 5, 10, 20, or 60 pulses of nitrogen-NTAPP at the energy of 0.75 J and then incubated for 48 hours. The cells were then incubated with 5 mg/mL of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 2 h. The MTT solution was removed, and then, 200 μL of DMSO was added to dissolve the formazan crystals. The optical density was measured at 540 nm using a spectrophotometer.

2.4. Immunocytofluorescence. SZ95 sebocytes were grown on coverslips, fixed in acetone, permeabilized with 0.1% Triton X-100 (Sigma Aldrich, MO, USA) in PBS, and incubated with a rabbit anti-mouse Ki67 (1 : 200; Abcam, MA, USA) antibody for 60 min at 37°C. Slides were incubated with a goat anti-rabbit FITC-conjugated secondary antibody (Santa Cruz Biotechnology, CA, USA) for 30 min at room temperature, and nuclei were visualized with propidium iodide (PI) (Vector, CA, USA). They were examined using an LSM 780 confocal microscope (Carl Zeiss, Jena, Germany).

2.5. Lipid Detection. For the quantitative measurement of lipids, SZ95 sebocytes (15,000 cells/well) were cultured in 96-well plates and then treated with T/LA with or without NTAPP treatment. Supernatants were then discarded and 100 μL of 1 μg/mL Nile red (Sigma Aldrich, MO, USA) was added. Primary antibodies diluted in TBS containing 0.05% solution were added to each well. The plates were incubated at 37°C for 20 min, and the emitted fluorescence was measured using a Molecular Devices' FlexStation 384II fluorescence image microplate reader (Molecular Devices, CA, USA). The results are presented as percentages of absolute fluorescence units compared with the controls, at excitation and emission wavelengths of 485 and 565 nm, respectively, for neutral lipids. To detect lipid droplets, 4% PFA-fixed cells were incubated with BODIPY™ 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) (10 μg/mL in PBS; Thermo Fisher Scientific, MA, USA) for 7 min at 37°C. After incubation, the slides were washed with 0.5% BSA-PBS and imaged under a LSM 780 confocal microscope (Carl Zeiss, Jena, Germany).

2.6. Real-Time Quantitative Polymerase Chain Reaction. RNA was extracted from SZ95 sebocytes using TRIzol reagent, according to the manufacturer's protocol, and cDNA was synthesized using a cDNA synthesis kit (Thermo Fisher Scientific, CA, USA). TaqMan real-time polymerase chain reaction (PCR) assays were performed to analyze mRNA levels (Applied Biosystems, CA, USA). TaqMan probe for PPARγ (Hs01115514_m1, Thermo Fisher Scientific, CA, USA) was used. The relative mRNA expression was normalized to the mean level of GAPDH mRNA (Hs02786624_g1, Thermo Fisher Scientific, CA, USA).

2.7. Statistical Analysis. All *in vitro* experiments were repeated at least three times with different batches of cells. Statistical significance was determined using two-tailed unpaired Student's *t*-test or Wilcoxon matched-pairs signed rank test. A P value less than 0.05 was considered significant. All statistics are reported in the figure legends. Data analyses were carried out using GraphPad Prism version 8.

3. Results

3.1. Argon- and Nitrogen-NTAPP Treatment Reduced Facial Sebum Excretion in Oily Human Skin. First, we investigated the effect of argon- and nitrogen-NTAPP pulses on sebum production in human facial skin. At the baseline, the mean (±SD) casual sebum levels on the forehead and both cheeks were 238.2 ± 58.18 and 191.7 ± 51.66 mg/cm^2, respectively. A significant reduction in casual sebum levels was found on the forehead and both cheeks at 2 and 4 weeks after NTAPP treatment compared to the baseline values. The reduction rates of casual sebum levels on the forehead and cheeks were 16% and 19.1%, respectively, after 2 weeks and 28% and 23.2%, respectively, after 4 weeks. But no significant difference was found between casual sebum levels at 8 weeks and the baseline (Figure 1(a)). In addition, the sebum excretion rate of the forehead decreased significantly by 26.6% at 4 weeks. There was also a significant decrease in sebum excretion rates of both cheeks by 15.5% and 22.9% at week 2 and week 4, respectively, compared to the baseline values (Figure 1(b)). Most subjects experienced mild temporary erythema, which spontaneously resolved within 24 hours. No treatment-related severe adverse events were observed during the 8-week follow-up period.

3.2. Porphyrin Index in the Seborrheic Area Decreased following Argon- and Nitrogen-NTAPP Treatment. *Cutibacterium acnes* (*C. acnes*) is a commensal bacterium that resides predominantly in the sebaceous gland-rich areas and has been implicated in the pathogenesis of acne. To examine the effect of argon- and nitrogen-NTAPP pulses on the abundance of *C. acnes* on the seborrheic area of the face, the content of porphyrins was analyzed in seven subjects at the baseline and week 2 using digital UV photographs. Treatment with argon- and nitrogen-NTAPP pulses resulted in a significant decrease in the average

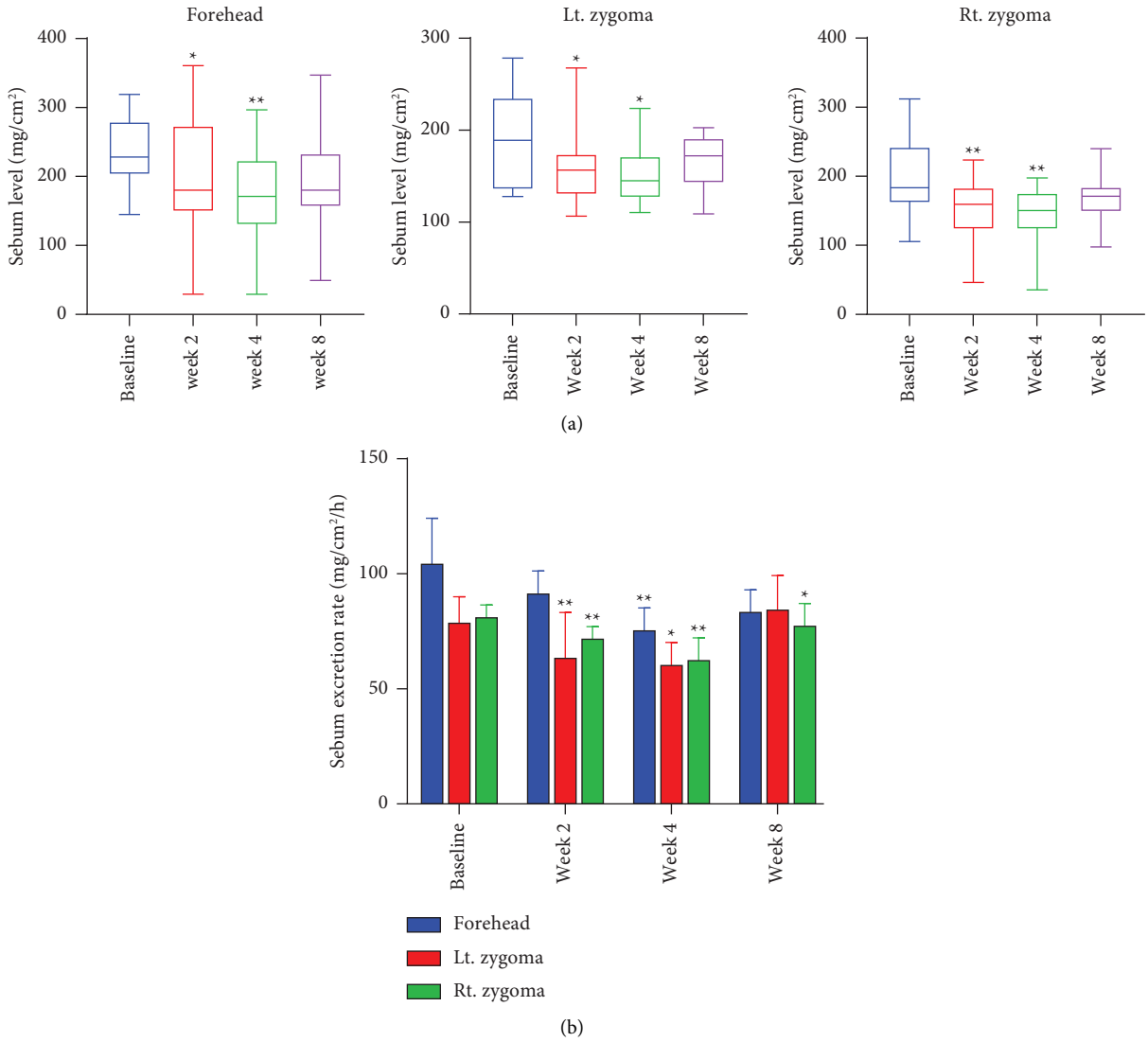

Figure 1: Effects of argon- and nitrogen-NTAPP pulses on facial sebum excretion in subjects with oily skin. The casual sebum level (a) and the sebum excretion rate (b) were measured using a Sebumeter at the baseline and at follow-up visits on weeks 2, 4, and 8. Values are presented as the mean ± standard error of the mean (SEM; $n = 14$). Data were analyzed by Wilcoxon matched-pairs signed rank test ($^{*}P < 0.05$, $^{**}P < 0.01$, vs. the baseline).

porphyrin index on the forehead and both cheeks by 27.7 and 37.7%, respectively, compared to baseline values at week 2 (Figure 2).

3.3. Argon- and Nitrogen-NTAPP Treatment Decreased Proliferation Marker Ki67 in the Basal Sebaceous Gland Cells and Epithelial Cells in Sebaceous Ducts in Oily Human Skin. To investigate the effect of NTAPP pulses on the microscopic structure of the sebaceous glands in the human skin, punch biopsies were performed from the zygomatic area of two volunteers at the baseline and week 2. There were no apparent coagulative changes in the sebaceous glands and surrounding dermis in skin samples taken at 2 weeks after treatment. Although hematoxylin-eosin staining did not reveal obvious changes in the number or size of sebaceous glands, the number of $Ki67^{+}$ cells in the basal layer of sebaceous glands and in the epithelium of sebaceous ducts significantly decreased at week 2 compared to the baseline, as determined by immunohistochemistry (Figure 3).

3.4. Argon- and Nitrogen-NTAPP Pulses Suppressed Lipid Production in Cultured Human SZ95 Sebocytes. To elucidate the mechanisms involved in the sebosuppressive effects of argon- and nitrogen-NTAPP pulses in oily human skin *in vivo*, we investigated whether the NTAPP treatment directly modulates the lipid synthesis in SZ95 sebocytes, which possess the major characteristics of human sebocytes [14]. We first examined the effect of argon- or nitrogen-NTAPP pulses on the viability of sebocytes by an MTT assay at the same energy settings performed *in vivo* on human skin. 5, 10, 20, and 60 passes of argon plasma pulses at the energy of 0.75 J or 5 and 10 passes of nitrogen plasma

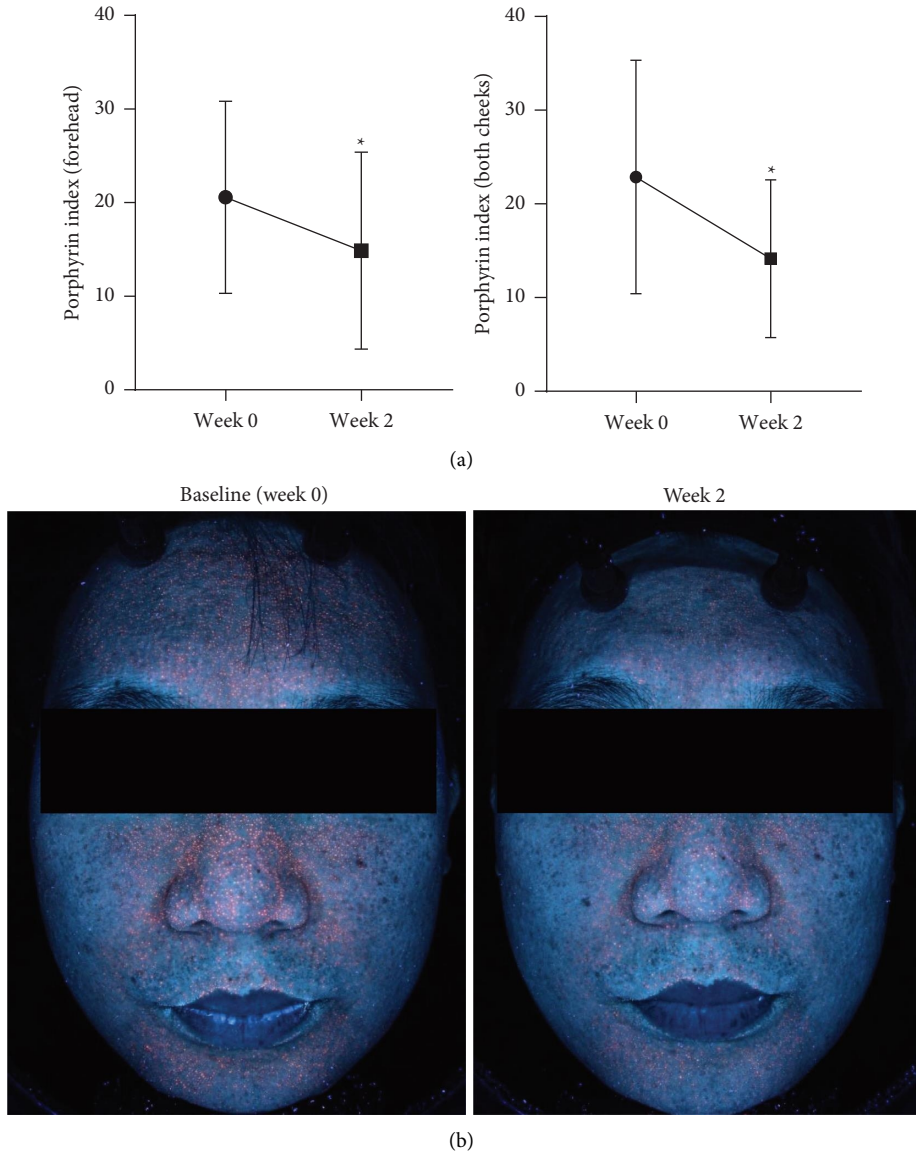

Figure 2: Argon- and nitrogen-NTAPP pulses decreased porphyrins produced by *C. acnes* in seborrheic areas of oily human skin. (a) Porphyrin index determined by a digital photography analyzer of ultraviolet photographs taken at the baseline and 2 weeks after argon- and nitrogen-NTAPP pulses treatment. Values represent the mean ± SEM ($n = 7$). Data were analyzed Wilcoxon matched-pairs signed rank test ($^*P < 0.05$ vs. baseline). (b) Representative ultraviolet photographs at baseline and week 2.

pulses at the energy of 0.75 J did not induce detectable changes in sebocyte viability over 48 hours, but 20 and 60 passes of nitrogen plasma pulses (0.75 J) slightly reduced the cell viability at 48 hours (Figure 4(a)). Therefore, subsequent experiments used 20 or 60 passes of argon plasma pulses at 0.75 J of energy and 5 or 10 passes of nitrogen plasma pulses at 0.75 J of energy. To investigate the effect of NTAPP on pro-acne agents-induced lipogenesis in sebocytes, we treated argon- or nitrogen-NTAPP pulses to SZ95 sebocytes immediately or 24 hours after treatment with a combination of testosterone and linoleic acid (T/LA), and then, the intracellular neutral lipid contents were assessed by BODIPY and quantitative Nile red staining. 20 or 60 pulses of argon-NTAPP at 0.75 J, immediately or 24 hours after the treatment with T/LA, significantly suppressed T/LA-enhanced lipid accumulation in sebocytes as assessed by BODIPY staining (Figures 4(b) and 4(c)) and quantitative Nile red staining (Figure 4(d)). In addition, 5 or 10 pulses of nitrogen-NTAPP pulses at 0.75 J also suppressed T/LA-induced lipogenesis in sebocytes when applied immediately or 24 hours after T/LA treatment (Figures 4(b)–4(d)). We also found that 60 passes of argon-NTAPP (0.75 J) or 5 or 10 passes of nitrogen-NTAPP (0.75 J) suppressed the T/LA-induced upregulation of mRNA levels of PPARγ, a potential modulator of sebocyte differentiation and lipid synthesis (Figure 4(e)). These results demonstrate that argon- and nitrogen-NTAPP suppresses lipogenesis in sebocytes and also suggest that the inhibition of PPARγ induction may partially contribute to the sebosuppressive effect of NTAPP.

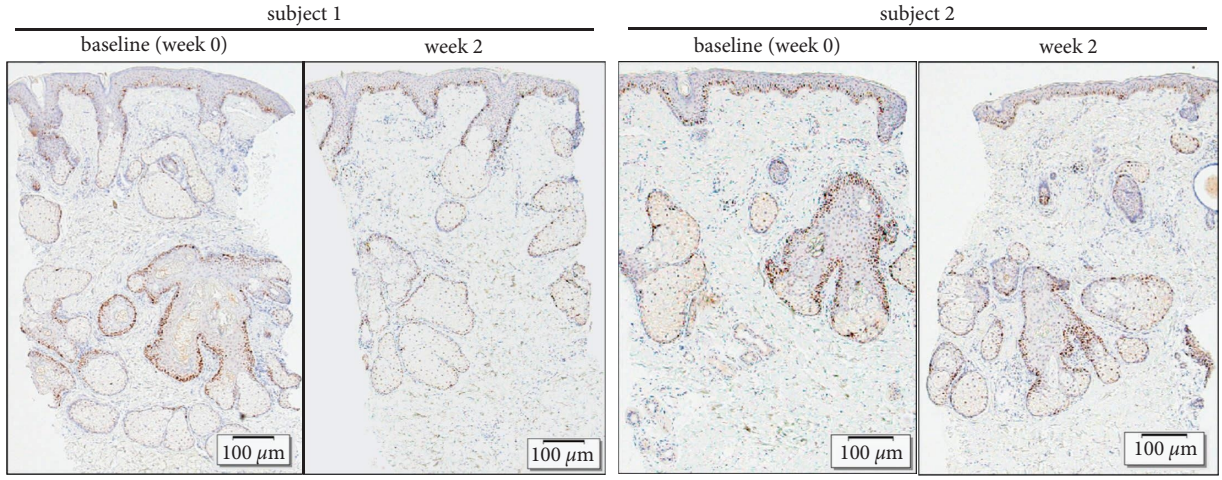

Figure 3: Representative immunohistochemistry of Ki67 in facial human skin at the baseline and 2 weeks after plasma treatment. Positively immunostained cells appear brown, and nuclei are blue. Scale bars: 100 μm.

3.5. Argon- and Nitrogen-NTAPP Pulses Inhibited Proliferation of SZ95 Sebocytes in vitro. Then, we examined the effects of NTAPP pulses on the proliferation of cultured SZ95 sebocytes by measuring $Ki67^+$ cells. As previously reported, T/LA treatment enhanced the proliferation of SZ95 sebocytes. The 20 or 60 pulses of argon-NTAPP treatments at 0.75 J or 5 or 10 pulses of nitrogen-NTAPP treatment at 0.75 J significantly attenuated the T/LA-induced increases in $Ki67^+$ cells when applied immediately or 24 hours after T/LA treatment (Figures 5(a) and 5(b)). Our data suggest that argon- and nitrogen-NTAPP pulses at these energy settings inhibit the proliferation of human sebocytes without inducing apoptosis.

4. Discussion

In this study, we demonstrated that argon- and nitrogen-NTAPP treatment significantly reduced sebum excretion in the oily facial skin, with a maximal effect at week 4. NTAPP treatment also decreased Ki67-positive proliferating cells within sebaceous glands in the oily skin. To the best of our knowledge, this is the first report that studies the effect of NTAPP on the sebum production in humans [22].

Our previous study, using a rat skin model, showed that five pulses of argon plasma at energy settings ranged between 0.35 J and 0.85 J or nitrogen plasma at energy settings ranged between 1.0 J and 2.0 J induced gray thermal tissue reactions in the sebaceous glands without remarkable carbonization [16]. In contrast, the histological examination of argon- and nitrogen-NTAPP-treated human skin revealed no apparent changes associated with the thermal tissue reaction in sebaceous glands in this study. This discrepancy may be attributed to differences in the energy settings and the number of pulses. Compared to the animal model, human skin was treated with lower energies and smaller number of pulses of argon (0.8 J/pulses and two passes per each session)- and nitrogen (0.75 J/pulses and two passes per each session)-NTAPP pulses for safety reasons. In addition, the effect of NTAPP at the same energy setting might be overestimated in rat skin because human skin is less permeable than the rat skin. Moreover, tissue changes in the rat skin were examined serially on days 1, 5, and 7 after plasma treatment, but human tissues were examined at 2 weeks after treatment; thus, the possibility of an early transient tissue reaction cannot be ruled out. Further time-course examination of the histological changes in the human skin following NTAPP pulses at various energy settings will be needed for future clinical applications. Taken together, our findings suggest that argon- and nitrogen-NTAPP treatment suppresses sebum production in oily human skin, likely through inhibiting ductal and sebaceous cell proliferation rather than thermal damage to the sebaceous glands.

Consistent with data from our clinical study, we found that argon- or nitrogen-NTAPP pulses suppressed the T/LA-induced lipogenesis in SZ95 sebocytes, suggesting that NTAPP can inhibit excessive sebum production induced by acne-promoting stimuli, such as testosterone and linoleic acid. Consistent with the histological changes in the NTAPP-treated human skin, we also found that both argon- and nitrogen-NTAPP pulses, in an energy setting that does not induce apoptosis, decreased $Ki67^+$ cells in T/LA-treated sebocytes. These findings suggest that antiproliferative effect of NTAPP on human sebocytes *in vitro* and *in vivo* may contribute to the reduction of sebum production. In addition, we showed that argon- and nitrogen-NTAPP pulses during the initial 24 hours of T/LA-induced differentiation inhibited the induction of PPARγ gene expression in sebocytes. These data suggest that inhibition of PPARγ signaling might partly be associated with NTAPP's sebosuppressive effect.

Notably, argon- and nitrogen-NTAPP treatment significantly reduced porphyrin levels in oily human skin. Given that porphyrins are mainly produced by *C. acnes*, these findings suggest a suppressive effect of NTAPP against

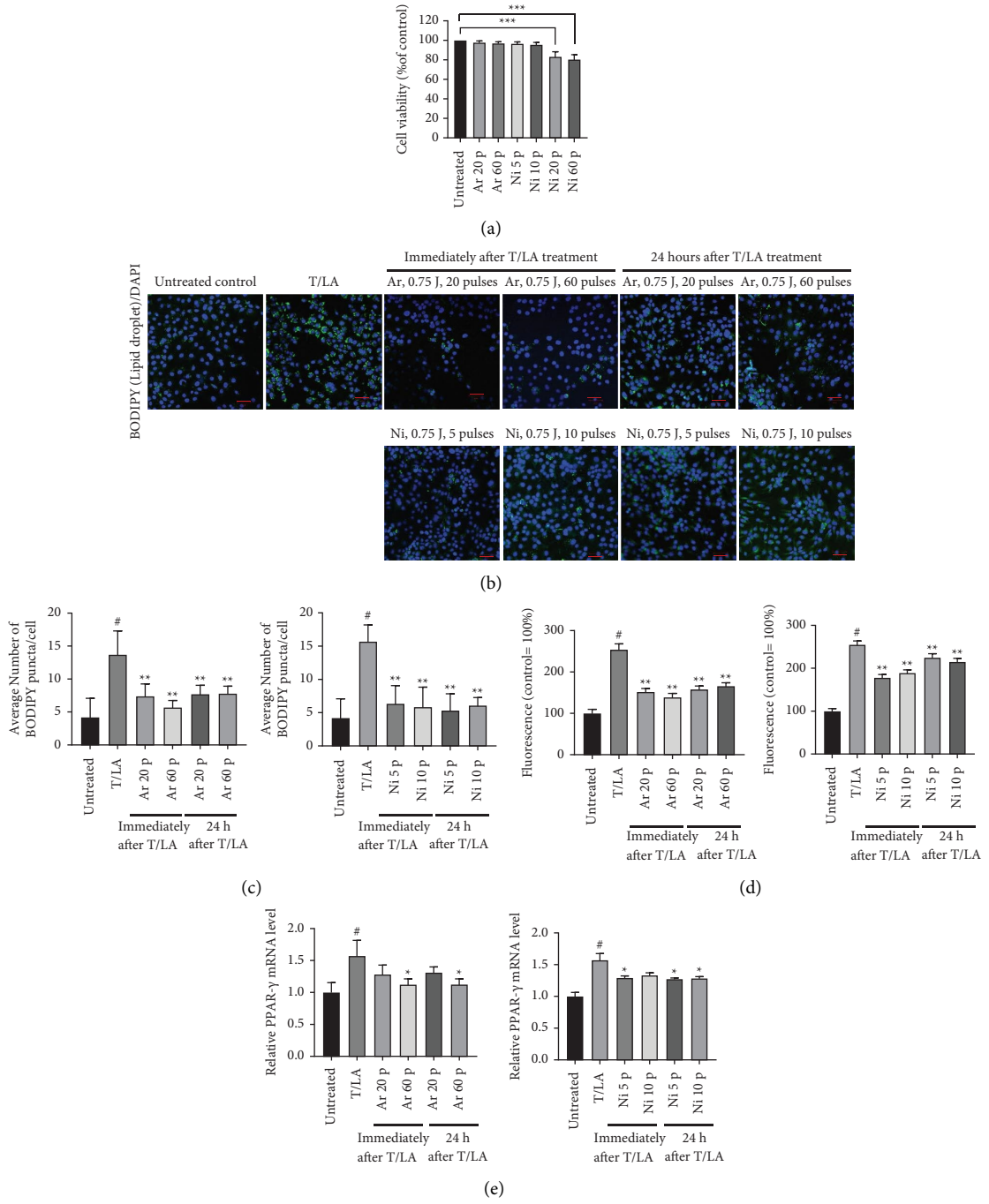

FIGURE 4: Effect of argon- and nitrogen-NTAPP pulses on the viability and lipid synthesis of human sebocytes. (a) SZ95 sebocytes were treated with argon- and nitrogen-NTAPP pulses for 48 hours and then subjected to an MTT assay. Data are shown as the mean ± SEM ($n = 3$). (b) Representative confocal immunofluorescence images of BODIPY in SZ95 sebocytes treated with T/LA alone for 48 hours or in combination with argon- and nitrogen-NTAPP pulses immediately or 24 hours after T/LA treatment. Scale bars represent 50 μm. (c) Average numbers of BODIPY-positive puncta in SZ95 sebocytes are shown as a bar graph. Data indicate the mean ± SEM ($n = 25$). (d) Neutral lipids were quantified by Nile red staining and then measured using a fluorometric imaging plate reader. Data indicate the mean ± SEM. (e) PPARɣ mRNA expression was analyzed by quantitative PCR analysis. Results are normalized to the internal control GAPDH and are shown relative to those in control cells incubated in the culture medium. Data are expressed as the mean ± SEM of three independent experiments. $^{\#}(P < 0.05)$ versus the untreated control and $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus T/LA-treated cells by two-tailed unpaired Student's t-test. T/LA, testosterone/linoleic acid; Ar, argon; Ni, nitrogen; p, pulses.

C. acnes. Antimicrobial effects of cold atmospheric plasma in the biological environment and on artificial surfaces are well-known [23, 24]. Therefore, further research is needed to determine whether argon- and nitrogen-NTAPP reduce the abundance of *C. acnes* by killing the organisms directly or by indirectly reducing the production of sebum.

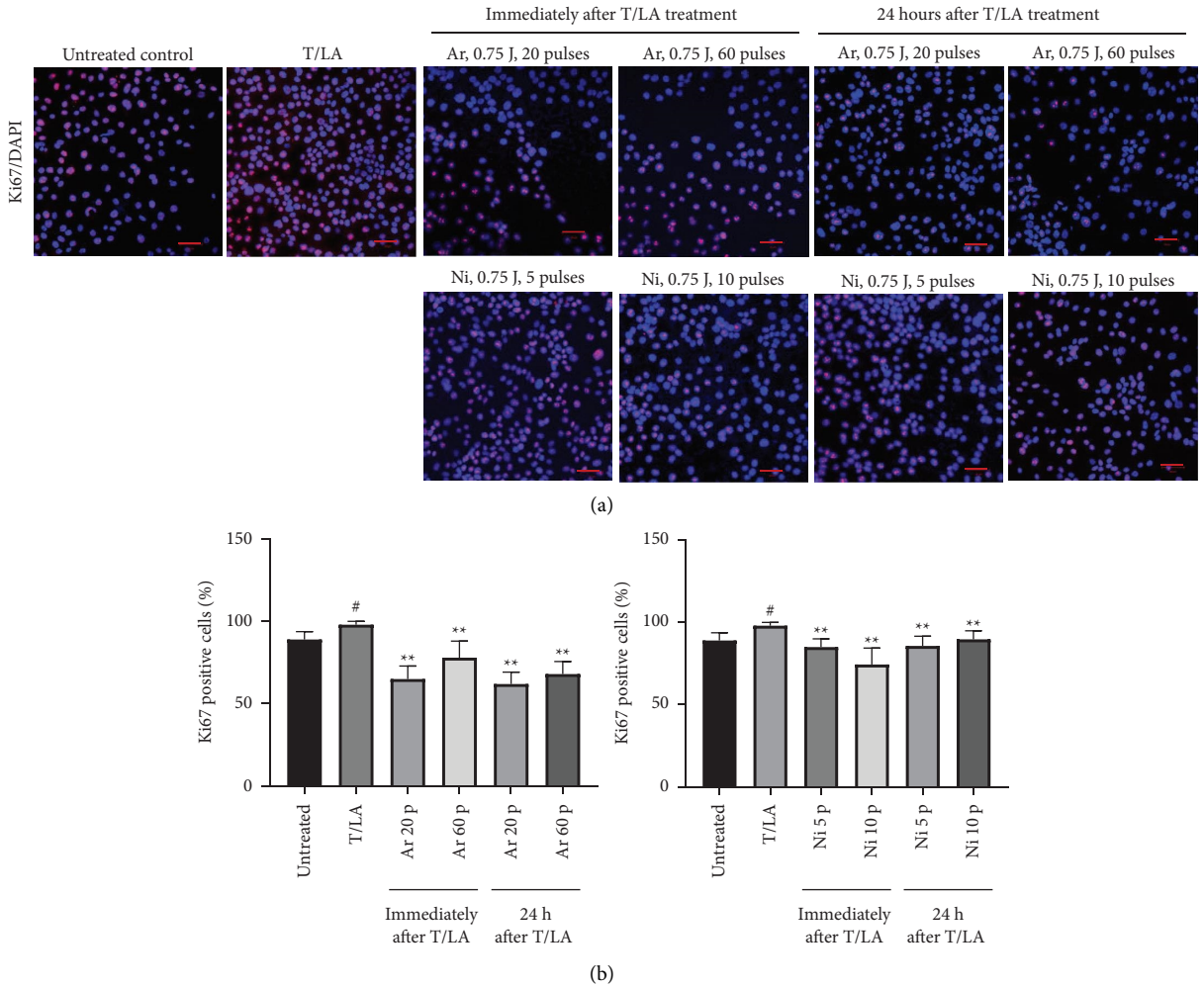

Figure 5: Argon- and nitrogen-NTAPP pulses regulate the proliferation of cultured SZ95 sebocytes. (a) Immunofluorescence of SZ95 sebocytes treated with T/LA alone for 48 hours or in combination with argon- and nitrogen-NTAPP pulses stained for Ki67. The scale bars represent 50 μm. (b) The percentage of nuclear Ki67 positive cells to the total DAPI positive cells in T/LA-treated SZ95 cells with or without co-treatment with argon- and nitrogen-plasma pulses. Data are shown as the mean ± SEM. $^{\#}(P < 0.05)$ versus the untreated control and $^{**}P < 0.01$ versus T/LA-treated cells by two-tailed unpaired Student's t-test. T/LA, testosterone/linoleic acid; Ar, argon; Ni, nitrogen; p, pulses.

Our study has several limitations, such as relatively small numbers of participants and being a single-arm design, which limits definitive conclusions regarding efficacy measures. However, given that the human study was conducted between February and May, it is unlikely that sebum secretion decreased due to the seasonal changes.

In conclusion, our study suggests that argon- and nitrogen-NTAPP can be a promising alternative energy-based therapy targeting sebaceous glands without inducing thermal tissue damage. Given its effects on sebum production and porphyrin levels in the oily skin, argon- and nitrogen-NTAPP pulses can be potentially applied for the treatment of acne vulgaris. Future clinical trials are needed to assess the safety and effectiveness of varied energies of argon- and nitrogen-NTAPP pulses in patients with acne vulgaris and hyperseborrhea.

Data Availability

No data were used to support this study.

Ethical Approval

This study was approved by the Institutional Review Board of the Yonsei University College of Medicine (IRB No. 3-2019-0239) and carried out in accordance with the Declaration of Helsinki and Good Clinical Practice as defined under the Republic of Korea Food and Drug Administration.

Consent

Written informed consent was obtained from all participants prior to inclusion in the study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Sung Bin Cho and Sang Eun Lee conceived and designed this project. Seungju Lee and Dae San Yoo were involved in the acquisition, analysis, and interpretation of clinical data. Song-Ee Kim performed and analyzed the experiments using sebocytes. Christos C. Zouboulis gave critical comments. Taehee Kim and Sang Eun Lee wrote the manuscript with the approval of all the other authors.

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