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Efficacy and Safety of Cold Plasma Vs. Low-Level Light Therapy for Treatment of Chronic Wounds in Patients with Recessive Dystrophic Epidermolysis Bullosa: A Pilot Randomized Controlled Study

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Abstract

Introduction: Diverse therapeutic options have been proposed to treat individuals with recessive dystrophic epidermolysis bullosa (RDEB), though no definite therapy has been established despite advancements in wound care. In this case series, we examined the effects of atmospheric pressure plasma jet (APPJ) and low-level light therapy in treating the cutaneous lesions of individuals with RDEB.

Methods: We evaluated wound healing in six RDEB patients in a pilot clinical case study carried out at the dermatology clinic of our institute in 2019. In each patient, two lesions were treated with low-level light therapy (LLLT) using a light-emitting diode device (660/850 nm) and another two lesions with APPJ. Also, one lesion, representing the control group, was dressed daily with Vaseline gauze. All patients received therapeutic modalities twice a week on Saturdays and Tuesdays for six therapeutic sessions.

Results: All patients experienced complete wound healing except one in the plasma group and four in the control group. Complete wound healing occurred after 3.44 ± 1.59 and 5.25 ± 2.18 half-weeks in the APPJ and LLLT groups, respectively ($P < 0.05$). The time to complete remission was significantly lower with APPJ than with LLLT. Both LLLT and APPJ decreased the wound area significantly. Furthermore, the wound area under APPJ treatment was reduced faster than the control.

Conclusion: Considering the satisfactory results of this study, both LLLT and APPJ are recommended in the treatment of chronic wounds in patients with EB, and future studies should focus on combination treatment.

Keywords: Cold plasma, Chronic wound, Epidermolysis bullosa, Low-level light therapy, Non-thermal atmospheric pressure plasma

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Introduction

No definite treatment has been recognized for individuals with recessive dystrophic epidermolysis bullosa (RDEB).[1,2] Various treatment modalities have been proposed, though clinical trials with a sufficient sample size are absent.[3] The priority for treating individuals with RDEB is managing complications related to the disease course, including wound care, infection prevention, surgical correction of contracture, and consideration of the long-term development of squamous cell carcinoma.[4,5]

One of the challenging issues in individuals with RDEB is the management of erosions and wounds. There are diverse modalities for wound care in RDEB, consisting of biological dressings, topical therapies, cultured epidermal autografts, allogeneic cultured dermal substitutes, and wound grafting.[6] In addition, cell therapy with intravenous or intradermal fibroblasts, human amniotic membranes, mesenchymal stem cells, or bone marrow transplantation is another proposed option for wound healing in RDEB.[1,7,8] Novel options for wound management in RDEB are gene and protein therapy.[4,9] Still, further investigation is necessary regarding treatment.

Plasma is appraised as a fourth state of matter with distinctive features setting it apart from solids, fluids, and gases. Plasma as a new modality has been employed in various fields, especially dermatology.[10,11] In dermatology, some of the indications of plasma are sterilization or disinfection, skin infection treatment, and skin rejuvenation.[12-14] Several studies investigated the efficacy of non-thermal plasma in wound healing.[15,16] Cold atmospheric plasma promotes wound healing through the generation of reactive oxygen and nitrogen species, which exert potent antimicrobial effects, modulate inflammation, and enhance keratinocyte and fibroblast proliferation. Moreover, it stimulates angiogenesis and extracellular matrix remodeling by upregulating growth factors and cytokines, thereby accelerating tissue regeneration and re-epithelialization.[11]

Furthermore, recent studies reported the advantage of diverse lasers in accelerating wound healing. Also, some researchers examine the efficacy of different lasers in treating cutaneous lesions in RDEB.[17,18] Mester et al. first established the biostimulatory action of low-level light therapy (LLLT) in the late 1960s. Theoretically, the absorption of coherent light of LLLT by dermal targets such as fibroblasts enhances wound healing.[19] Recent cell studies reported that LLLT reduces TNF-α concentrations and increases fibroblast proliferation, thereby accelerating wound healing.[20]

In this case series, we investigated the effects of cold plasma and LLLT compared with a control in treating the cutaneous lesions of individuals with RDEB.

Materials and Methods

Study Design

In this investigation, we evaluated wound healing in individuals with recessive dystrophic epidermolysis bullosa (RDEB) in a pilot clinical study carried out at the dermatology clinic of our institute in 2019. The study protocol, which was conducted in accordance with the Declaration of Helsinki, was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.SRC.REC.1394.13; *IRCT registration number: IRCT20160320027109N2*).

The required sample size was calculated via the paired t-test formula, employing the size of wound reduction.

Participants

Six individuals with chronic RDEB wounds (older than 6 months) were included in the study. The inclusion criteria were patients with confirmed RDEB who had at least five chronic wounds persisting for more than six months and who had not received active wound-directed therapies other than supportive wound care before study enrollment. We obtained written informed consent from each patient or their parents where appropriate. The diagnosis of RDEB was established based on clinical features and histopathological findings.

Patients receiving prior treatments for their cutaneous lesions, except supportive wound care, were excluded.

Interventions

Five wounds persisting for more than six months were selected in each patient. In patients presenting with more than five eligible chronic wounds, five wounds were selected using a random number allocation method. Four wounds were randomly (block randomization) chosen to be treated with APPJ (two lesions) or LLLT (two wounds). Also, one wound was selected randomly to be dressed daily with Vaseline gauze as standard wound care (SWC) in the control group.[21] Antibiotic ointments (mupirocin) were prescribed for all groups on an as-needed basis.

All wounds were debrided, cleaned, and measured in terms of area and presence of exudate during each session. All patients received therapeutic modalities twice a week on Saturdays and Tuesdays for six therapeutic sessions. For the evaluation of cutaneous lesions, we considered a half-week interval (from Saturday to Tuesday) between each therapy session, over six consecutive treatment sessions.

The primary study outcomes were (1) percentage reduction in wound area, calculated as [(baseline wound area − post-treatment wound area)/baseline wound area] × 100, and (2) time to 50% wound healing, defined as the time point at which at least 50% of the baseline wound area had healed.

Furthermore, during each session, we evaluated possible complications, including the formation of blisters and a sense of burning or pruritus, for each treatment modality. After three months, we evaluated every patient concerning recurrence or possible complications.

Devices and Techniques

Atmospheric Pressure Plasma Jet (APPJ) Setup

The APPJ setup is shown in Supplementary file, Figure S1. APPJ included two 20 mm-diameter copper disks with seven 4 mm-diameter holes used as high-voltage and

grounded electrodes with a distance of 20 mm apart, with seven 4 mm-diameter quartz tubes inside them.

An AC high-voltage power supply was driven at 22 kHz and 4.5 kV peak to peak. A mass flow controller (Alicat) delivered helium gas with a purity of 99.99% at a flow rate of 6 slm.

Plasma was produced between two electrodes and exposed to the surfaces of the wounds. In this study, each wound was treated with APPJ for one minute per square centimeter. The APPJ was located approximately 5 mm above the skin surface. Temperature and reactive species were monitored when plasma was applied.

Plasma Characterization

Optical emission spectroscopy (OES) is an established laboratory diagnostic technique for identifying and monitoring the intensity of chemical species and the frequency of plasma. In this study, we used an AvaSpec 3648 spectrometer (Avantes Co., Lasers Med Sci Netherlands) to identify the intensity of molecules and reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) exposed to the tissue.

Plasma Temperature

An infrared thermal imaging camera (FLIR E4; 80 x 60-pixel resolution & < 0.15°C thermal sensitivity) measured the temperature of the atmospheric pressure plasma jet (APPJ) device and the tissues to ensure the treatment was safe regarding thermal damage.

Electron Density and Plasma Frequency

As helium exits APPJ (Supplementary file, Figure S1a), it collides with the surrounding air and produces ROS and RNS. As shown in Figure 1a, the typical optical emission spectra and excited species produced by APPJ (flow 6 slm, frequency 22 kHz, and voltage 4.5 kV peak to peak) included short- and long-lived reactive species, such as OH radicals (at 308.96 nm as a result of H_2O dissociation), O_3 (at 315.31 nm), N_2 (at 337.13, 357.64, and 380.53 nm as the dominant component of the ambient air), helium (as the working gas), and O (at 777 and 845 nm as a result of O2 and H2O dissociation).

Electrical Analyses

Figure 1 depicts typical voltage, current, and power waveforms. The voltage waveform produced by the power supply was sinusoidal, and the amount of voltage was 4.5 kV peak to peak (Figure 1b). The typical distortion in the current waveform due to discharges is shown in Figure 1c, where the amount of current is less than 6 µA. The reactor power was measured using the Lissajous curve method,

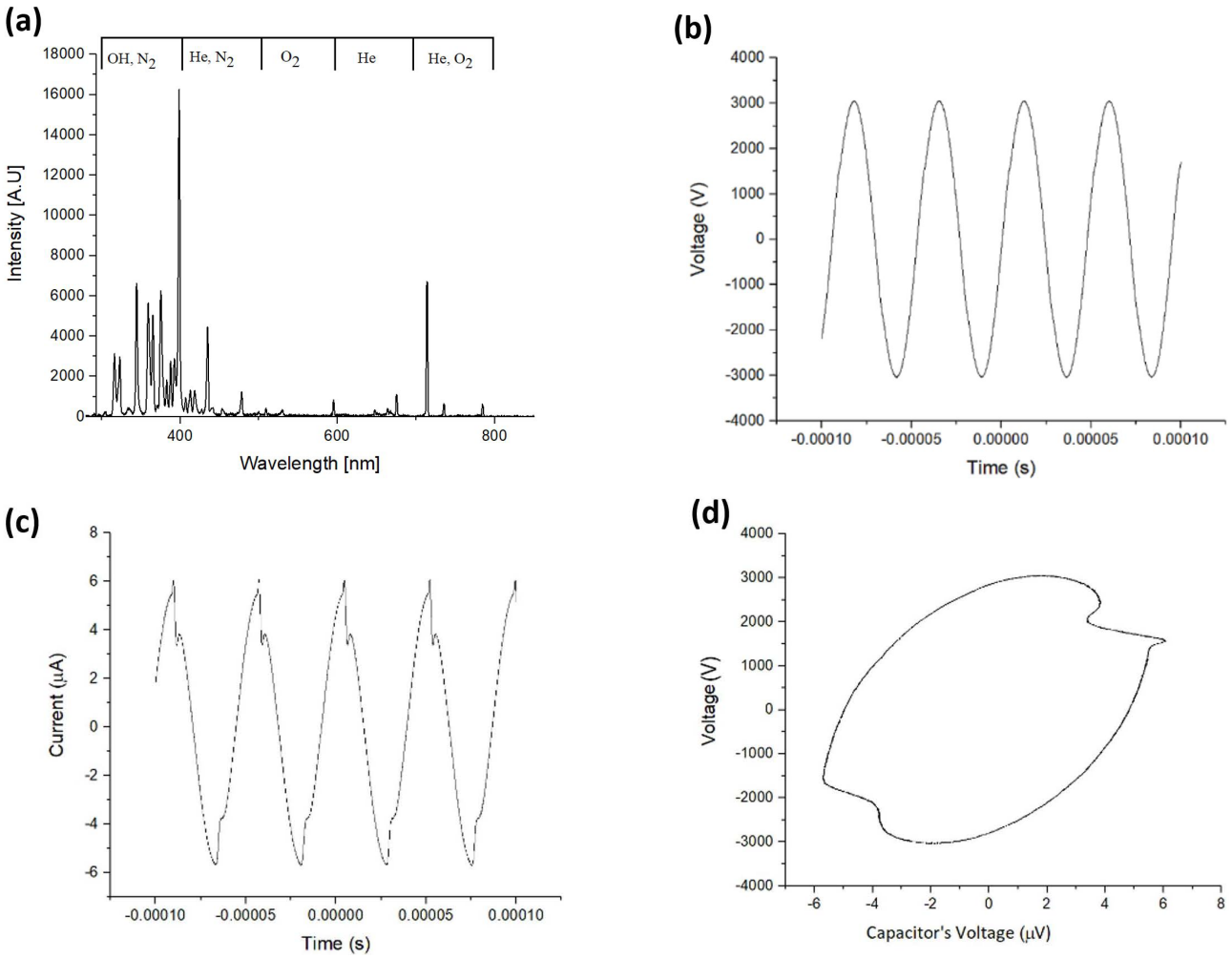


Figure 1. **(a)** The optical emission spectrum; **(b)** Typical voltage waveform; **(c)** Typical current waveform; **(d)** Lissajous curve

reflecting an APPJ power of 0.6 W (Figure 1d).

Low-Level Light (LLL) Device

Supplementary file, Figure S1 depicts the low-level light (LLL) device used in this study (THOR Laser Company, UK). It consisted of a cluster of light-emitting diode (LED) probes, including 34 × 660 nm and 35 × 850 nm wavelengths, with an active area diameter of 63 mm and a total power of 1390 mW. The control unit (voltage 230 V) contained an optical power meter, a timer (5 seconds – 5 minutes), and 11 pulse frequencies from 2.5 Hz to 20 kHz (continuous), with a weight of 3 kg and dimensions of 195 * 125 * 350 mm. In this study, the device was set at a 2.5 Hz pulse frequency and positioned approximately 5 mm from the skin. Each wound was treated for two minutes.

Temperatures

The room temperature was preserved at 25 °C; the patients were settled at room temperature for 10 minutes before we applied the treatment modality.

Wound surfaces were cleaned and dried with sterile gauze. In each session, we used a thermal camera (FLIR E4; 80 x 60-pixel resolution & < 0.15°C thermal sensitivity) to measure the temperature of wounds and predict the progress of the healing process.

A thermal camera monitored the temperatures of APPJ and LLLT. Figure 2a shows that the temperature of APPJ therapy was 37.6 °C, while Figure 2b demonstrates that the temperature of LLLT was 38.2 °C.

Wounds Temperature

Figure 2c-h shows that the wound surface had different temperatures (32 and 34.1 °C) after treatment with APPJ and LLLT. To ensure the safety of applying APPJ and LLLT, the wound and skin temperatures were checked after each therapeutic session.

Statistical Methods

For descriptive purposes, categorical data are expressed as frequency (percent), and quantitative variables are described as mean ± standard deviation (SD). To compare different response variables in three intervention groups, we first used univariate tests such as the chi-squared and Kruskal-Wallis tests (because of the non-normal distribution of quantitative outcome variables according to the Shapiro-Wilks test). We also used marginal modeling and generalized estimating equations (GEE) methodology to compare the mean trends of repeated measures

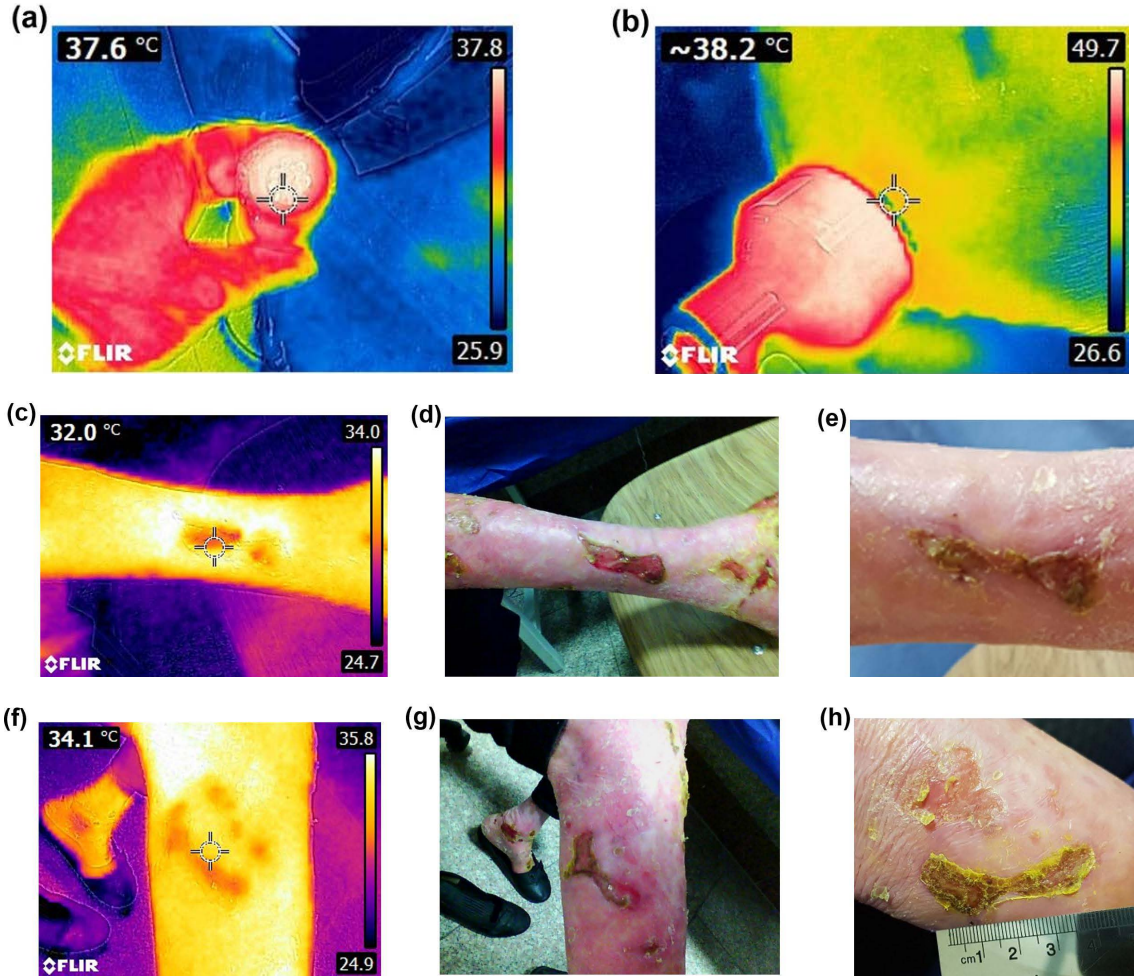


Figure 2. The treatment temperature of **(a)** atmospheric pressure plasma jet (APPJ) and **(b)** low-level light therapy. A chronic wound on the left leg of a female with recessive dystrophic epidermolysis bullosa: **(c)** FLIR thermal image after the first session; **(d)** the actual wound; **(e)** the same wound during the next session. A chronic wound on the right leg of a female with recessive dystrophic epidermolysis bullosa: **(f)** FLIR thermal image after the first session; **(g)** the actual wound; **(h)** the same wound during the next session

(longitudinal) outcomes between the study groups. SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY) was utilized for the data analysis, and *P* values of less than 0.05 were considered statistically significant.

We evaluated the time of 50% wound healing, which means the time at which 50% or more of the wound area had healed. Also, the percent of the decrease in wound area size was calculated as [(baseline size - post-treatment size)/ baseline size] x 100.

Results

Effects of Cold Plasma and Low-Level Light Therapy

This study enrolled six patients (four females and two males). Supplementary file, Table S1 describes the basic characteristics of the participants.

A total of 29 lesions were assessed, of which six were assigned to the control group, 12 to LLLT, and 11 to plasma therapy. One cutaneous lesion was excluded from the assessment due to trauma (self-manipulation) after the third session of plasma therapy.

The mean time of 50% wound healing was 2.00 ± 0.89, 2.92 ± 0.90, and 4.33 ± 2.08 half-weeks in the plasma, LLLT, and control groups, respectively ($P = 0.023$). Three lesions in the control group did not achieve 50% improvement and were excluded from this analysis.

The mean decrease in wound area size was 99.29 ± 1.83, 100.00 ± 0.0, and 43.42 ± 34.65 percent in the plasma, LLLT, and control groups, respectively ($P < 0.0001$ on the Kruskal-Wallis test).

One patient under plasma treatment and four in the control group did not experience complete wound healing. Furthermore, one patient in the control group experienced complete wound remission after two weeks, but the wound recurred a week later. Complete wound healing occurred after a mean of 3.44 ± 1.59 and 5.25 ± 2.18 half-weeks in the plasma and LLLT groups, respectively. The time to complete healing was significantly lower with plasma treatment than LLLT ($P = 0.049$) (Table 1).

We used the marginal model in generalized estimating equations (GEE) analysis to compare treatment effects on the size of the wound area. Table 2 shows the estimates from fitting this model. At baseline, the patients in the laser group had significantly smaller wound size areas than those in the control group ($P = 0.013$), while there was no significant difference between the plasma and control groups ($P = 0.462$). The estimate of -0.187 for the time variable shows a non-significant downward trend for the outcome variable during the study period for patients in the control group ($P = 0.174$). Finally, the estimate of parameters for the interaction between the therapy group and time shows that patients in the plasma group experienced lower area values than those in the control group. In other words, patients in the plasma group had a lower wound size (by about 1 cm) than patients in the control group during the study period ($P = 0.045$). In addition, we found no significant difference in the trend of the mean wound area between patients in the laser and control groups ($P = 0.075$). Figure 3 displays the study groups' mean trend of the wound area.

The presence of wound exudate at baseline was associated with a higher wound healing time ($P = 0.005$). None of our patients reported any complications during the application of each treatment. In addition, after three months of follow-up, no patient reported any complication or recurrence of treated cutaneous lesions.

The healing process of each wound was assessed according to its size during each session (Figure 4). For instance, Figure 4a-f shows the healing process of a chronic wound on the scalp of a 37-year-old female treated with APPJ, while Figure 4g-m demonstrates the improvement of a chronic wound in the right leg of a 35-year-old female patient treated with LLLT.

Discussion

Various therapeutic modalities have been used to treat individuals with recessive dystrophic epidermolysis bullosa (RDEB), but a definitive treatment is yet to be established.[22] The cornerstone of management for RDEB patients is wound care and controlling disease-related complications.[4] Local wound care, systemic treatments, cell therapy, and gene therapy have been examined in individuals with RDEB.[3,23,24] Different types of fractional (Er: YAG and CO_2 laser) and low-level light therapy (LLLT) have also

Table 2. GEE results for comparing the mean trend of the wound area between the study groups

Variable	Category	Estimate	SE	*P* value
Group	LLLT	-2.99	1.20	0.013
	Plasma	2.06	2.81	0.462
	Control	Reference category		
Time	-	-0.187	0.14	0.174
Group*Time	Laser	-0.68	0.38	0.075
	Plasma	-1.01	0.50	0.045
	Control	Reference category		

Abbreviations: GEE, generalized estimating equations; LLLT, low level light therapy.

Table 1. The wound area (cm^2) during each treatment session across the three groups of lesions

Group	Treatment session									*P*
	Baseline	1	2	3	4	5	6	7	8	
Control	9.57 ± 6.81*	8.72 ± 7	7.77 ± 6.85	7.68 ± 7.19	6.8 ± 7.1	6.43 ± 6.95	7.18 ± 6.8	7.02 ± 7.14	6.53 ± 6.8	0.006
LLLT	5.76 ± 6.02	4.51 ± 4.9	3.42 ± 3.98	2.3 ± 3.07	1.71 ± 2.69	1 ± 1.55	0.53 ± 0.91	0.09 ± 0.29	-	< 0.001
Cold Plasma	9.93 ± 10.96	5.43 ± 6.1	2.81 ± 5.02	1.95 ± 4.51	1.51 ± 3.77	0.94 ± 2.42	0.45 ± 1.51	0.18 ± 0.6	0.05 ± 0.15	< 0.001

*Mean ± SD. Abbreviations: LLLT, low level light therapy.

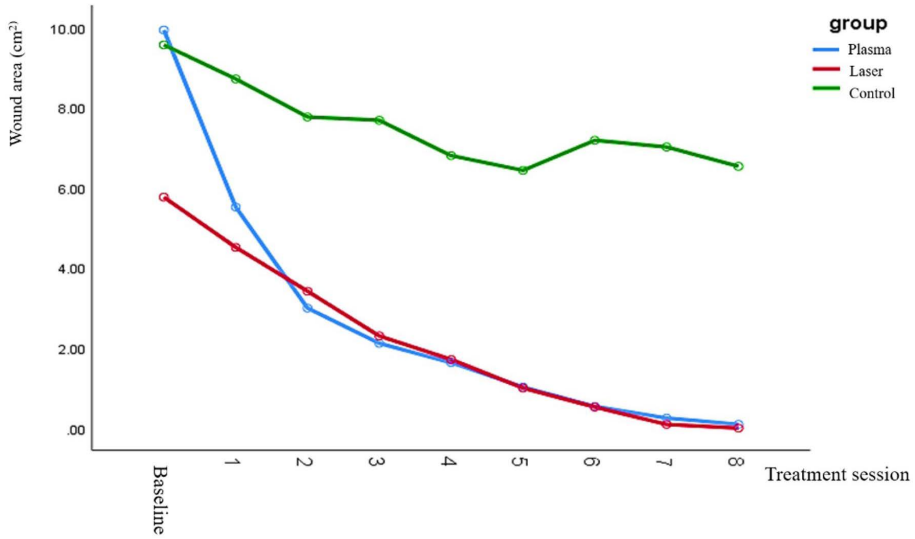


Figure 3. The mean wound area during each therapy session

been employed for wound management in these patients.[17] In this case series, we concentrated on novel local wound care treatments: LLLT and atmospheric pressure plasma jet (APPJ) therapy.

There are several notable characteristics of LLLT, including its ability to stimulate biological processes known as photobiomodulation, improve wound healing (by promoting cell proliferation and increasing nucleic acid and collagen synthesis), and enhance oral lesions in autoimmune diseases.[25,26] Photobiomodulation therapy (PBMT) is a form of light therapy that utilizes various light sources, including lasers, light-emitting diodes, and other non-ionizing light sources operating within the visible and near-infrared spectrum, to modulate biological processes and cellular responses. PBMT has demonstrated beneficial effects in the treatment of chronic wounds, burns, venous ulcers, pressure ulcers, and diabetic foot ulcers.[26,27] Notably, Minicucci et al. reported successful treatment of EB lesions using a 660-nm diode laser,[27] whereas studies on chronic wound healing have employed a range of red and near-infrared wavelengths, typically between 600 and 950 nm.[26]

Minicucci et al. reported a case of RDEB in a child with cutaneous erosions and blisters treated with LLLT. The cutaneous lesions improved completely with LLLT.[27] Also, Ho et al. described an individual with RDEB with clinical improvement (EBDASI score decreasing from 53 to 37; damage score decreasing from 42 to 28) using LLLT.[28] Other studies proposed LLLT as a beneficial therapeutic modality in treating oral lesions of individuals with EB.[29,30] LLLT has been widely studied for treating diabetic foot wounds, venous ulcers, and chronic wounds.[31] Kaviani et al. reported that LLLT is an accelerator of wound healing in patients with diabetic foot and may minimize the time needed to improve the wound.[31]

Several mechanisms are suggested in in vitro and in vivo examinations, including the reactivation of oxygen species, activation of enzymes like ATPases, and stimulation of Ca, mitosis, mRNA, and protein formation. Other beneficial effects of LLLT for wound healing include stimulating collagen synthesis, increasing fibroblast and keratinocyte proliferation, promoting capillary proliferation and microcirculation, and enhancing epithelialization.[27,32] In this study, LLLT significantly healed the cutaneous lesions of individuals with RDEB compared with control lesions. Notably, all lesions treated with LLLT improved completely.

Plasma, as a fourth state of matter, has been used broadly in medicine, including wound healing, bacterial infection treatment, and cancer therapy.[33] Several characteristics of cold plasma are important in wound healing. These include anti-inflammatory and anti-bacterial effects, generation of reactive oxygen and nitrogen species inducing tissue regeneration, stimulation of cell proliferation (fibroblasts), neovascularization, and epithelialization. Cold atmospheric plasma (CAP) is employed to treat acute, chronic, venous, diabetic, traumatic, and pressure ulcer wounds.[33,34] Thiem et al. reported two cases of RDEB treated with CAP, where the cutaneous lesions improved significantly over five months.[35] We used APPJ to treat the cutaneous wounds of individuals with RDEB. Different gases are employed to apply APPJ. In this investigation, we utilized helium gas to treat cutaneous lesions, and the patients experienced no burning sensations or discomfort. Even so, it is an expensive gas. Other gases, such as nitrogen and argon, can also be applied but may be associated with discomfort, according to our experience. All cutaneous wounds completely resolved, except for two lesions in one patient, which showed significant improvement but did not completely heal. Compared with LLLT, the time to complete remission was significantly lower with APPJ. Our findings are consistent with previous clinical studies demonstrating that cold atmospheric plasma accelerates chronic wound healing through reduction of microbial burden, modulation of inflammation, and promotion of tissue regeneration.[36] Similarly, although both APPJ and LLLT reduced wound area in this study, the more rapid healing observed with APPJ may reflect the broader biological effects of plasma-derived reactive oxygen and

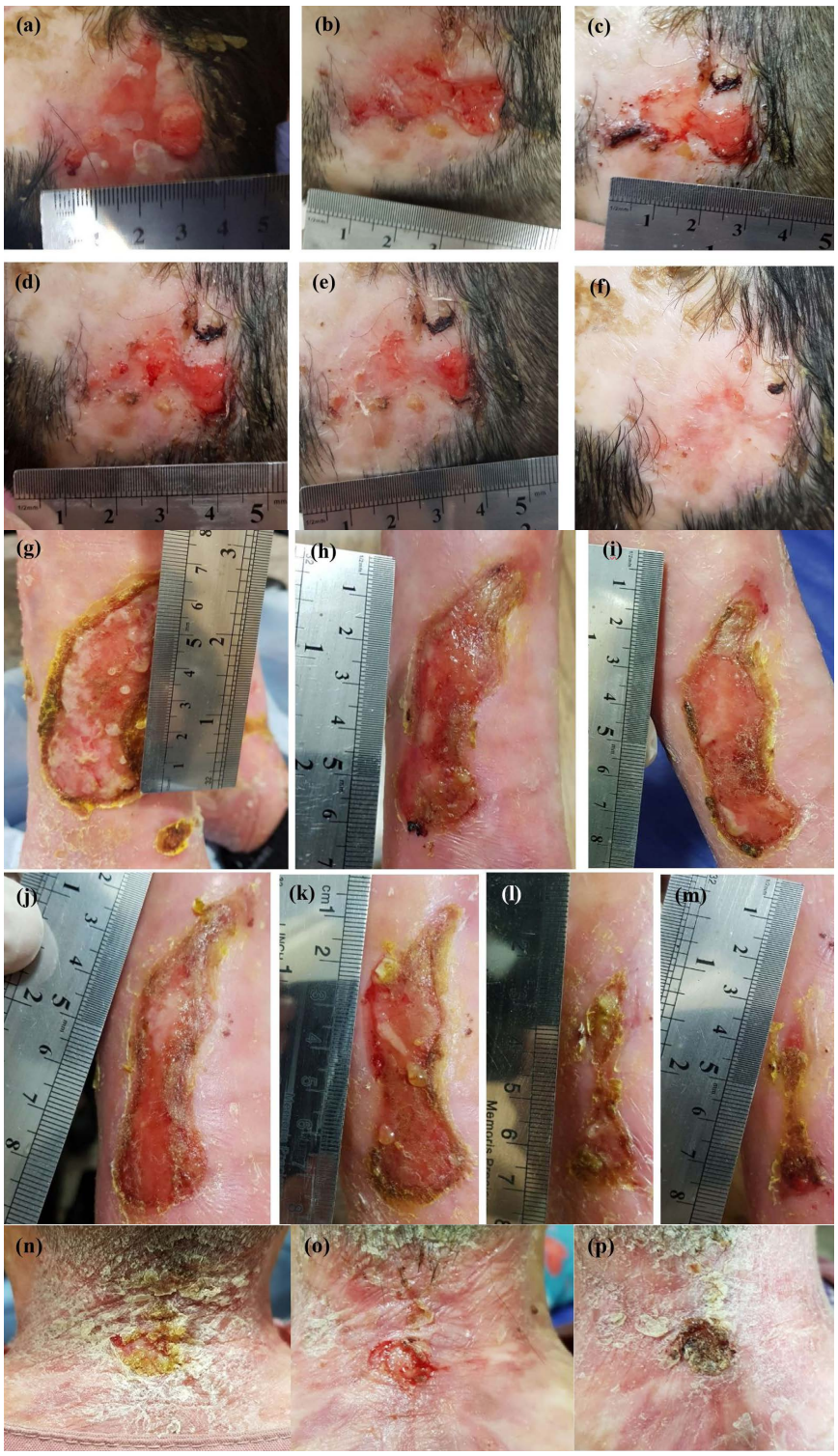


Figure 4. Changes in the wound area of a chronic wound on the scalp of a 37-year-old female with recessive dystrophic epidermolysis bullosa across different sessions of atmospheric pressure plasma jet (APPJ) therapy. **(a)** At baseline, **(b)** after second session, **(c)** after fourth session, **(d)** after fifth session, **(e)** after sixth session, and **(f)** after eighth session**.** Changes in the wound area of a chronic wound on the right leg of a 35-year-old female with recessive dystrophic epidermolysis bullosa across different sessions of low-level light therapy. **(g)** At baseline, **(h)** after second session, **(i)** after third session, **(j)** after fourth session, (k) after sixth session, **(l)** after seventh session, and **(m)** after eighth session**.** A chronic wound on the neck of a 37-year-old female with recessive dystrophic epidermolysis bullosa located on the back of her neck. This wound was dressed with Vaseline gauze as the control treatment, with photos showing it **(n)** at baseline, **(o)** after two weeks, and **(p)** after four weeks

nitrogen species on angiogenesis and cellular proliferation as well as wound-bed decontamination.[36,37]

The main limitation of this study was the small sample size because of the limited number of eligible RDEB patients presenting with multiple chronic wounds. Therefore, our findings should be considered preliminary, and larger studies with longer follow-up periods are required to confirm both the efficacy and long-term safety of these therapeutic modalities. In addition, the baseline size and anatomical location of wounds varied among patients, which may have acted as potential confounding factors. Furthermore, blinding of patients, treating clinicians,

and outcome assessors was not feasible because of the visible nature of the interventions. Moreover, patient-reported outcomes, including pain relief and quality-of-life measures, were not systematically evaluated and should be incorporated into future studies.

In our study, both LLLT and APPJ treatment decreased the wound area. Furthermore, the wound area of lesions treated with APPJ was reduced faster compared to the control.

In conclusion, considering these satisfactory results, we recommend both LLLT and APPJ; future studies should look into combination therapy as, in our experience, better results may be achieved by applying plasma initially and then combining it with LLLT in subsequent sessions. We recommend further investigations to achieve standard and optimal treatment protocols with LLLT and cold plasma for wound healing in individuals with RDEB.

Conclusion

Considering the satisfactory results of this study, both LLLT and APPJ are recommended in the treatment of chronic wounds in patients with EB, and future studies should focus on combination treatment.

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Competing Interests

The authors declare no conflict of interest.

Ethical Approval

The study protocol, which was conducted in accordance with the Declaration of Helsinki, was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.SRC.REC.1394.13; *IRCT registration number: IRCT20160320027109N2*).

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Supplementary Files

Supplementary file 1 contains Figure S1 and Table S1.

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