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Healing of wounds in lower extremities employing a non-thermal plasma

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ABSTRACT

Purpose: Venous, neuropathic, and mixed (venous and arterial) ulcers are complex chronic wounds that have a tremendous long-term impact on the mortality, morbidity, and quality of life of patients. In this work it was evaluated the efficacy of the non-thermal helium plasma at atmospheric pressure applied in patients with this suffering.

Methods: Using three elements: a RF generator, a needle-type reactor, and helium gas flow, for non-thermal plasma generation were used. The power density on the tissue of patients was of 0.6–0.67 W/cm^2. The application time was 30 s/cm^2 daily, at 5 to 10 mm of distance from the wound.

Results: The thirty-two patients with venous and mixed ulcers indicated that they had a decrease progressively of the pain with the therapy, without the need to continue with analgesics. Besides, the ten patients with chronic wounds showed 100% healing in neuropathic ulcers. For eight patients with venous leg ulcers, the percentage was reduced to 75% healing. While for fourteen patients with mixed leg ulcers, the rate of healing was 59%.

Conclusion: The non-thermal plasma generated in a needle reactor with helium gas is a promising candidate for clinical and therapeutic use in the treatment of neuropathic leg ulcers, with time reduction healing, and it can be applied everyday. In the study conducted, it was found that the non-thermal plasma applied to patients with autoimmune diseases clinically was not beneficial.

1. Introduction

The World Health Organization (WHO) states that leg and foot ulcers are a public health problem that affects a high percentage of the world population. That is why it is a constant concern of doctors who treat chronic leg ulcers difficult to heal, and in some cases, become complicated by further infections. This type of leg ulcer contributes significantly to the morbidity and mortality of patients, and representing a very high economic investment for the health centers. The most common kinds of ulceration are vascular (venous, arterial, lymphatic, vasculitis), neuropathic (diabetic neuropathy, Hansen's disease, etc.), metabolic, neoplastic, hematologic, infectious and parasitic, traumatic, and indefinite. Conventional therapies could not provide enough cell growth factors necessary to maintain the wounds healing process, involving a severe medical problem due to risk factors such as wound infection, sepsis, or amputation. Therefore, doctors and scientists have been given the task of developing new and novelty alternatives for the healing of chronic wounds, mainly leg ulcers such as vascular and those due to diabetes.

The conventional treatment for the healing of chronic neuropathic ulcers in legs and feet consists of the surgical cleaning and debridement, and later, the use of dressings and local agents [1,2]. At present, other recent alternative therapies to traditional methods for the healing of neuropathic leg ulcers are those including: physical activity and exercise [3], antibiotic [4], laser [5-7], stem cells [8,9], platelet-rich plasma [10], extracorporeal shockwave [11], amniotic membrane [12], insulin [13], acellular dermal matrices [14], biological [15], dressing [1,7], oxygen system [16,17], artificial dermis [18], and others.

The treatment of venous leg ulcers consists in the correction of the hemodynamic alteration; the recommended treatment by medical

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specialists is using a compression therapy, walking and leg elevation [19,20], or by invasive procedures [21]. Compression therapy is the first treatment option; here, it is important to choose the most effective compression modality [22]. There is evidence indicating that the higher the compression, the faster the healing [23]. In this regard, there is information that affirms greater effectiveness of the elastic stockings or the elastic bandage in comparison with the inelastic material [23]. When the venous leg ulcer presents greater damage, and the previous therapies no longer work, it is necessary to resort another type of treatment. Currently, some of the different therapies are: based on extracellular collagen dressings [24], electrical stimulation [25], drugs [26], hyaluronic acid [27], stem cells [9,28], laser [29], skin graft [30], polymers [31], local warming [32], and photonic technology [33,34].

In the last decade, a new research line in the clinical field has been developed using dielectric barrier discharges non-thermal plasmas (DBD-NTP) at atmospheric pressure [35,36]. When this type of plasma is applied slightly above to the skin surface, reactive oxygen species (ROS) such as O_2^-, OH, and reactive nitrogen species (RNS) like NO are generated [37]. Both ROS and RNS can produce physiological mediators that act as vasodilators that stimulate the production of endogenous compounds that, in turn, stimulate the endogenous growth factor [38,39].

Considering all above, a DBD-NTP was applied in patients for the treatment of chronic wounds in lower extremities through case studies earlier reported [40,41]. Based on these previous studies and results, a medical protocol was approved to apply a helium DBD-NTP for the healing of chronic wounds of the type neuropathic, venous, and mixed (venous and arterial) leg ulcers inside the Department of Angiology and Vascular Surgery at The Medical Center ISSEMyM in the State of Mexico.

2. Method

2.1. Set-up description

For the treatment of patients, a dielectric barrier discharge non-thermal plasma reported in previous studies was used [40,41]. Briefly, it was composed by a RF generator (13.56 MHz) functioning as a source of polarization for a plasma reactor. Fig. 1 shown the whole plasma source used in this study, including the NTP reactor using helium as working gas.

If it is assumed that exist an excellent electric coupling between the RF generator and the load through the matching box, 50% of applied load power is transmitted to the cable and the NTP reactor. Thus, as the real-time applied load power provided by the RF generator was in the range of 18 to 20 W and considering an 80% of electrical efficiency of the whole set up, the resulting load power was comprised in the range of 7.2 to 8 W. Considering the total transmission area determined by the RF cable (11.774 cm^2) and the needle-type reactor (0.097 cm^2), the estimated power density applied was between 0.6 and 0.67 W/cm^2; this value is <4 W/cm^2. This condition guarantees that there is not biological risk, according to the data of the International Commission on Non-Ionizing Radiation Protection [42]. In the treatments, the helium gas flow was from 0.8 to 1.5 liters per minute (LPM), the application time was 30 s/cm^2, and the distance from the skin surface for plasma application was around 5–10 mm, this depended on the roughness of the wound. The equipment is portable, and the treatments can be applied in practically any place, such as an examination table.

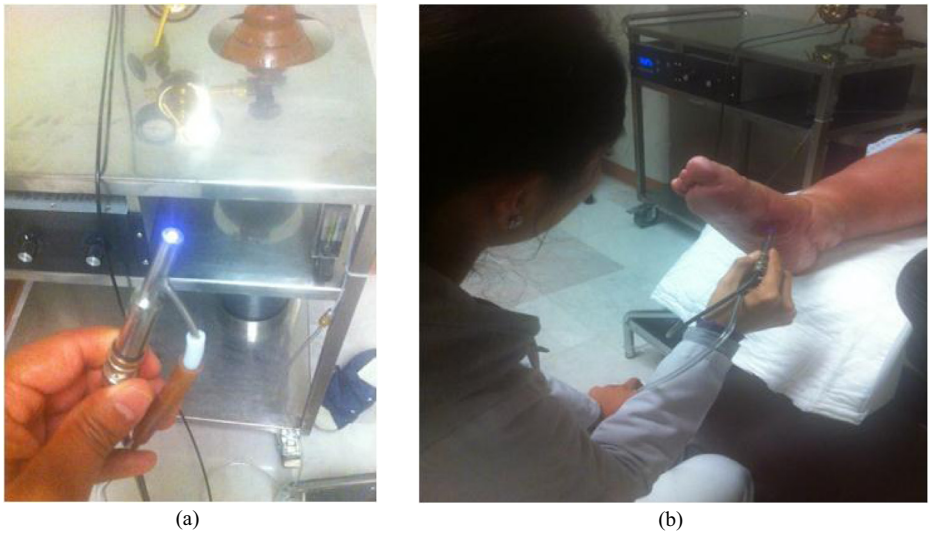
2.2. Patients

The corresponding medical protocol was approved by the Ethics in Research and Health Research Committees of the Medical Center ISSEMyM; being this a third level hospital in the State of Mexico. The criteria for patient selection were those with chronic peripheral vascular type wounds, chronic venous insufficiency, and ischemic vasculitis that were attended in the Department of Angiology and Vascular Surgery of the Medical Center ISSEMyM. The inclusion criteria were patients regardless of their age, body mass index, and health state. Patients who agreed to participate were ambulatory for a period of 8 months (August 2017 to April 2018), and all of them signed the corresponding informed consent. The exclusion criteria were those patients with psychiatric disorders, a clinic history of seizures, or those who receive drugs with known effects on the central nervous system, in addition to patients who did not accept to participate in the study. Finally, the elimination criteria were those patients who decided to withdraw their consent or who suspended the treatment.

2.3. Therapy

Once the plasma was established inside the reactor, the reactive flow propagated outside the reactor and expanded around it. With this, an increase in the temperature of one degree was obtained by applying the plasma close to the wound, i.e., the temperature in the injury was approximately 38 °C, and as indicated in Section 2.1, this plasma was within the safety interval for medical applications.

The therapy protocol was performed as follow: a) Debridement of wound, every time that the patient required the procedure for



(a) (b)

Fig. 1. Non-thermal plasma source: a) side view showing the plasma needle, and b) plasma applied to the patient.

eliminating diseased and non-viable tissue. b) Subsequently, the wound should be washed with distilled water and gauze; the wound should be perfectly cleaned, avoiding the bleeding as much as possible. c) Patients received an application of non-thermal plasma daily, with a variable treatment time between 60 and 300 s depending on the size of the wound.

2.4. Assessment of the wound size

In the treatments, the characteristics of the non-thermal plasma were considered as an independent variable. The tool used in this study for measuring the chronic wound was the "RESVECH scale" [43,44] that includes the dimensions of the wound, depth of affected tissues, edges, perilesional skin maceration, tunneling, tissue type in the wound bed, exudate, infection/inflammation, and frequency of pain (in the last ten days). The measurement was accomplished weekly.

3. Results

The care provided in the treatment of venous, neuropathic and mixed leg ulcers by non-thermal plasma started from the clinical examination of the patients. This phase consisted of the identification of the etiology, location, exudate characteristics, edema, and the presence of pain in the lesion. This initial assessment was essential for a proper follow-up of ulcer development and to provide a quality care. The study involved a total of thirty-two patients with ulcer problems, of which eight patients were with a venous leg ulcer, ten patients with neuropathic leg ulcers, and fourteen patients with mixed leg ulcers. The clinical results are shown below.

3.1. Treatment of patients with venous leg ulcers

Table 1 shows information about the wounds of each of the patients with venous ulcers. Of the eight patients, two of them decided to abandon the treatment (patients 7 and 8, Table 1). From the analysis of the data shown in Table 1, the population can be considered pre-obese according to the World Health Organization (WHO) and with an average of 54 years old.

From the information on table 1, it was observed that the wounds on patients were healed in less than 30 days. As an example, Fig. 2 shows a series of photographs from patient number 2, which presented a chronic wound (Fig. 2.a). In this wound, it can be observed a wet exudate, inactive edges, and the patient reports to have too painful. In Fig. 2.b shows the progress until the seventh day of treatment and yet the exudates still followed, the edges are activated, and the patient reveal does not have pain; and finally, Fig. 2.c shows the wound healed on the fourteenth day of the treatment application showing the efficacy of the non-thermal plasma. The patients manifested a gradual reduction of pain from the beginning of the treatment even without the use of analgesics and signs of infection in the wound were not observed, showing a clinical increase in their daily life.

3.2. Treatment of patients with neuropathic ulcers (diabetic foot)

The ten patients with neuropathic ulcer problems healed; this was in very variable periods and depended mainly on the size of the ulcer. The characteristics of each one of the patients with chronic wounds of neuropathic ulcers are summarized in Table 2. In this case, all the patients showed a sense of relief within minutes of having applied the treatment; some patients recovered sensitivity in the wound area without the need for medication.

An example of the clinical results of a chronic lesion of diabetic foot (first patient on table 2) is shown in the sequence of photographs of Fig. 3. In this case, a culture of a sample of the wound was carried out, and the bacteria *Aerococcus viridans* was found. The initial treatment in this particular case was with intravenous antibiotic Piperacillin/Tazobactam for seven days; subsequently, for only fifteen days, the antibiotic was changed to Trimethoprim/Sulfamethoxazole, and also non-thermal plasma was applied, the latter until the end of the treatment. In this case, it was essential to evaluate and treat the infection promptly to avoid proceed to the amputation and hospitalization of the patient. Before the application of the non-thermal plasma, the wound was washed with distilled water, debrided, and disinfected with a cationic detergent. The revascularization and the integration of the edges of the wound were accomplished in a relatively short time (Fig. 3.b), where considerable improvement was observed. After this, the wound evolved favorably until that it was healed in 118 days (17 weeks).

The series of photographs shown in Fig. 4 correspond to a 60-day postoperative patient with ulcers on the fifth finger position (the fifth finger was previously retired by amputation) and a plantar wound, with an area of 38 cm^2 (Fig. 4.a). The patient, due to his neuropathy, did not present symptoms of pain, which is why the wound grew without control. Also, the wound on the fifth finger position, when the treatment was started, had an approximate depth of 2.5 cm (Fig. 4.b). Due to this, it was decided to perform a combined procedure for this particular wound, that is, a silver dressing was placed (one per week) in the entire surface of the fifth finger position, and the non-thermal plasma was applied daily. The dressing was placed throughout four weeks of combined treatment, after that the wound presented a depth of approximately 1 cm (Fig. 4.d); and next only non-thermal plasma was applied until the wound healed completely, this after 68 days of treatment (Fig. 4.f). Finally, the plantar wound healed in 105 days of treatment (Fig. 4.e).

Table 2 shows the main characteristics of chronic wounds of neuropathic leg ulcers. From this table, it is observed that 100% of patients healed. The sampling population can be considered to be of working age since the average age is 56.6 years. According to the body mass index classification, 50% is obese, and the other 50% is normal. Also, in this case, the average time healing is less than a month, except for the two cases analyzed foregoing, in which the wounds were greater than 20 cm^2. Moreover, regardless of gender, this problem affects men and women equally.

Table 1
Characteristics of chronic wounds of venous leg ulcers.

Patient	Gender	Age	Body mass index [kg/m^2]	Wound dimensions [cm^2]	RESVECH scale score	Healing time [days]/ observation
1	Female	60	29.4	< 4	6	23
2	Male	45	26.3	< 4	6	14
3	Male	45	27.0	< 4	6	15
4	Male	46	29.4	< 4	17	13
5	Male	46	29.4	< 4	17	14
6	Male	64	26.8	4–16	17	29
7	Male	51	34.2	4–16	18	90/suspended
8	Male	74	27.6	< 4	14	97/suspended
Average		53.87	28.76		12.62	

(a) (b) (c)

Fig. 2. Patient with chronic wound of venous leg ulcer: (a) Original wound, (b) on the seventh day of treatment, (c) wound healed on the fourteenth day of treatment.

Table 2
Characteristics of chronic wounds of neuropathic leg ulcers.

Patient	Gender	Age	Body mass index [kg/m^2]	Wound dimensions [cm^2]	RESVECH scale score	Healing time [days]
1	Male	38	22.7	16–36	17	118
2	Female	65	22.8	4–16	13	46
3	Female	65	23.2	<4	12	14
4	Female	65	23.6	<4	6	18
5	Male	38	23.5	<4	6	8
6	Female	65	36.7	16–36	20	105
7	Female	65	34.9	4–16	6	68
8	Female	65	33.0	<4	6	14
9	Male	50	27.7	<4	11	4
10	Male	50	28.4	<4	11	2
Average		56.6	27.65		10.8	

3.3. Treatment of patients with mixed (venous and arterial) leg ulcers

In this type of wound, the healing was very variable. Fig. 5 shows a patient (patient 6, Table 3) with a 4 months mixed leg ulcer. This wound had been treated with different medications without achieving healing successful; on the contrary, it was growing. When the treatment with non-thermal plasma was started, the wound presented a surface area of 12.5 cm^2, and in the inner side a well of approximately 2 cm deep, in 100 days of treatment the wound healed. The evolution of reepithelialization and tissue dynamism in the ulcer was evident and culminated in accelerated healing by treatment with non-thermal plasma. In the series of photographs shown in Fig. 5, the improvement in the wound healing can be attributed to the proliferation of fibroblasts both at the edge and in the center of the ulcer, favoring the contraction of the ulcer and promoting the repair of the tissue since they are responsible for the synthesis of collagen [38,45,46].

Fig. 6 shows the photographs of a patient (patient 3, Table 3) with a mixed leg ulcer. In this case, she presented the following symptoms on admission: pain, burning, inflammation, and heaviness. From her diagnosis and as seen in Fig. 6.b, there was an increase in the granulation of the ulcers, which accelerates the angiogenic potential and its effect on the attraction of fibroblasts, until the ulcer is eliminated. In contrast, the edge of the ulcer showed a significant increase in inflammatory cells from day 30 after the first treatment. This fact can be related to the higher number of cells involved, such as keratinocytes and Langerhans cells, which could activate more chemotactic substances for inflammatory cells and, consequently, reactivation of the healing process [38,47].

3.4. Adverse events

Table 4 shows the results of three critical cases, an older adult (85 years old) and two young adults (40 years old), with mixed leg ulcers and autoimmunity diseases and who were clinically discharged. Fig. 7 shows the case of a chronic wound with an area of 15 cm^2 (Fig. 7a). After 30 days of treatment with non-thermal plasma, the progress in closing the wound was only 2 cm^2, and it presented dry bed, fibrin, and exophytic hyperplasia. The patient was directed to the immunology service for evaluation. While the medical report was obtained, the non-thermal plasma was continued, and after 57 days of treatment, it was decided to suspend the treatment.

In Fig. 8, the case of another patient considered that had a mixed leg ulcer is presented. Here, vasculitis due to Antiphospholipid Syndrome

(a) (b) (c) (d)

Fig. 3. Evolution of a neuropathic ulcer: (a) Upon admission of the patient, (b) after 25 days of treatment, (c) after 60 days of treatment, and (d) after 118 days of treatment.

Fig. 4. Multiple neuropathic ulcers of the right foot and fifth amputated finger.

(APS) was diagnosed. This patient, after non-thermal plasma therapy, showed a different situation to the cases in which the others patients were healed. In this particular case, the patient manifested an increase in pain in the area of the wound, edema, increase in size of the wound with exudative tissue, and fibrin.

The summary of the treated patients is shown in table 5. From this, it can be observed that the treatment of chronic wounds had a 100% effective in neuropathic ulcers. In the case of venous leg ulcers, it proved to be effective in 75% of the cases, and the percentage was due in part to the patients being very irregular in their medical appointments. Finally, in the case of mixed leg ulcers, wounds healed in a percentage around of 59%. Approximately 24% of patients irregularly attended the treatment and finally stopped it. The procedure did not work efficiently in patients with mixed leg ulcers and presenting autoimmune disorders because 100% of all them their wounds did not heal satisfactorily.

4. Discussion

Different research groups have shown that non-thermal plasma at atmospheric pressure has been useful in the elimination of bacteria and biofilms so that it can be applied in the clinical environment [48-51].

A significant barrier to the healing of chronic wounds, and particularly in patients with a weakened capacity to overcome the infection is the formation of microbial biofilms [52]. The creation of biofilms reduces the capacity to the penetration of beneficial active agents, and microorganisms resistant to them become increasingly problematic, inducing that chronic wounds were unable to heal. In this sense, non-thermal plasma has shown to be effective against a wide range of microbial biofilms [53,54]. Other factors, such as poor perfusion, limit to furnish of systemic antibiotics to the wound and increase the complexity in the treatment. That is why new therapeutic options could aid to prevent and control the infection of chronic wounds, such as those caused by diabetes or venous insufficiency, which can cause leg ulcers

Fig. 5. A mixed chronic wound in the heel a) Original wound, b) at 30 days of treatment, c) at 100 days of treatment.

Table 3
Characteristics of mixed leg ulcers.

Patient	Gender	Age	Body mass index [kg/m^2]	Wound dimensions [cm^2]	RESVECH scale score	Healing time [days]/ observation
1	Male	76	28.1	<4	6	4
2	Male	60	25.3	<4	8	5
3	Female	78	21.9	4–16	22	135
4	Female	78	21.4	<4	6	14
5	Female	77	32.9	<4	10	16
6	Male	68	28.6	16–36	14	85
7	Male	61	26.6	<4	10	58
8	Male	63	25.6	<4	6	4
9	Female	42	28.2	<4	19	30
10	Male	80	24.5	<4	11	4
11	Female	66	37.2	16–36	15	16/suspended
12	Male	54	23.0	<4	11	86/suspended
13	Male	57	23.5	<4	9	69/suspended
14	Male	55	36.3	4–16	16	90/suspended
Average		65.4	27.4		11.6	

difficult to heal, and that can finally be the cause of amputation of the lower extremities.

On the other hand, since the discovery of the nitric oxide molecule (NO) with application in the field of medicine [55,56], different authors have stated that a deficiency of nitric oxide characterizes diabetes at the site of the wound [57,58]. Based on previous studies of our team [51,59], it was shown that non-thermal plasma generated with helium gas produced, among other chemical species, nitric oxide. Through the action of this compound, and based on the methodology established and presented in this paper, it can be said that diabetic foot problems can be treated successfully.

From this, it is seen that the procedure had positive effects on the different processes involved in the healing of the wounds, since, based on the treated cases, the healing process of the chronic cutaneous wounds was improved. The above can be attributed to the promotion of angiogenesis, cell migration [60], and increasing tissue perfusion [61] to promote the healing process. In this study, the effects of non-thermal plasma were examined, which, as a final effect, significantly accelerated the wound healing, this being a painless, effective, and mainly safe procedure.

The results obtained with autoimmune patients are contrasted with studies presented by other authors such as [62,63]. The above may be attributed to a lot of ROS generated by the non-thermal plasma on the wound and able to cause oxidative damage [64,65]. There is also significant research, which suggests monitoring ultraviolet radiation in patients with autoimmune diseases and immune pathophysiology [66,67]. In both cases, it can lead to disease progression and intensify complications in this group of patients. Thus, it must to be determined whether these effects may be a protective factor in some autoimmune diseases or a risk factor for their induction.

5. Conclusions

The application of non-thermal plasma, regardless of the place of use, either at the edge or in the wound bed, was clinically safe and effective in the treatment of venous or diabetic foot ulcers. Based on the results presented, it is concluded that the treatment by non-thermal plasma is highly effective in patients with neuropathic ulcer diseases, and it can be affirmed that the effectiveness is 100%. In the case of patients with problems of venous leg ulcers and mixed leg ulcers, it is essential that patients follow the medical indications. From experience acquired in this research period, a commitment on the part of patients to stay at rest is necessary to improvement their health condition, as well as avoiding to stop the treatment implemented. An important fact to emphasize is that in people with autoimmune diseases, the procedure was not entirely functional because the characteristics of the ulcers were decaying after a few sessions of the treatment. Therefore, others alternatives must to be found to treat these diseases. A significant result of this work is that all ulcers gradually were lowering the pain with plasma therapy, without analgesics required. Therefore, from this study, the effects of the non-thermal plasma were examined demonstrating that the main effect is to accelerate wound healing process significantly.

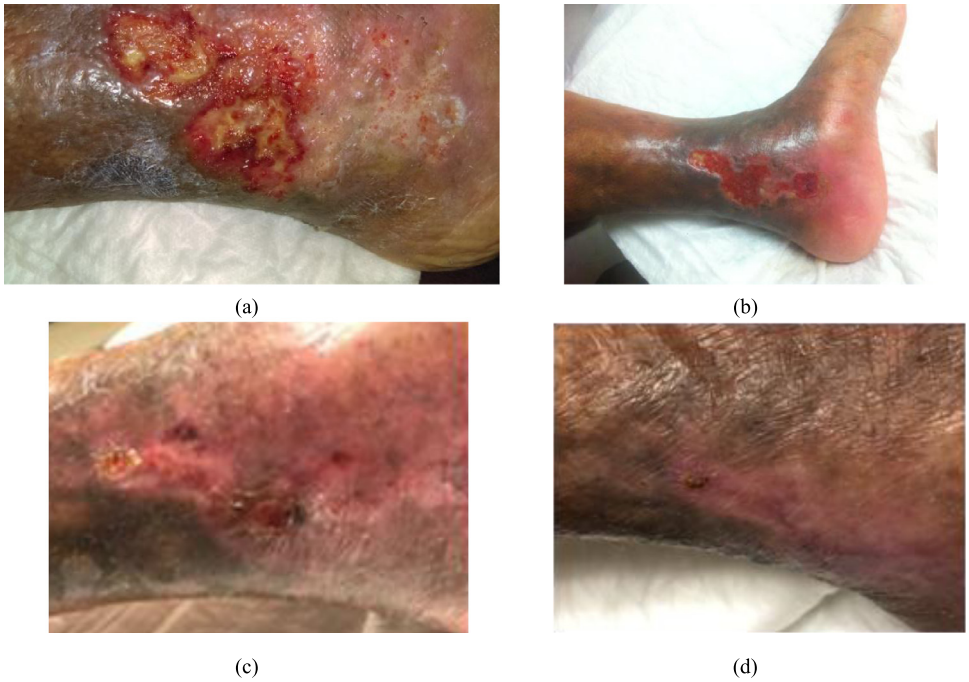
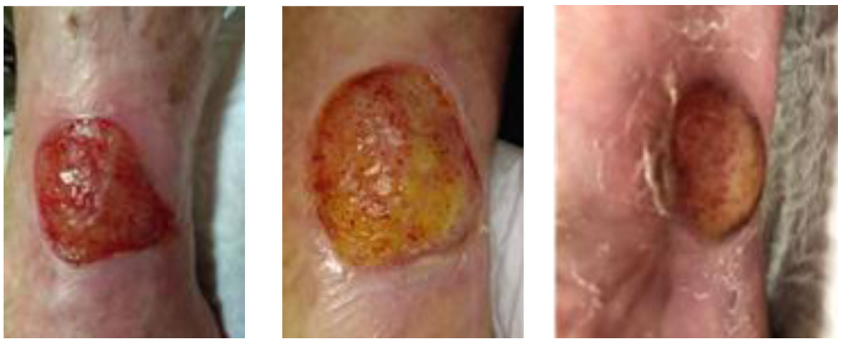


Fig. 6. Mixed leg ulcer complicated in the healing: a) Original wound, b) after 90 days of treatment, c) 120 days of treatment, and d) 135 days of treatment, healthy wound.

Table 4
Characteristics of mixed leg ulcers of patients with autoimmunity diseases.

Patient	Gender	Age	Body mass index [kg/m^2]	Wound dimensions [cm^2]	RESVECH scale score	Healing time [days]/ observation
1	Female	85	20.3	4–16	12	57/suspended
2	Female	40	24.6	16–36	14	8/suspended
3	Male	40	42.1	4–16	10	120/suspended



(a) (b) (c)

Fig. 7. Mixed leg ulcer wound with autoimmunity diseases, a) Original wound, b) 30 days of treatment and c) 57 days of treatment, after that the treatment was suspended.

(a) (b) (c)

Fig. 8. Patient with APS disorder. a) Original wound, b) after 3 days of treatment, and c) 8 days of treatment.

Table 5
Summary of patients treated with non-thermal plasma.

Wounds type condition	Venous ulcers	%	Neuropathic ulcers	%	Mixed ulcers	%
Medical discharge	6	75	10	100	10	58.82
Treatment suspended by the patient	2	25	0	0	4	23.53
Treatment suspended by the doctor	0	0	0	0	3	17.65
Total of patients treated	8	100	10	100	17	100

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Declaration of Competing Interest

None.

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