Demonstration of the high efficiency of an air plasma jet combining electric field and RONS in the treatment of chronic wounds

Osvaldo Daniel Cortázar, Ana Megía-Macías, Bernardo Hontanilla and Hernán Cortázar-Gallicchio

***Abstract*—A Cold Atmospheric Air Plasma Jet (CAAPJ) for the treatment of skin injuries in medicine and veterinary medicine is presented with experimental evidence that point to the electric field inside the plasmas jet could be a determinant therapeutic mechanism. The device is characterized by producing a cold atmospheric air plasma jet compatible with living tissues at a low heat transfer rate with a temperature on the skin surface below 40 °C. It has a practical design to be used by physicians and veterinaries during daily practice, with a special focus on the treatment of skin injuries and unhealed ulcers. Plasma diagnostics, including currents-voltage signals, UV-VIS spectroscopy, IR images of the skin, and electric field measurements in the air cold plasma jet are presented. The last is made for the first time in this type of plasma, and them can justify the induction of local electric currents on the wound surface to accelerate healing by highlighting the possible synergy with Reactive Oxygen and Nitrogen Species (RONS) as a decontaminant agent for bacteria (including resistant), fungi and viruses without damaging healthy tissue. A remarkable clinical case study example is reported.**

***Index Terms*—Plasma Medicine, Cold Air Atmospheric Plasma Jet, Wound Healing, Ulcers, Tissue Regeneration**

I. Introduction

COLD atmospheric plasmas (CAPs) are increasingly popular in diverse applications, including medicine, veterinary medicine, agriculture, and food science [1]–[7]. The use of CAPs is supported by its efficacy as a decontaminant tool, the potential to promote wound healing, and the capacity to induce cancer cell death. Because they are highly unbalanced thermodynamically, they transfer a minimal amount of thermal energy to other bodies or materials. There is a general worldwide agreement that RONS administered by CAPs are successful in neutralizing bacteria, fungi, cancer cells, and viruses with a minimal impact on healthy tissue [8]–[14]. The active principle of oxidative stress generated by RONS is responsible for weakening microorganisms [15]–[19]. Mammalian cells possess a greater threshold for oxidative stress tolerance as compared to bacteria, fungi, and viruses due to their structural properties and stimulation of inherent self-repair mechanisms [20]–[22]. Although several research groups have contributed significantly to the development of cold atmospheric pressure plasma jets (CAAPJs) that can be used in direct contact with living tissues [23]–[27], in the device presented in this study, the air overheating has been solved by two patented innovations and its level of development is such that it can be used in hospitals by physicians and nurses in a practical and simple manner [28]–[30].

In addition, plasma diagnostics, comprising current-voltage signals and UV-VIS spectroscopy, are presented. Moreover, electric field measurements were performed on our *air* cold plasma jet. To the best of our knowledge, the measurements published so far were performed in Helium and Argon. We provide for the first time experimental evidence of electric field in this type of *air* plasma. This could justify the induction of local electrical currents at the wound surface as an important mechanism to accelerate healing, being the hypothesis that we will develop here.

II. COLD AIR PLASMA JET DEVICE

A Cold Atmospheric Air Plasma Jet (CAAPJ) device developed by the company MEDICAL PLASMAS® [31], denominated PlasmAction Med™, was used for the research described in this article. A general view photograph of the device is presented in Fig. 1 where its main components are indicated, and Fig. 2 shows a schematic block diagram with the interrelationship between subsystems. One silent diaphragm non-oil compressor (1) takes the room air and introduces a 10 l/min flow through the system. A first stage of cooling (2) reduces air temperature to by reaching 5-6 ºC producing a water condensation (3) before injecting the flow into transport hose (4) to finally reach the manual applicator (5) where it is expelled. Once air flow reached a set of predetermined conditioning parameters, the HV power supply of 15 kV RMS at 30 kHz (6) can be activated by means a the plasma activation pedal (see Figure 1), so a plasma jet (7) is produced

This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Spanish Agency of Drugs and Medical Devices (AEMPS) file 823/20/AE on January 23, 2020.

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at the tip of manual applicator. The transport hose is a coaxial arrangement of a shielded HV cable and an external flexible silicon hose where electrical current flows in the center and air in the space between both. A build-in computer is in charge of the general control system (8) and interaction with the user is made by means of a touch screen (9) where treatment time and number of sessions can be set.

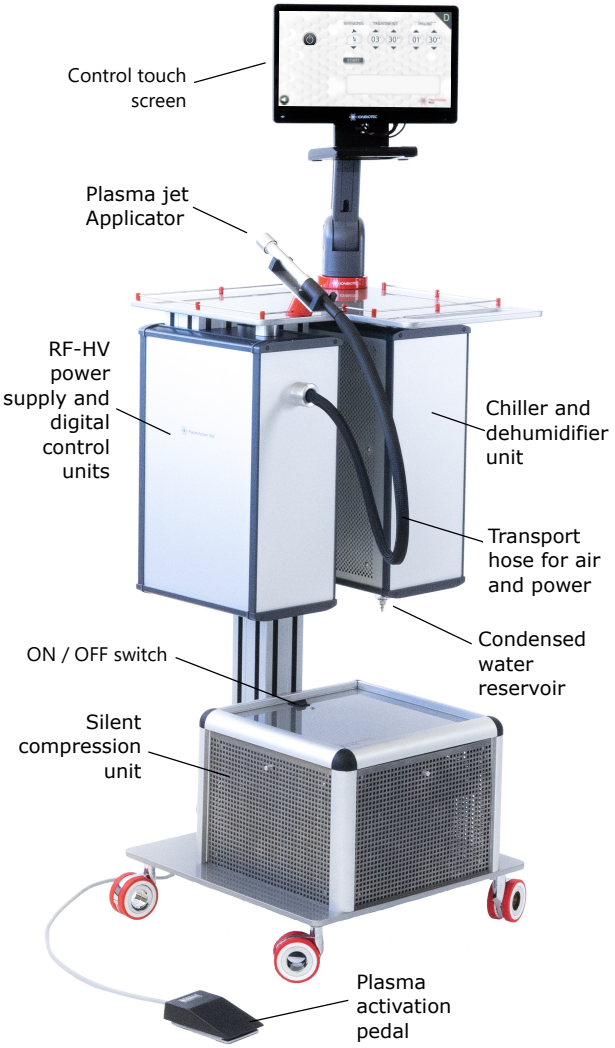

Fig. 1. General view of the plasma source device.

Fig. 3 shows a cross-sectional view of the hand applicator tip where its structure is detailed and its main components are indicated by numbers in round brackets. The plasma discharge is produced between a pair of concentric and opposing electrodes separated by a gap of approximately 2 mm. Positive electrode (10) has a frustoconical shape with a passing-trough orifice from where air flow (11) is introduced in the gap where plasma is produced (12). The flat ground electrode (13) has a centered orifice that conducts to a series of plasma expansion chambers (14) composed by a stack of different alternating inner diameter washers. An output orifice of 2 mm diameter

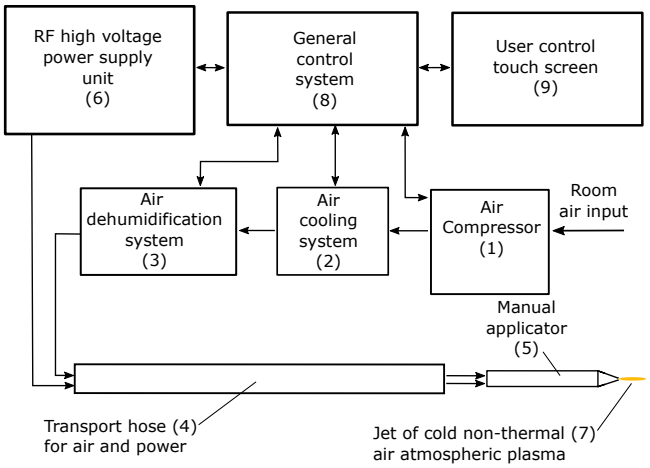

Fig. 2. Block diagram of the plasma source with subsystems.

(19) determines the jet formation in the external atmosphere.

Inside of transport hose (4), the electrical current flows at center by a HV shielded cable and air around; but in the applicator this distribution must to be inverted by using the flow exchange piece (16). This is necessary in order to air flow (11) to pass through the hole in the center of the positive electrode (10) and establish the discharge in the gap between it and the flat ground electrode (13).

A removable sleeve made of stainless steel (26) covers the applicator head to prevent cross-contamination between patients through accidental direct contact of the applicator head with the wound surface during treatments.

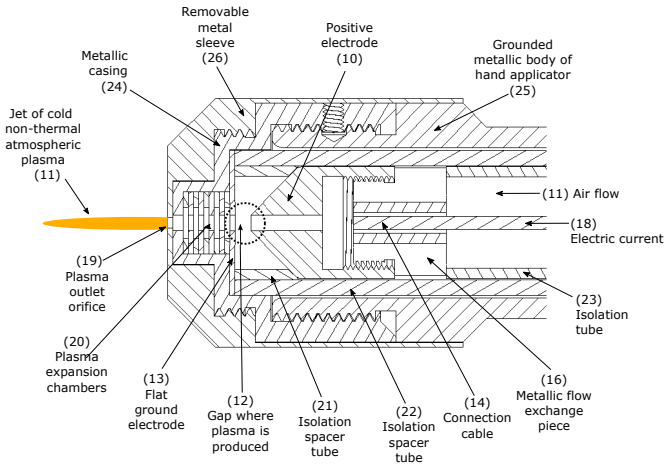

Fig. 3. Cross section of manual applicator head.

III. PLASMA CHARACTERIZATION

A. *Electrical characterization and Electric Field Measurement*

The Voltage signal was measured with a HV probe by Tektronix model P6015A with a temporal response of 4 ns and a maximum rate of 40 kV of peak value. The current signal was measured with a TA189 current probe (Pico Technology) with a range between 2 mA and 30 A and 100 ns resolution. This is a clamp type inductive coil probe for scope measurements

that was placed around the HV cable with a proper insulating centering piece at the output of HV power supply.

The electrical field in the sine of the cold air plasma jet was measured by using a electro-optical probe Kapteos, eoProbe™ with the Optic to Electro converter eoSense™. This electro-optical system has a low permittivity $\approx$ 3.6, sensitivity 250 mV/m, up to 10 MV_{rms}/m, bandwidth 10 Hz to 12 GHz, spatial resolution of 1 mm according with data provided by the manufacturer. The probe is connected with an optical fiber cord to an interface (Kapteos, eoSense LF 100U-1) that convert the optical signal and voltage that can be recorded in a scope. The ET5-air is a Pockels effect based probe that use the changes in a laser beam polarization produced by electric fields [32]–[34]. Fig. 4 shows a picture of the electro-optical probe during a measurement. The measurements of current, voltage and plasma electric field signals as a function of time were performed simultaneously. Fig. 5 shows the typical scope capture of the current (a), voltage (b) and electric field (c). High voltage and current probes were connected to the output of high voltage (HV) source that feeds the plasma generator while it is running showing that excitation is at 30 kHz with RMS voltage and current of 2.5 kV and 5 mA respectively. The resulting RMS power delivered to the plasma is 15 W. However, it is important to note that the inductance of the secondary of the high voltage transformer and the capacitance of the coaxial cable and other components of the plasma jet applicator form an LC circuit that must be taken into account. In fact, we experimentally adjust the working frequency to match the resonant frequency $1/2\pi\sqrt{L.C}$ to make the transmission line losses negligible. Note that the current used to produce the plasma between the positive (10) and ground (13) electrodes shown in Fig. 3 has no direct relation to that which can be induced on the surface of a wound as we will discuss later in Section V.

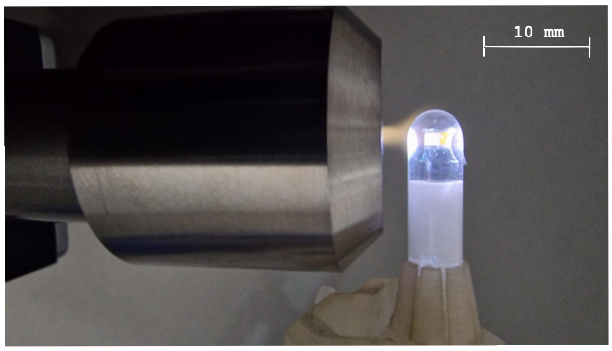

Fig. 4. Electric Field Electro-Optical Probe during a measure at 6 mm from the plasma nozzle.

The signal in Fig. 5(c) corresponds to a radial direction measurement at 6 mm of distance from plasma nozzle, as is shown in Fig. 4, and it follows current and voltage signals. In addition, the axial and radial components of the electric field were measured as a function of distance from the plasma jet output to determine its range. Fig. 6 illustrates such a spatial distribution where significant peak field values were detected up to a distance of 20 mm although the ones of more interest for us are those obtained from 8 to 16 mm because it is in the

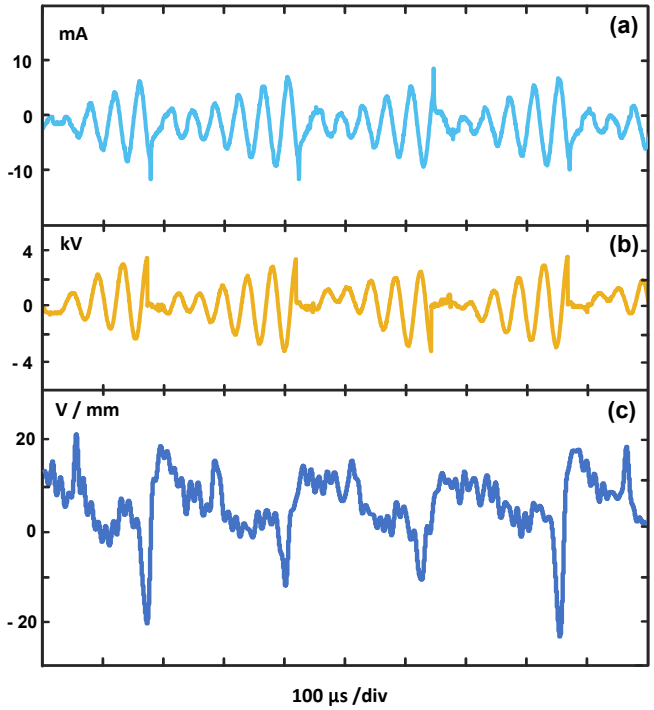

Fig. 5. Electric current intensity in the plasma jet (a) with RMS value of 5 mA, voltage (b) with RMS value of 2.5 kV and electric field signals (c) with peak values 20 V/mm.

range where it is applied in the treatment of wounds. Observe that E-fields peak values from 10 to 30 V/mm are typical.

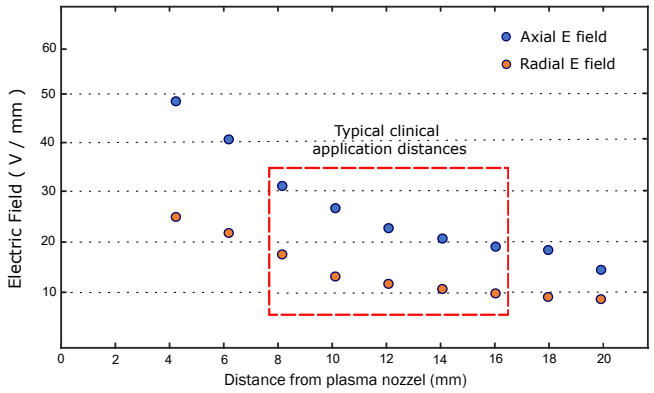

Fig. 6. Axial and radial plasma peak electric field vs. distance from the applicator nuzzle.

B. UV spectral emission measurements

Although only a few RONS can be observed by UV spectroscopy, it remains as a valuable tool for its simplicity and accessibility to observe the presence of important line groups associated to biological uses. Special attention is paid to UV range (200-400 nm) to observe the relative RONS abundance because its importance for biological applications. A fiber optics spectrometer from BWTEK Inc. Models Exemplar-LS BRC115P-U-UV (200 to 400 nm, resolution $\geq$ 0.6 nm) was use connected to an Ocean Insight model QP1000-2-SR (200 to 900 nm) fiber optics patch.

Fig. 7 shows a UV spectrum obtained at axial normal incidence (direct frontal) at 20 mm distance from the plasma nozzle, with a detailed molecular and radical emission from RONS like NO, OH and N_2 lines. The assignment of spectral lines in the spectrum of Fig. 7 has been carefully made taking into account the results of emission spectroscopy in cold atmospheric plasmas by other authors published in a wide range of plasma generator devices [35]–[41].

We can see four packages of lines associated to molecular and atomic emissions that are inside of doted line boxes in the spectrum of Fig. 7. Nitric monoxide (NO) lines are mainly recorded between 200 and 280 nm from the molecular transition $A^2\Sigma^+, v' - X, v''$. Hydroxide anion (HO^-) lines are mainly recorded between 280 and 310 nm from the molecular transition $^2\Sigma^+ - {}^2\Pi$ with the exception of some molecular N_2 lines around 296 nm. The main emission package of N_2 lines is between 310 and 380 nm from molecular transition $^3\Pi_\mu - {}^3\Pi_g$ and $^2\Sigma^+_\mu - {}^2\Sigma^+_g$. On the right side of the spectrum there an overlap of some O, O^+ and N_2^+ emission bands in the range of 380-400 nm is observed that cannot be resolved by our instrument [35], [39], [42], [43]. Moreover, two copper lines from the electrode contribution are also observed at 324.8 and 327.4 nm. The presence of NO lines between 200 and 280 nm is notable respect to other devices [44]–[46].

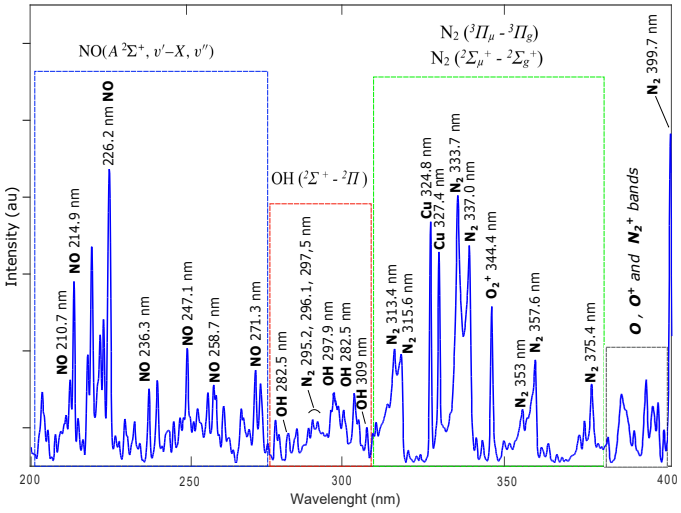

Fig. 7. UV spectrum from plasma jet in normal incidence from 200 to 400 nm.

On the other hand, and considering the clinical application on living human and animal tissues, safety aspects are relevant. In this sense, we have paid special attention to the UV emission that can be projected on the tissue from the plasma jet because it could be a serious limitation for clinical application, as was opportunely observed by *Nosenko et al* for Argon jet devices [47]. In addition, UV emission has been suggested as one of the physical decontamination factors in cold atmospheric plasma devices and it must be resolved by measurements for our case [48]. To clarify this issue, a direct integrated measurement using a PCE Instruments UV radiometer and dosimeter model PCE-UV34 was used to measure the UV irradiance of the plasma source for safety monitoring with a resolution of 0.01 mW/cm^2 in a bandwidth of 260 to 390 nm and an accuracy of 4 %. The radiometer was placed directly in front of the plasma jet at a distance of 10 mm at normal incidence of the detector surface. The obtained value is an upper limit of irradiance that does not take into account the sensitivity of living tissue to the different wavelengths as defined in Directive 2006/25/EC of the European Parliament and of the Council [49] where it can be seen that the weighted value will always be lower than that measured directly by the UV radiometer, which gives us an additional safety margin for the intended use of the cold atmospheric plasma equipment used here. The peak value recorded with the radiometer was 0.074 W/m^2. Considering that the upper exposure limit established by the aforementioned directive is 30 J/m^2, then the maximum exposure time of the patient's skin to the UV emission of the plasma jet is 6.76 min. Taking into account that the treatment time never exceeds 1 min/cm^2, the observed area of illumination with normal incidence at 10 mm distance is 1.5 cm^2, the angle of incidence with which the plasma jet is applied to the surface is 45º and the above mentioned straight measurement of the radiometer, we can say with a wide safety margin that the use of this cold atmospheric air plasma jet is safe.

C. Macroscopic tissue heating effect

Cold atmospheric plasma jets are strongly out of thermodynamic equilibrium, however, once CAPs are interacting with some material a process of thermalization takes place and the measurement of temperature on the material surface makes sense as representative of its internal energy change ΔU. Moreover, in our specific case it is of great interest because it points directly on the use for which the device is intended.

An Optris PI640 - 640 x 480 pixels IR camera with a 12º x 9º IR microscope lens (f = 44 mm) was used to obtain the IR images. Fig. 8 shows two pictures showing the interaction between air plasma jet and a alive finger tip skin. Left image (a) is a visible picture and right (b) was taken by a IR camera. Note that the IR emission of the jet itself is not detected by the IR camera, only its effect on tissue surface is observed by temperature increasing. A doted line is superposed on the right picture to indicate the approximated jet dimensions. Maximum temperature in the skin is below 40 °C with no burning consequences on living tissue.

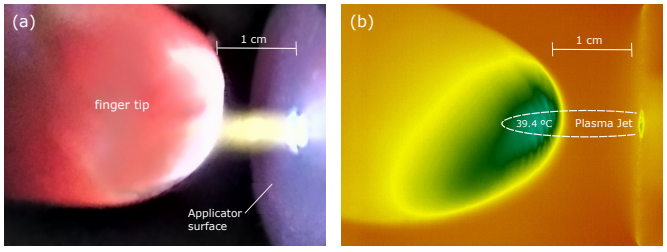

Fig. 8. Optical (a) and IR (b) pictures obtained during the interaction of air cold plasma jet with skin. IR emission form the jet can not be detected by IR camera until plasma touch the skin.

Our point of view for this analysis is macroscopic and it is based on the most recent review on the threshold of burn tissue damage in humans skin. It establishes that the perception of

pain in the skin of an adult is just around 43 ºC and that tissue damage occurs when 44 ºC is exceeded [50]. Therefore, the limit of 40ºC that we have established for ourselves is very prudent and is supported by our own evidence of the lack of discomfort and pain on the part of the patient during the entire treatment applied.

IV. Case study: use in healing torpid ulcers

Preliminary medical tests have been carried out under the compassionate use protocol approved by the Spanish Agency of Drugs and Medical Devices (AEMPS) file 823/20/AE of January 23^{rd}, 2020. A 94 years old female patient with previous pathologies of arterial hypertension due to arteriosclerosis, hypertensive heart disease, multifunctional anemia due to chronic disorder, folate deficiency and monoclonal gammopathy with ulcers in both feet was treated with the cold air plasma jet before described.

The protocol for preclinical treatment starts with a first stage where all tissue with necrosis is removed by scalpel and/or surgical scissors while full wound surface is cleaned with abundant saline solution and dried with sterile gauze. This operation is denominated debridement. After a final drying of the wound with a sterile gauze the cold air plasma jet is applied. The duration of each session was established depending of the wound surface, and this is calculated by using the criterion of 1 min/cm^2 of wound. Once the treatment is over, a sterile bandage is applied as usually in the wound cure. This protocol was repeated two times a week during nine weeks.

Fig. 9 shows a sequence of images with the evolution of an ulcer on the right foot instep from a surgical dehiscence associated with graft rejection where exposed tendons and bone without periosteum are observed. At the start of treatment, the ulcer had remained open for more than eight months with a poor prognosis, despite being treated regularly with a combination of pressure dressings and hydrocolloid patches.

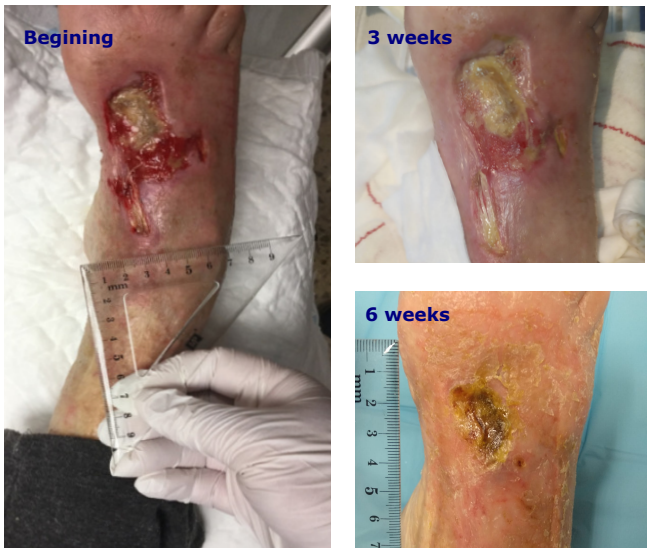

Fig. 9. Sequence of pictures obtained during the compassionate treatment of a ulcer in a 92 years old patient.

Fig. 10 shows the sequence of same patient but with other torpid ulcer of 3 cm diameter on her left foot which reached one centimeter depth. During the first three weeks of treatment a decrease in inflammation, pain and redness was observed with a notable re-vascularization showing the formation of granular tissue. In subsequent weeks, tissue recovery occurred until complete closure in the ninth week.

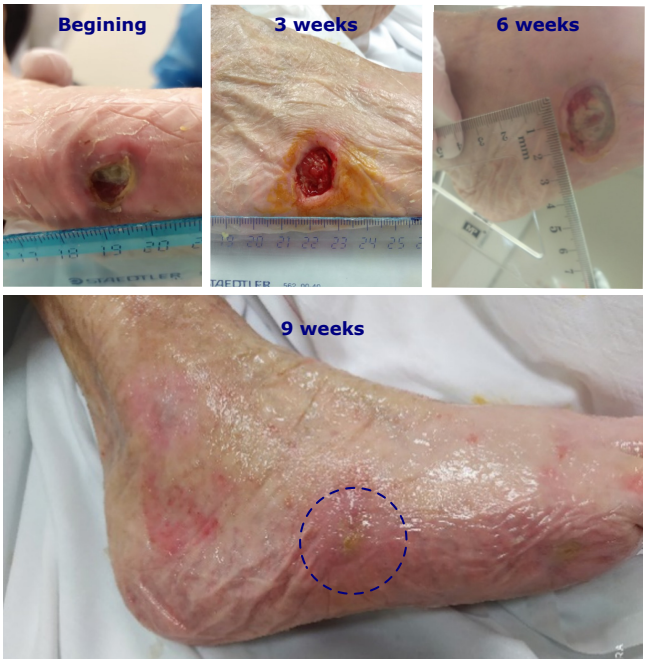

Fig. 10. Sequence of pictures obtained during the compassionate treatment of a ulcer in a 92 years old patient.

V. DISCUSSION

A new original equipment for the production of a cold air plasma jet which is intended for the treatment of chronic wounds is presented including electrical and plasma characterization measurements. A clinical case study on a patient with a negative prognosis of evolution after intensive testing of conventional treatments is also presented showing a remarkable result. In addition, first time electric field measurements within the air plasma jet induce us to consider its role as an important physical therapeutic mechanism.

The competition between physical and pharmacological effects of CAPs applied to wound healing acceleration is a current issue in the plasma medicine community. *Kramer et al* published a comprehensive review considering only the physical effects of heat transfer and UV emission as decontamination agents in competition with the pharmacological decontaminant effect of RONS [51]. In our case, both heat transfer and UV emission are too low to be seriously considered as is shown in sections III-B and III-C. Another important reference has been made by *Bekeschus et al.*, where redox-based biochemistry is pointed out as the main driver of wound healing [52].

On the other hand, an important experimental evidence of the electric field produced in the sine of atmospheric plasma jets was recently published by *Nastuta* and *Gerling* with a

laboratory device of helium and argon jets [73]. The values obtained range from 50 to 300 V/mm for helium and from 25 to 320 V/mm for argon. These fields were measured with a spatial resolution of 1 mm and a temporal resolution of $\leq$ 1 μs. The signals obtained show that the electric field wave follows the voltage and current waves generated by the plasma. Moreover, we have presented herein our own measurements using the same electro-optical probe system, which show a similar behavior of the V-I signals and with values of electric field ranging from 10 to 30 V/mm, as can be seen in Fig. 6.

An important question that emerges is whether these electric field values can be considered as being applied to the wound surface when the plasma interacts with the wound. For this we must take into account that these fields are time-varying following a waveform as shown in Fig. 5. This electric field wave is inevitably associated with a magnetic field wave (which we have not measured) which are indissoluble from each other according to Maxwell's equations. So, we can reduce the above question to the case of the interaction between this electromagnetic wave with a target surface and the consequences of this interaction on the values of both electric and magnetic fields.

For the case of a metal target [74], the charge carriers in the surface are free electrons that possess enormous mobility due to their small mass and the metal crystalline potential environment. These free electrons can react by canceling and reflecting any tangential electric field of an electromagnetic wave incident on the metal surface. The frequency response of these electrons to avoid penetration has a very wide range and reaches very high frequencies well above those used in plasma devices. In fact, this phenomenon is the basis of all passive electromagnetic shielding devices such as the well-known Faraday's Cage as a classic example. The fact that the tangential transmitted electric field on the metal surface vanishes is well described by solving Maxwell's equations and is not surprising. Regarding the penetration of the normal components of the wave, it decays exponentially and its penetration will depend on the conductivity of material. However, the wave is capable of inducing surface currents that circulate in closed loops on the surface. This is how induction stoves and other devices work by Eddy currents regardless of whether they are grounded or not.

In contrast, when the target is an open wound the surface conditions are completely different. The wounds that are wet from their own exudative fluids present a surface with cells in liquid suspension and the presence of dissolved salts that allow both negative and positive molecular charge carriers to coexist in solution. Due to the size of these carriers and the medium in which they are found, its mobility is very low compared to the free electrons of a metal. Indeed, from a macroscopic point of view, this is observed with the appearance of an electrical resistance (measured in ohms) and the ability to cancel an external tangential electric field is very limited. In the case of dry chronic wounds these same physical arguments make the same behavior but even closer to a dielectric material where the tangential transmitted component of an external electric field remains intact, the penetration of normal components is deeper although the availability of carrier should be even lower. From these arguments, which can be verified in physics and chemistry textbooks, we consider that we can transfer the values measured in the plasma to the wound surface with considerable confidence.

In addition to this, with no apparent link with CAPs, the effect of electric fields to accelerate wound healing has been clearly demonstrated by the well proven technology of Wound Healing Electro-Stimulation Devices (WHESD). WHESDs apply electric fields to unhealed wounds using a pair of electrodes attached to opposite edges of the wound and connected to a power source [53]–[62]. There has been a wealth of literature on the benefits of accelerating wound healing using WHESD for over two decades, with positive results in accelerating the wound healing process by speeding up the migration of key cells [63] enhancing the migration of lymphocytes [64], fibroblasts [65]–[67], macrophages [68] and keratinocytes [69]. In fact, recent results in the use of electric fields for the regeneration of damaged tissue in spinal cord and transcranial lesions have been published by *Matter, Shaner* and *Lu* [70]–[72]. The importance of electric fields in tissue regeneration processes has strong theoretical, experimental and clinical supporting evidence. For the specific case of chronic ulcers we are concerned with here, the typical parameters used by WHESD can be obtained from the work published by *Kloth* in 2014 [58]. The WHESD systems that are called High Voltage Pulsed Current (HVPC) use short pulses of $\leq$ 50 μs and voltages ranging from 75-150 V with maximum values reaching 500 V. Given a typical ulcer size of 10 to 50 mm, the applied electric field can be easily estimated by dividing the applied voltage by the distance between the electrodes placed at the wound edges, giving values from 1.5 to 50 V/mm.

Table I shows the typical electric field values as they can be delivered on the wound surface in order to compare the technologies under discussion.

Device Type	Pulse duration (μs)	Electric Field (V/mm)
HVPC Wound Healing Electro Stimulator Devices [58]	50	1.5 -50
Cold Helium Plasma Jet [73]	60	50 - 300
Cold Plasma Argon Jet [73]	60	25 - 320
Cold Air Plasma Jet (See Fig. 5)	50	10 - 50

TABLE I
ELECTRIC FIELDS VALUES THAT CAN BE DELIVERED ON A WOUND SURFACE BY WHESDS AND CAPJS: *Helium, Argon and Air*.

Unfortunately, we cannot directly measure the circulation of these induced currents, in contrast to WHESDs where the circulating current is easily measured because the wound is part of the circuit. However, the fact that the currents measured with WHESDs exist, directly implies that the induced currents also exist because the physics involved is the Ohm's law, $\mathbf{j} = \sigma\mathbf{E}$ where $\mathbf{j}$ is the current density vector, σ the conductivity and $\mathbf{E}$ the electric field. So, the agreement showed in Table I between WHESD and the CAAPJs is significant because it implies the circulation of locally induced currents in the wound area where the jet is applied driven by the electric field and controlled by the local wound conductivity.

When a WHESD is used, the stagnation of wound healing is broken and the tissue reacts, showing a micro-revascularization

that leads to a closure, with no doubt above that the breaking factor is the electric field. In the case of CAPs, the main mechanism of action that breaks the stagnation of chronic wounds in favor of a final closure remains under discussion. One extended explanation relates the biochemistry of nitric oxide (NO) with the angiogenesis observed in the tissue after plasma application [75]–[77]. However, the role of the electric field has been little mentioned, even in the case of dielectric barrier discharge (DBD) plasma devices, where its presence is evident [78], [79] and despite the fact that recent studies with DBD plasmas have shown that the improvement of microcirculation outlasts treatment time by measurements of oxygen saturation [80]–[83]. But, on the other hand, we have deduced here that our cold plasma jets can induce currents in the same range as WHESD. Thus, the role of electric fields in helping to break stagnation in chronic wounds by stimulating microcirculation should be reviewed in the light of its possible synergy with the decontaminating selectivity of RONS. In our opinion, this synergy could explain the resounding success demonstrated by CAPs as a unique tool for the treatment of chronic wounds in medicine.

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AUTHOR CONTRIBUTIONS STATEMENT

- Conceptualization: ODC & AMM
- Methodology: ODC, AMM, BH & HCG.
- Investigation: ODC, AMM, BH & HCG.
- Resources: ODC, AMM & BH.
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