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# Mimicking of human body electrical characteristic for easier translation of plasma biomedical studies to clinical applications

A. Stancampiano, T-H. Chung, S. Dozias, J-M. Pouvesle, L. M. Mir, E. Robert

**Abstract**— Non-thermal plasma (NTP) medical applications are now well established but the translation from *in vitro* plasma effects to *in vivo* effects remains far from being intuitive. Among various possible reasons, the translation may be disturbed by a loose control over the electrical characteristics of the different targets (e.g. cell culture dish, animal models) met during the development process, a parameter generally neglected and often unmentioned in papers. The aim of this study is to raise consciousness on how target electrical parameters (e.g. conductivity, electric potential...) play a major role in determining plasma treatment conditions. This effect is of particular relevance in plasma medicine where we move from treating small *in vitro* samples to humans, passing by animal models. The plasma conditions on targets with different electrical characteristics are compared by means of electrical measurements, optical emission spectroscopy and basic liquid analysis. Commonly tested *in vitro* targets induce the generation of a plasma significantly different from that produced in contact with a human body. We demonstrate how by means of a basic and easy to implement electrical circuit it is possible to “compensate” the electrical differences between *in vitro* models, mice and human body and in such a way to reach more reproducible treatment conditions between *in vitro* and *in vivo* targets. The proposed method for the control of the target electrical parameters could greatly favor the transition from *in vitro* and *in vivo* models to patients for many plasma devices developed for biomedical applications.

**Index Terms**— plasma medicine, atmospheric pressure plasma, compensation circuit, potential, targets

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## I. INTRODUCTION

THE antitumour effect of non-thermal plasmas (NTP) is now well established. The list of studies demonstrating plasma action on tumour cells *in vitro* is too long to be reported here but we can estimate that more than fifty cell lines of most types of cancers have already been tested [1–3]. Similarly, the number of studies demonstrating other *in vitro* plasma medical applications, such as wound healing and decontamination, is nowadays significant and constantly increasing [4]. Nevertheless, studies involving patients are still limited and the exact plasma action mode is far from being fully understood [5, 6]. In general, the translation from *in vitro* plasma effects to *in vivo* effects remains far from being intuitive. This task is even more challenging due to the physical and chemical complexity of NTP interactions with liquids/cells/tissues, which depend on a number of variables such as for example the applied voltage, pulse duration, pulse repetition frequency, gas mixture, distance to the target, time of exposure, protocols of application, physical and chemical characteristics evolution of the target during the NTP delivery.

Amongst these parameters, the electrical characteristics of the target are often neglected and usually go unmentioned, especially in papers which are more focused on the biological effects of the plasma. Nevertheless, as already reported in the literature, the target nature can strongly influence the plasma characteristics [7–11]. Physically, during a direct NTP treatment, the target (e.g. the medium bathing the cells in culture or the living treated tissue) is part of a ‘transient electrical circuit’ that is formed between the high voltage electrode and the ground (physical electrical connection to the earth). This means that target electrical parameters (e.g. conductivity, potential, total impedance...) play a major role in determining treatment results. Even with all the other parameters fixed, different targets can lead to very different plasma characteristics and therefore very different plasma effects. In this study, we demonstrate that even the same target (water solution in a multiwell plate) but under different electrical conditions (grounded or at floating potential) or set on different supports (dielectric plate vs metallic plate) can lead to very different treatment results.

The variation of the NTP treatment conditions due to target characteristics is of extreme relevance, especially

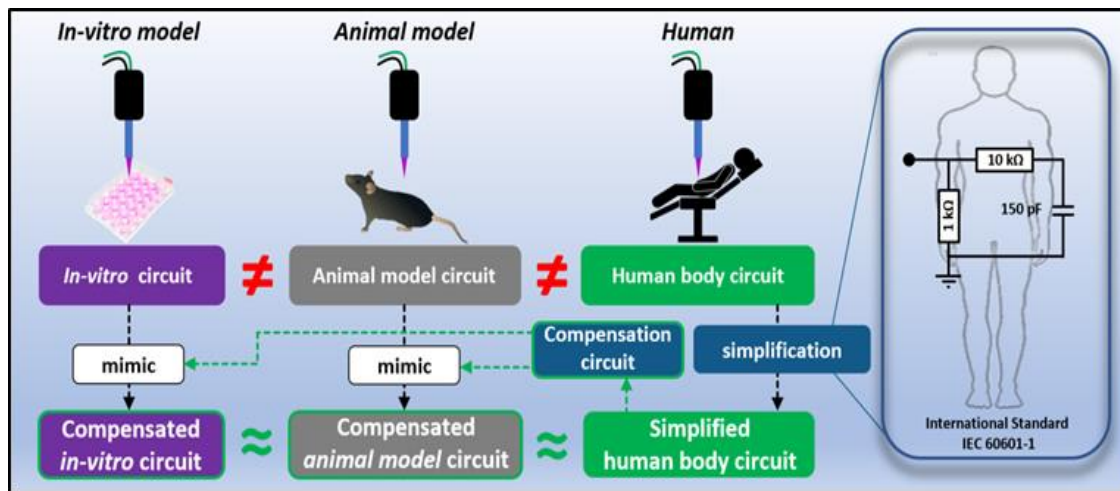


Fig. 1 . Graphical representation of the proposed method to make *in vitro* and *in vivo* models look more similar to the reference substrate for plasma medical studies, the human body.

in the plasma medicine field where we move from treating small *in vitro* samples to humans passing by various animal models.

The standard approach for medical validation that foresees the sequence *in vitro* model  $\rightarrow$  *in vivo* model  $\rightarrow$  patients may not be the best option if applied in a straightforward manner to plasma medical studies. The plasma generated on a small *in vitro* sample, characterized by a high conductivity and a low capacitance, can definitively not be compared with the one produced on a human body, with typically a lower conductivity and a much higher capacitance. This in turn ends up potentially affecting the reliability of the results achieved on *in vitro* and even *in vivo* models and their possibility to be translated to patients beside plenty of biological reasons that already make the passage far from being trivial.

Very recently, the issue has been partially addressed by some studies proposing feedback control systems based either on the real time tuning of the thermal effect and the intensity of plasma jets [11] or on the dynamic response of cancer cells to NTP based on mathematical model developed starting from experimental results [12]. Another proposed approach is based on the development of a basic material target that mimics the same dielectric properties of the human body and that could be used for the study of the plasma properties in more realistic operating conditions [13].

An improved control over plasma treatment conditions will also help to better investigate the impact of physical and electrical stimuli possibly induced by plasma. Within the recent past, the importance of electrostimulation at weak currents and the impact of the plasma-associated electric field were being rediscovered and investigated [14, 15].

In this study, we propose and successfully test a simple and versatile solution to reduce the degree of variation encountered in NTP studies translating from *in vitro* and animal models to human body. The main idea is to “compensate” the electrical differences between the models and the human body by means of a small and affordable electrical circuit that can be applied on both animal and *in vitro* models (see Fig. 1). The approach is significantly different from those found in the literature as it

is based on a non-invasive modification of the targets, with special attention to common biomedical models. Acting on the target electrical characteristics rather than on the plasma source control, the compensation method tackles the issue from a completely different angle and is potentially complementary to feedback-based control systems as those already proposed by Graves *et al* [11] and Keidar *et al.* [12]. Moreover, being based on a human body equivalent circuit derived from international standards for safety evaluation of electrical equipment, this approach could encourage the development of plasma sources complying with the requirements for basic safety and essential performance [16]. The same circuit may also be valuable for modelling studies trying to simulate targets close to those in actual plasma medical treatments.

## II. MATERIALS AND METHODS

### A. Plasma source

The atmospheric plasma jet used in this work is a Plasma Gun (PG) already described in detail in [8]. Briefly, the PG is a coaxial DBD reactor with a quartz capillary flushed with helium and powered by a micropulsed high voltage generator. The 12 cm long capillary was tapered at the outlet ( $\varnothing_{in} = 1.5$  mm,  $\varnothing_{ext} = 3$ mm). The plasma source was operated in two sets of operating conditions. In the first part, concerning only *in vitro* experiments, the source was flushed with a helium flow of 1 slm, powered by 4  $\mu$ s duration voltage pulses of + 12 kV peak with a 1 kHz repetition rate and maintained at a distance of 10 mm from the target. In the second half of the work that included *in vitro*, animal targets and humans, the PG was operated at 0.5 slm of helium, + 10 kV, 1 kHz and at 15 mm from the target. The different sets of parameters were selected in order to on one side emphasise reactive species production in *in vitro* liquid samples (so to ease their detection) and on the other side to limit the current crossing *in vivo* targets.

### B. *In vitro* targets

To represent *in vitro* targets we used a common 24-multiwell plate (Nunclon® Delta Surface, Thermo Fisher Scientific, DK) and a bigger custom-made well with a rectangular base (W 2 cm x L 5 cm x H 0.9 cm, material: PVC). The liquid adopted for the test was high purity water (distilled, conductivity <math>< 1\mu\text{S}</math>, by Chem-lab) with dissolved NaCl (Fisher Scientific, UK) to adjust its conductivity to the desired values of 10 and 20 mS/cm (reasonable values for common culture medium as those adopted in electroporation studies).

The conductivity of the solution was measured by means of a liquid conductivity probe (InLab Conductivity Probes 51344030, Mettler Toledo). A volume of 1-3 or 9 mL was introduced respectively in the multiwell plate and the custom well before each experiment. The multiwell plate was either positioned on a conductive plate (aluminium, 3 mm thick) electrically connected to ground or on a dielectric plate (low density polyethylene LDPE, 15 cm thick) that was itself positioned on the conductive plate (Fig. 2). Both plates were chosen large enough to completely cover all the multiwell plate bottom.

**Floating potential (FP):** the liquid at floating potential was not in contact with any conductive material.

**Ground potential (GR):** the liquid in the multiwell plate was connected to ground by means of a small stainless-steel plate (3.6 x 7 x 0.5 mm; Fig. 3a) while in the custom-made well grounding was processed through a stainless-steel plate of bigger dimensions (19 x 8.1 x 1 mm). Both connection plates were positioned inside the liquid close to the wall of the well, so to be as far as possible from the plasma impinging point at the centre of the well. While possible, electrolysis was regarded as negligible in this configuration as no visible bubbling or

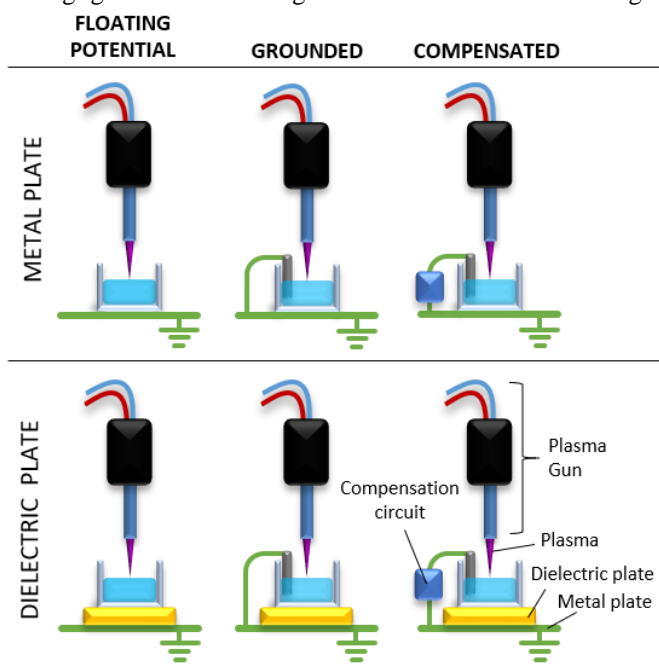


Fig. 2. Schematic summary of the investigated *in vitro* conditions for the PG impinging on a 24-multiwell plate filled with salted water.

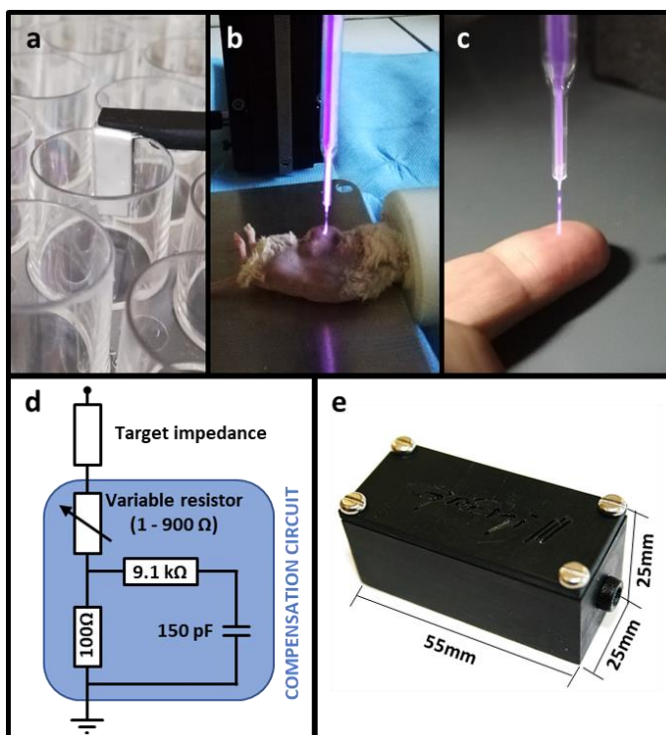


Fig. 3. a) Ground connection for the 24-multiwell plate; b) NTP treatment on an animal model on a grounded plate; c) NTP treatment on a human fingertip; d) Compensation circuit electrical scheme; e) photo of the compensation circuit with dimensions

erosion of the electrode was detected in any of the investigated conditions. Evaporation accounted for a liquid volume variation lower than 3% and was therefore considered negligible in this case.

### C. *In vivo* targets

To represent *in vivo* targets we adopted mice (BALB/cJ) with or without tumour (CT26 murine colon carcinoma subcutaneously at 500.000 cells/100  $\mu\text{L}$  on each flank, 8 days before treatment). Treated flank of the mice was shaved and depilated the day before the treatment. Mice were anesthetised and positioned on a metal heating plate connected to the ground to maintain a constant body temperature of the mice during the treatment (Fig. 3b). To represent the human body, the Authors volunteered as samples. The chosen treated area was the fingertips of the index finger of one hand (Fig. 3c). Ground connection was realized by means of a hand-hold metal cylinder. No other grounded surfaces were in contact with the person during tests.

### D. Electrical measurements

Voltage measurements were performed by means of a high voltage passive probe (Tektronix P6015A) on the high voltage cable connecting the PG and the generator. Current measurements were made by a current passive probe (Pearson 6585). For tests comparing *in vitro* and *in vivo* targets with human body, the current probe was positioned on the cable connecting the target to the ground. For tests comparing *in vitro* targets in different electrical conditions, since no ground

connection was available for the cases at floating potential, the current probe was positioned around the plasma source capillary (5 cm away from the outlet).

### E. Reactive species in liquid evaluation

Semi-quantitative chemical analyses of peroxide, nitrate and nitrite concentrations produced in the plasma treated liquid were performed to support the understanding of the influence of the target electrical conditions. For these semi-quantitative measurements Quantofix<sup>®</sup> test strips (Macherey-Nagel GmbH & Co. KG, DE) were used. Tests were repeated in triplicate.

### F. Optical emission spectroscopy (OES)

Plasma spectra were acquired by means of a spectrometer (MayaPro2000, integration time 200 ms, 8 averaged spectra for each acquisition) connected to a 1 mm in diameter VIS and UV optical fibre (14° full angle aperture). The optical fibre inlet was positioned horizontally 2 mm away from the plasma jet axis. The fibre vertical position was varied by means of a millimetric manual displacement system in order to scan the emission along the jet axis. All acquisitions were done within 10 s from the start of the treatment on a new sample. The spectra were analysed to extract the line intensity (a.u.) with no sensitivity correction for the chosen reference lines associated with species significant from a biological point of view: NO\* (236,3 nm), OH\* (308,8 nm), N<sub>2</sub>\* (B-A) (775 nm), O\* (777.2 nm).

### G. Compensation circuit

The human body is a very complex system and presents electrical characteristics that can greatly vary from one person to another due to various factors as for example age, size, sex and health conditions. Moreover the total body impedance is frequency dependent on and vary with the characteristics and position of the electrodes/contact points [17]–[19]. Even the same person may present very different total impedances if is connected to the ground through his bare feet, one hand or not at all. For safety reasons, in general in medical practice patient are unlikely to be completely insulated (at floating potential) and are more often connected to ground potential or to the return electrode of the electrical medical instrument in use (e.g. in electro-surgery) [20], [21]. In this study, we decided to adopt as a reference circuit for the human body the electrical model (see Fig. 1) reported in the International Standard IEC 60601-1 for medical electrical equipment [22]. This standard has been chosen as internationally recognized and widely adopted [16], [23].

In practice, the circuit is realized starting from the one proposed in the IEC 60601-1 (Fig. 1) and reducing both resistors (becoming 100 Ω and 9.1 kΩ) so to have some margin to take into account the target impedance (Fig. 3d). In first approximation, due to the short duration of the current pulse (0.7 μs) with respect to the characteristic time of the circuit (RC time constant = 1.5 μs) we can assume the 150 pF capacitance to be as a closed circuit. In this way we can calculate the equivalent impedance as the results of resistors in parallel. The equivalent impedance of the human body circuit (Fig. 1) after

the simplification results in 909 Ω while that of the compensation circuit, without taking into account the variable resistor, is 99 Ω. The difference between the two circuits after the simplification is therefore of 810 Ω. If we position the compensation circuit in series between the target and the ground, we will ideally end up with three resistors connected in series: the target, the variable resistor and the equivalent impedance of 99 Ω calculated above. In order to make the target-compensation circuit look similar to the human body circuit, it is sufficient to adjust the variable resistor so that, summed with the target natural impedance (that can be measured or estimated), will give a 810 Ω impedance. The proposed method is certainly affected by strong approximations but on the other hand presents a very simple structure and is easy to implement. Moreover, being composed of widely available electrical components, the total cost of the circuit results very low and its dimension can be further reduced starting from this first prototype (Fig. 3e).

## III. RESULTS AND DISCUSSION

### A. To ground or not to ground

As already mentioned, the electrical characteristics of *in vitro* targets are often neglected or considered as marginal. In this first part of the study we demonstrate how even by positioning the same liquid (1 mL salt water at 10 mS/cm), in the same 24-multiwell plate, but on a conductive grounded plate (e.g. biosafety cabinet) or on a dielectric plate (e.g. standard lab table) we can greatly influence the treatment conditions. Considering the cases with the liquid at FP, we see that moving the plate from a dielectric support to a conductive one significantly modify the current going through the plasma in the capillary (Fig. 4). For these FP cases, the current peak (28mA on dielectric plate and 35mA on conductive plate) that takes place after the impact of the primary plasma front and the formation of a conductive channel between the high voltage electrode inside the capillary and the target [8] is limited by the capacitance associated to the *in vitro* target. Therefore, increasing this capacitance by moving the plate from a

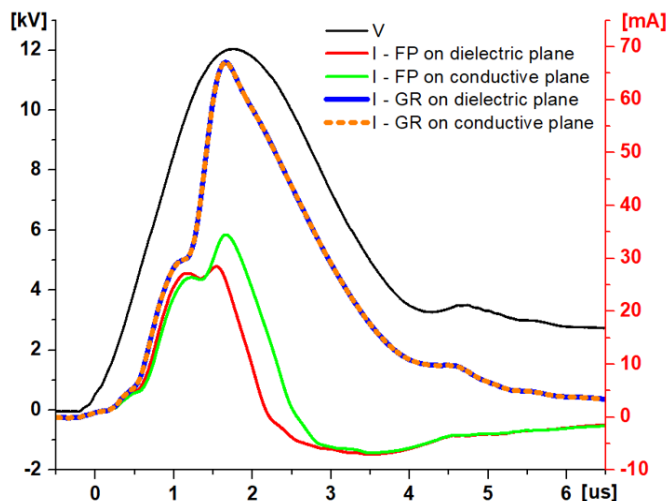


Fig. 4. Voltage (V) and current (I) waveforms during direct treatment of water in 24-multiwell plates at different electrical potentials (floating FP or grounded GR) and on different supports (dielectric or conductive). Gap distance 10 mm.

dielectric plate to a conductive grounded plate may increase the associated current.

A variation of the treatment conditions moving from one support to another is also testified by the analysis of the treated liquid (Tab. 1) where we measure that a significantly greater amount of peroxides and nitrates are produced when the support is conductive. Therefore, we are led to believe that two biological *in vitro* assays, performed one inside a biosafety cabinet and the other on a dielectric table would supposedly produce different results.

The same test was repeated with the liquid set at GR. In this case the recorded currents resulted virtually identical between the two supporting plates (Fig. 4). Grounding the liquid is like connecting a resistor in parallel to the capacitance we already discussed above. Since the conductivity of the tested liquid (as well as that of most common culture media) is relatively high, the equivalent resistance results small. This in turn favours the passage of a greater conduction current compared to the cases at floating potential (Fig. 4) and greatly reduces the impact of the capacitance associated with the plate underneath. These observations were confirmed by the liquid analysis where the shift from floating to grounded potential induced the production of higher concentrations of reactive species (Tab. 1) and hindered the influence of the supporting plate. In fact no significant difference could be detected between the two grounded cases. The differences between the FP and GR reactive species production in liquid can potentially be

TABLE 1

Concentrations of H<sub>2</sub>O<sub>2</sub>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> generated in 1 mL of water in 24-multiwell plates for different target electrical conditions. Treatment time 5 min. Gap distance 10 mm.

Target potential	Support	H <sub>2</sub> O <sub>2</sub> [mg/l]	NO <sub>3</sub> <sup>-</sup> [mg/l]	NO <sub>2</sub> <sup>-</sup> [mg/l]
Floating	Dielectric	2 - 5	10	<1
	Conductive	10	10-25	<1
Grounded	Dielectric	25	100	20
	Conductive	25	100	20

attributed also to different mechanism of charges accumulation on the liquid surface (reduced in GR cases) and the influence of induced current on the liquid chemistry.

Also OES acquisitions (Fig. 5) show a significant higher intensity emission for the GR cases (since they were identical, only one is reported for the simplicity) compared to the cases at FP. Emission of the NO\* system and nitrogen first positive system N<sub>2</sub>(B-A) almost only occur for the GR cases. This is in agreement with already published studies on the Plasma Gun

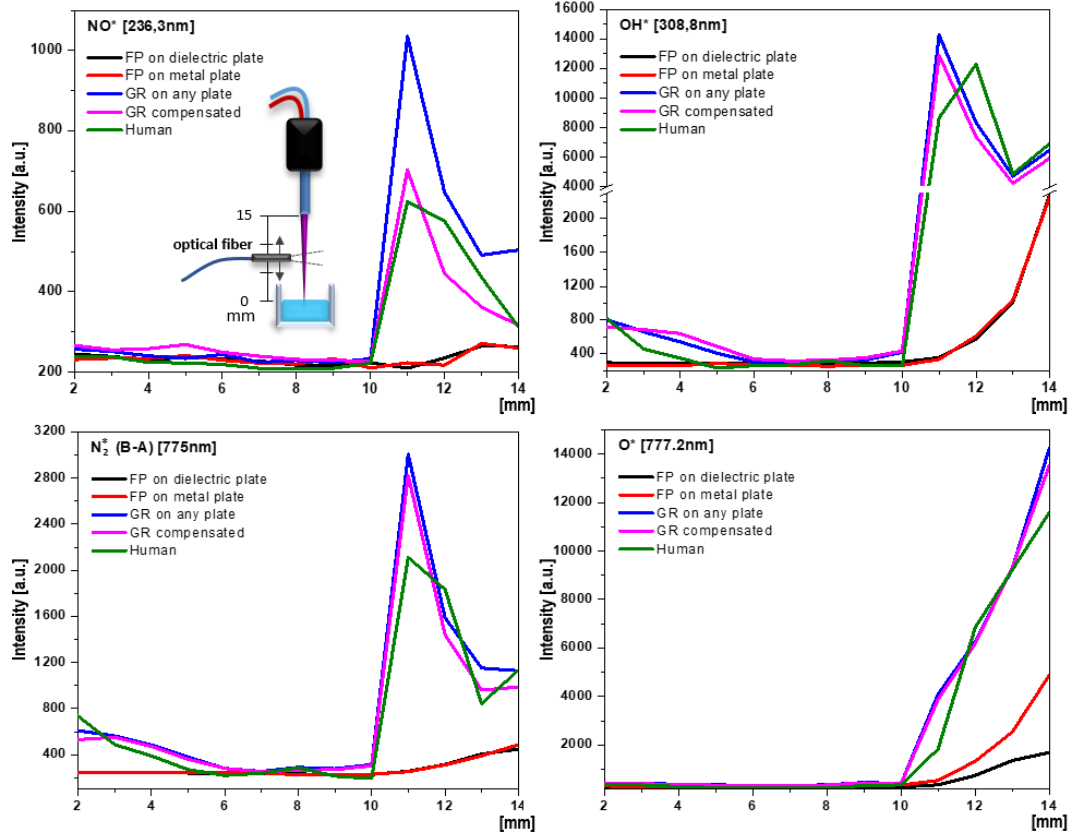


Fig. 5. Plasma gun (0.5 slm, 10 kV, 1 kHz, 15 mm gap) emission intensity (expressed in arbitrary unit) for selected spectral lines and for different electrical conditions of an *in vitro* target (FP: floating potential, GR: grounded) and a grounded human body. Acquisition were collected at different distances from the surface of the target.

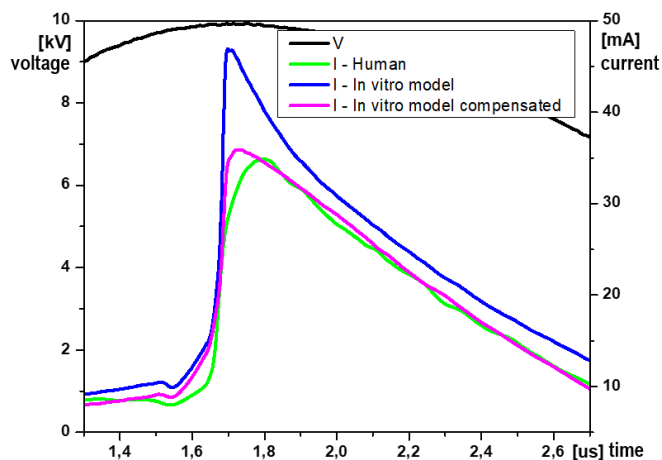


Fig. 6. Voltage (V) and current (I) waveforms during direct treatment on grounded human body and on grounded salt water (10 mS/cm) in 24-multiwell plate with and without compensation circuit. Gap distance 15 mm.

comparing free jet condition with those impinging on a grounded metallic target [8].

The differences between the FP and GR cases are particularly emphasized in proximity of the plasma source outlet. While the FP cases exhibit a constant decrease of the emission intensity moving away from the outlet, the GR cases present a peak for the  $\text{NO}^*$ ,  $\text{OH}^*$  and  $\text{N}_2(\text{B-A})$  at 11 mm from the liquid surface (4 mm from the capillary, see Figure 5). This is probably due to fluid dynamic reasons related to the mixing of the helium flow with the surrounding air. Interestingly, for some species ( $\text{OH}^*$  and  $\text{N}_2(\text{B-A})$ ) there is an evident difference between the FP and GR cases also in close proximity of the target surface. While the FP present nearly no emission in close proximity to the target surface, the GR cases show some significant emission. This is probably the consequence of a secondary ionization front propagating from the target to the high voltage electrode as already reported in previous studies [8] and that may be more energetic in the GR cases in virtue of a higher degree of ionization in the noticed plasma channel left by the primary ionization front. It must be recalled that while reactive species with relatively long lifetime (e.g.  $\text{NO}^*$ ,  $\text{O}_3$ ,  $\text{H}_2\text{O}_2$ ) generated in the plasma channel can be transported to the liquid surface by the gas flow, other with sub-millisecond lifetime such as the  $\text{OH}^*$  can reach the target only if generated in close proximity to the interface [24].

In this perspective, it is important to underline how the emission spectra of the *in vitro* GR case results significantly more similar to that of a human body than both *in vitro* FP cases. Several reactive species playing important roles in plasma medical applications, such as in our case  $\text{NO}^*$  for the promotion and regulation of wound healing [25], may not be produced on an *in vitro* FP target or are produced in significant lower amounts when compared to a human body target (Figure 5).

These results suggest that grounding *in vitro* samples may be a good option to achieve more realistic operating conditions in plasma medical studies. Summing up this first part of results, we may conclude that grounding *in vitro* models may beneficially affect the plasma treatment making it more stable

and independent of the supporting plate nature while increasing the amount of reactive species, especially the nitrogen ones, produced both in gas and in liquid phase.

### B. Electrical compensation of models

In the second part of the study, the proposed compensation circuit described in the materials and methods was assessed. As mentioned, the goal of the circuit is to adjust the total impedance of the investigated models to match that of a human body. As a first step, the current on the ground connection of a human body treated by the PG (Fig. 6) was recorded. This measurement was adopted as a reference for later tests.

Then, the current going through 3 mL of salt water (10 mS/cm) in a 24-multiwell plate and connected to the ground was measured. The same was repeated for the same setup but after the connection of the compensation circuit between the liquid and the ground. As shown in Figure 6, the current peak going through a liquid sample (not compensated) is significantly higher than that through a human body. This is due to the considerably lower resistance and capacitance characterizing the *in vitro* sample. The introduction of the compensation circuit greatly reduces these differences so that the current measured on compensated *in vitro* targets is much closer to that measured on a human body.

Since the recorded current depends on the characteristics of the plasma, we can assume that with the compensation circuit, the current generated on the *in vitro* sample being more similar to the one generated on a patient, the plasma effects would thus be more similar. This is partially confirmed by the OES results (Fig. 5) where it is observed that for the emission associated to  $\text{NO}^*$  the compensation circuit helps the plasma generated on the *in vitro* sample to resemble that on the human body. The emissions associated to the other species are less affected but on the other hand no negative effect is observed due to the introduction of the compensation circuit. Especially, it is worth noting that if compared to the more common condition featuring an *in vitro* sample at FP, the proposed condition with a grounded and compensated sample significantly helps the generation of a plasma similar to the one found when treating a human body.

TABLE 2

Concentrations of  $\text{H}_2\text{O}_2$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  generated in water in a 24-multiwell plate (3 mL) and in the custom well (9 mL) with and without the compensation circuit. Gap distance 10 mm.

Compensation	Well	Treatment time [min]	$\text{H}_2\text{O}_2$ [mg/L]	$\text{NO}_3^-$ [mg/L]	$\text{NO}_2^-$ [mg/L]
no	24-multiwell	5	2	10-25	1
	Custom-made	15	5	25	1-5
yes	24-multiwell	5	2	10	1
	Custom-made	15	2	10	1

Only for the human body and GR cases, we observe a significant production of nitrogen reactive species, absent in the FP cases. The same holds for OH\* that looks similar, as well as for the magnitude of the O\* emission which is the same.

The same tests were repeated with similar results (data not shown) changing the geometry (use of the custom-made well) or the liquid conductivity (20 mS/cm instead of 10 mS/cm). Interestingly, the circuit, originally designed to make the *in vitro* samples to behave more similar to the human body, may also be used to homogenize the behaviour of different *in vitro* samples (e.g. different well or liquid) from an electrical point of view. This is confirmed in Tab. 2 where the compensation leads to the production of similar amounts of reactive species for two different well geometries and liquid volumes. In this case, not only the compensation circuit was adapted but also the treatment time so to maintain a constant ratio of 5 min treatment every 3 mL of liquid. Such setting of the ratio between treatment duration and liquid volume to reach the same reactive species concentration is very often used but may not be accurate enough as expected without the compensation circuit. This was measured with our set up for the compensated cases for which moving from 5 to 15 min of treatment when increasing the liquid volume, resulted in a drastic change of the reactive species concentration (first two rows of Tab. 2)

As a further validation of the compensation method, the same approach was repeated on animal targets. Due to a lack of supporting literature concerning equivalent electrical circuit for mice and the complexity of effectuating accurate measurements of the mice properties, in this case we approached the compensation through successive iterations. The best results in this case were achieved assuming a mouse equivalent impedance of 200  $\Omega$ . This is certainly an approximation that may be affected by several factors such as skin condition and mouse to ground connection (in this case processed through the metal plate supporting the mouse). Preliminary results show that the electrical characteristics of a mouse are, as expected, already closer to those of a human body than those of an *in vitro* sample. Even if on a smaller scale, also in this case the addition of the compensation circuit contributed to increase the similarity both in the rising and falling front of the current waveform (Fig. 7). The same experiment was repeated on mice with grafted tumours (Fig. 8). For this target, the compensation resulted less satisfactory probably in virtue of the different resistivity of the tissues exposed to the plasma (healthy skin and tissues vs a thinner skin and a more conductive tumour mass below the skin). Indeed, tumours present a conductivity that is usually higher than the healthy tissue [26], meaning that the equivalent circuit also will be different. Perhaps it should have to be compared to the reference circuit for a human body affected by the same pathology.

#### IV. CONCLUSIONS

In the first part of this study, the importance of taking into account and control the electrical characteristics of the *in vitro* samples commonly employed in plasma biomedical studies was demonstrated. When the target is at floating potential, even the

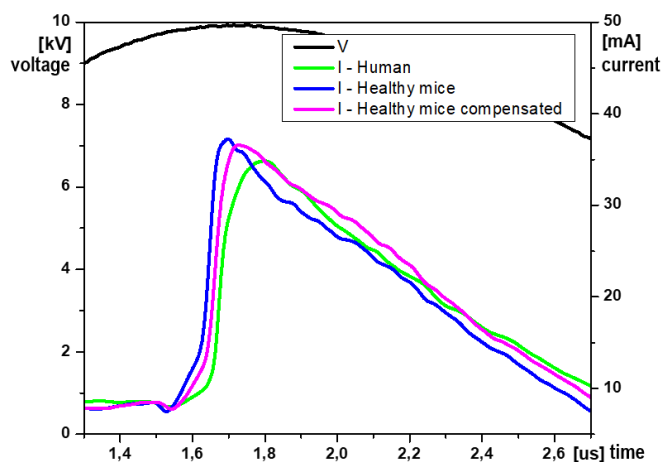


Fig. 7. Voltage (V) and current (I) waveforms during direct treatment of human body and healthy mice with or without compensation circuit. Gap distance 15 mm.

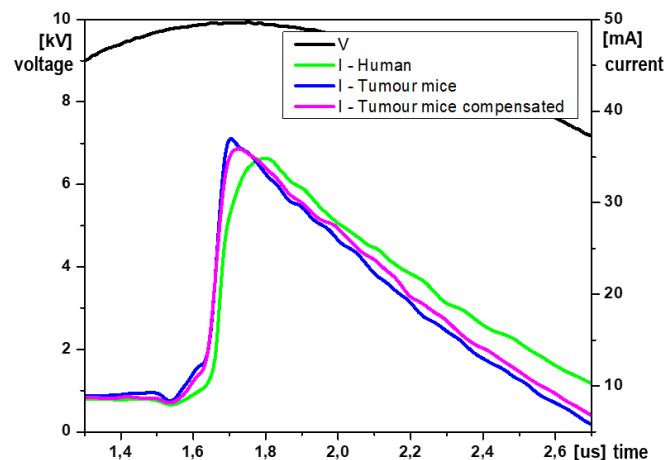


Fig. 8. Voltage (V) and current (I) waveforms during direct treatment of human body and mice with grafted tumour and with or without compensation circuit. Gap distance 15 mm.

nature of the plate supporting the sample can influence the treatment. For example, different concentrations of reactive species in the treated medium can be the result of treating the sample on a dielectric table rather than inside a biosafety metallic cabinet. For this reason, it may be advisable to impose the potential of the target to ground level in order to reduce the influence of the surroundings and increase the reproducibility of the experiments. Nevertheless, a well-controlled floating potential condition can still be more appropriate for specific cases where for example the risk of contamination does not allow the use of submerged electrodes into the liquid.

In the second part of the study, we proposed a new method aimed at the reduction of the degree of variation encountered in NTP studies moving from *in vitro*/animal models to human body. Keeping in mind that the final target of plasma medicine studies are human patients, we propose to adopt a small and affordable electrical circuit to “compensate” the electrical differences between models and human body and, as result, have the same NTP effects on very different targets, including humans.



The use of the compensation method was proven to lead to not only closer current waveforms through different targets but also to a closer reactive species generation in liquid solutions. Also, emission spectra analysis confirmed that the plasma characteristics on a compensated *in vitro* sample are closer to that found on a human body if compared with those generated on a more common FP configuration.

Results therefore confirm that the circuit helps maintaining the same treatment effects from one target to another.

Moreover, the mimicking circuit could also help to keep the same treatment conditions when there is a change from one *in vitro* support to another (e.g. from a well plate type to another).

In spite of the promising results, the compensation circuit alone is clearly not sufficient to transform *in vitro* samples into perfect replica of a human body. Other factors not related to the electrical properties such as surface evaporation and morphology inherently induce differences between models and human body. For these aspects, other studies have proposed other methods to mimic various human body properties [27]. Is not to be excluded that in the future these methods could be implemented with the electrical compensation method here described to provide even better results.

In the meantime, while being not perfect, the proposed compensation method could significantly improve the representativity of *in vitro* plasma experiments in a practical and simple manner that at present show no drawback that could prevent its use.

We therefore hope that this solution would help to optimize the transition from preclinical to clinical studies in the plasma medicine field.

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