IL NUOVO CIMENTO **42 C** (2019) 232
DOI 10.1393/ncc/i2019-19232-5

COMMUNICATIONS: SIF Congress 2018

A plasma medicine tool for accelerating blood coagulation

G. DE MASI ([1])([2])([*])

([1]) *Consorzio RFX - Padova, Italy*
([2]) *Dipartimento di Scienze Mediche e Chirurgiche, Università degli Studi "Magna Graecia" Catanzaro, Italy*

received 25 March 2019

Summary. — Plasma medicine is an emerging field among the broad spectrum of the plasma science and technologies. In this contribution, we focus on the application of Cold Atmospheric Plasmas (CAPs) for accelerating blood coagulation. A review of the state of art on the topic is discussed in the first part. In the second part, the main features of the Plasma Coagulation Controller, a plasma source recently developed at Consorzio RFX, are presented together with the *in vitro* tests on blood samples withdrawn from patients following anti-coagulant therapy. Blood clots are clearly visible after treatments even shorter than 1 minute.

1. – Plasma medicine: An introduction

Plasma medicine is an emerging research field, which deals with the use of plasma for several medical purposes [1, 2]. It can be really considered a multidisciplinary topic, involving competences in the fields of physics, chemistry, biology and medicine. Plasmas are ionized gases in which part of the gas molecules are split into ions and electrons, giving rise to noteworthy physical and chemical effects that have been well-known and exploited for different applications for many decades (from nuclear fusion to plasma etching). Also in the medical field, some plasma technologies delivering significant power to the target (*e.g.*, the Argon Plasma Coagulator, APC [3]) have been used for tissue cauterization, blood coagulation and/or in substitution of the traditional scalpels. However, the basic idea of plasma medicine is to get a therapeutic effect on the living substrate through a very localized and non-thermal (few watts of power) treatment. In this respect, the establishment of the plasma medicine is much more recent and can be identified in the seminal works on plasma needles for surface treatment of biomaterials [4] at the beginning of the 2000s. Since then, this emerging science has rapidly grown and gained interest thanks

([*]) On behalf of the Brains2South Plasma Medicine Research Group.

to the ambitious prospect to deliver a therapeutic action minimizing the side effects. At present, many international research groups involving physicists, engineers, biologists, chemists and medical doctors are working on different plasma medicine applications. Very promising results are reported in dental cavities treatments [5], tissue disinfection [6, 7], wound healing [8], blood coagulation [9-12], dermatological diseases [13], but we may cite also the more pioneering studies on the possibility to selectively trigger the apoptosis of cancer cells [14, 15].

1˙1. *Cold Atmospheric Plasma sources.* – Plasmas can generally be divided into thermal plasmas, where all the components (ions, electrons and neutral particles) are heated to very high temperatures, and non-thermal plasmas, where only the electrons achieve a very high temperature. Commonly, high power generated plasmas (fusionistic plasmas, arc plasmas: lightnings, APC, etc.) are thermal since accelerated electrons and ions through frequent collisions get the thermal equilibrium. On the other side, non-thermal plasmas are obtained by applying lower power and using different techniques to prevent thermalization. Plasma medicine applications concern these latter plasmas, typically produced at low temperature and at atmospheric pressure. Several schemes of CAP (Cold Atmospheric Plasma) sources have been developed and can be roughly divided in Dielectric Barrier Discharge (DBD) and radio-frequency (RF).

In the DBD concept, short (in the range of ns to 100 ns) high voltage (in the range of tens of kV) pulses are applied between two electrodes separated by a dielectric layer (glass, quartz, pyrex, ceramic). The applied electric field ionizes part of the neutral gas (air, helium, argon or other mixture are used) flowing between the electrodes but the presence of a dielectric layer limits the current and prevents the arc transition. By exploiting the same concept, different modifications have been proposed using a double dielectric layer on both electrodes (double DBD) or replacing one electrode with the substrate to be treated (Floating Electrode DBD [11]). In the RF concept, a sinusoidal radio-frequency (from MHz to 100 MHz) voltage is applied between the electrodes to ionize the main gas. Depending on the source type, electrodes geometry and kind of coupling, voltage can sensibly vary from tens of volts ([6]) to few kV (in the case of the plasma jets [16, 17]). Regardless of the scheme used, however, plasma produced through a CAP involves many components: charged particles (ions and electrons), visible and UV radiation, (small) currents and reactive species. These latter come from the ionization of the air molecules mixing with the main gas when operating at atmospheric pressure. The presence of Reactive Oxygen and Nitrogen Species (RONS) has been studied with particular interest [18], also because RONS are already involved in several biological processes (see for instance [19]). A therapeutic effect can also result from the produced UV radiation (UVB and UVA radiation phototherapy is for instance an approved clinical treatment in dermatology [20, 21]), even if the most energetic component UVC is not desirable due to DNA mutagen properties [22]. The role of charged particles, especially in blood coagulation acceleration, has been highlighted in other studies [23]. Investigation is still ongoing to understand the interaction with living matter of the different plasma components, all of which, probably, have a synergistic action in realizing the desired therapeutic effect.

2. – Applications on blood coagulation

Coagulation is the first step of haemostasis and occurs immediately after damages to the blood vessel to prevent bleeding from the injured vessel and to seal the area to

protect it from infection. The mechanism of coagulation involves both platelet activation and coagulation factor cascade. It is a complex process, which follows two pathways: the extrinsic or the intrinsic pathway. The first one is triggered by the release of tissue factor from the site of injury, while the second is stimulated by the contact with a negatively charged surface. Both these pathways lead to the same crucial reactions that stimulate the conversion from inactive fibrinogen to active fibrin [24].

Several studies have been published describing the ability of non-thermal plasmas to induce accelerated blood coagulation, both *in vitro* and on animal models. The group at Advanced Industrial Science and Technology (AIST) in Tsukuba (Japan) has shown that a helium plasma jet based on the DBD layout could stop bleeding from a femoral artery in a mouse animal model in less than 10 s [9], while keeping the treated surface at less than 40 °C; furthermore, they showed that the blood clots produced by the plasma action comprised eosinophilic fibrous membrane-like structures produced by both platelets and erythrocytes [25]. The group at Drexel University (USA) has demonstrated, again using a dielectric barrier discharge, a strongly accelerated coagulation, both *in vitro* and on *ex vivo* human tissue samples and organs, again keeping the substrate near room temperature, and has also initially proposed a possible mechanism based on calcium ion concentration change induced by a redox reaction [11]. This mechanism was later revised, based on new experimental observations, and an alternative mechanism based on the activation of some coagulation proteins, *e.g.*, direct conversion of fibrinogen into fibrin, was put forward [26]. Kuo and associates in New York University (USA), using a concentric electrode magnetized plasma torch powered at 60 Hz AC and operating in air on a pig animal model, also found rapid coagulation, with a reduction of the bleeding time from 190 s to 18 s in a simple straight cut and from 4 min to 13 s in a cross cut [12]. In these experiments the proposed mechanism was the interaction of atomic oxygen produced by plasma with water in the blood, producing oxidants, which activate erythrocyte-platelet interactions and influence plug formation [27]. Finally, blood coagulation induced by a CE-certified plasma jet was investigated quantitatively *in vitro* by measuring haemoglobin absorbance by another research group [28].

3. – Plasma Coagulation Controller

In this section, we present the main features and the results obtained *in vitro* of the Plasma Coagulation Controller (PCC). PCC [10, 29] is a cold atmospheric plasma source prototype based on the traditional DBD scheme and specifically designed for accelerating blood coagulation. Using helium or argon as a main gas, PCC produces a stable plasma plume (fig. 1) at a low temperature and suitable for applications on the human body. Different tests have been performed in order to characterize the power supply circuit in terms of power delivered to the target, of chemical composition of the produced plasma and, finally, to highlight the ability of the PCC to accelerate blood coagulation on blood samples.

3˙1. *Electromagnetic characterization.* – According to the DBD concept, in the PCC, plasma is formed by applying a time variable high voltage electric field between two close electrodes. To make the system extremely compact, portable and flexible, PCC is composed of two main modules: a control unit and a plasma source head.

Figure 2 shows a scheme of the main components layout: the source head (where the plasma is formed), a step-up transformer (embedded in the source head but shown for simplicity as a stand-alone module) and a power supply embedded in the control unit.

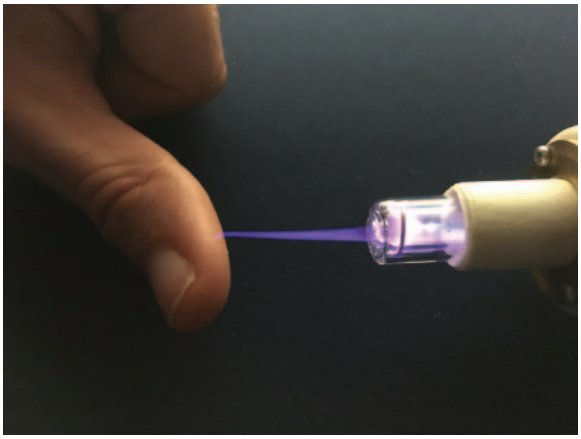

Fig. 1. – The Plasma Coagulation Controller: a cold atmospheric plasma plume is produced using helium as a main gas.

The control unit is also provided with an Arduino microcontroller and a graphic interface that allow to easily set the discharge repetition rate (1–20 kHz) and the applied voltage (2–8 kV) depending on the kind of application requested.

The source head (where plasma is created) is connected to the gas tank and a flow meter used to set the gas flow rate. In more detail, in fig. 3, the geometry of this component is shown: a pyrex tube (1 mm diameter), closed on one side, houses the high voltage copper electrode (connected to the step-up transformer); two concentric pyrex tubes, closed on one side, host in the middle the grounded ring electrode; between the two pyrex component there is a channel (8 mm diameter) in which the gas is injected. At each discharge, a short (∼100 ns) high voltage pulse is applied between the electrodes and the gas flowing in the channel is ionized and a plasma plume is obtained (see also fig. 1).

Figure 4 shows the behaviour of the applied voltage (measured through a passive high voltage probe) between the electrodes as a function of the discharge repetition rate

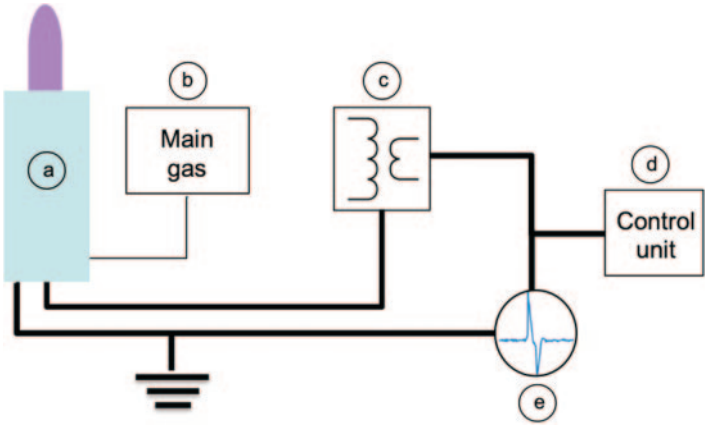

Fig. 2. – Schematic of the main PCC components: (a) source head; (b) gas line and flow meter; (c) step-up transformer (embedded in source head); (d) control unit and (e) power supply connected to the standard electricity.

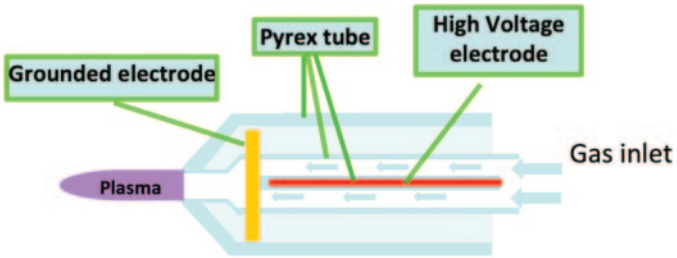

Fig. 3. – PCC head components. The neutral gas is injected from the back hole and is ionized between the electrodes when a high voltage pulse is applied.

and the circuit opening time (the time in which the capacitor in the driver circuit is charged). Since the circuit scheme involves also a ballast resistor, the relation between circuit opening time and applied voltage is not linear, but displays an optimum value at each discharge repetition rate. In order to compare the effect on blood coagulation, we chose three operational scenarios according to the applied voltage, named high (8 kV, 2 kHz), standard (6 kV, 5 kHz) and low (4 kV, 10 kHz). Plasma current has been also measured through an AC current probe on a metallic grounded target placed at a distance of 1 cm from the source head. In all operational scenarios, the effective current averaged over 1 ms of application does not exceed 1 mA, making the treatment of living substrates suitable and safe.

3˙2. *Spectroscopic analysis*. – As described in sect. **1**˙1, the possible candidates driving the interaction with living matter are the chemical species produced upon the ionization process of the helium/air mixture. The chemical composition of the plasma plume was evaluated through the emission spectroscopy analysis.

For this purpose, a diagnostic setup involving a PI IsoPlane spectrometer of 320 mm focal length equipped with a 300 and a 2400 g/mm gratings, and coupled to a PIXIS camera with 2048 × 512 square pixels of 13 μm was used. A metallic target was placed at 1 cm from the PCC nozzle and the camera was placed perpendicular to the PCC-target axis with the idea of investigating the composition of the plasma impinging on the target. The entire spectrum between 330 nm and 800 nm has been acquired and the

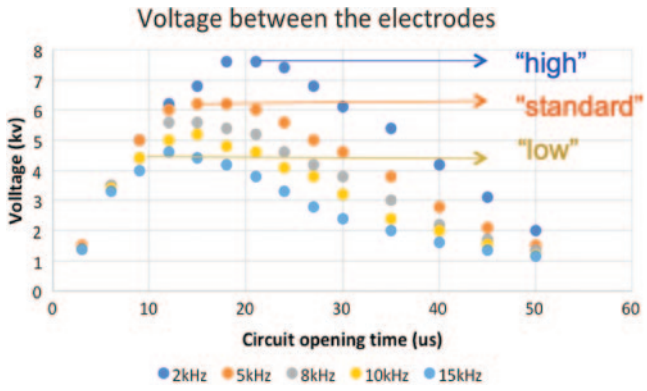

Fig. 4. – Behaviour of the maximum applied voltage as a function of the circuit opening time and discharge repetition rate.

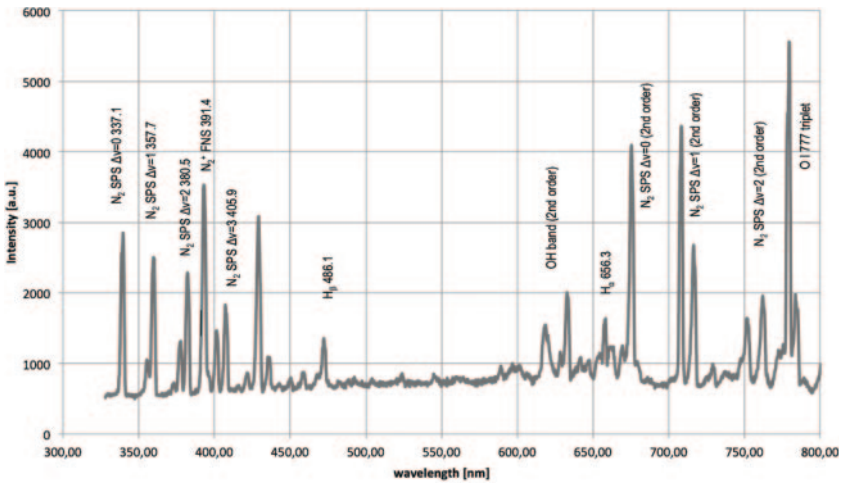

Fig. 5. – Emission spectrum of the PCC plasma impinging on the target as collected by the camera.

result is shown in fig. 5. Reactive species of nitrogen (N_2^+ and N_2) and oxygen (OH^-) are clearly visible together with helium excited states (He I), the oxygen triplet and the H_α and the H_β lines.

Following the method used in [10, 30], the rotational spectrum of the N_2 molecule at 670 nm (second positive system) has been used to evaluate its rotational temperature. This estimate is obtained by simulating the expected N_2 rotational spectrum and minimizing the difference with the experimental one (the best match is reported in fig. 6). The rotational temperature of the N_2 molecule within the PCC plasma plume ranges around 279 ± 27 K, thus very close to the room temperature. This confirms that the produced plasma is non thermal (electrons, accelerated by the high voltage electric field, are supposed to have a much higher temperature).

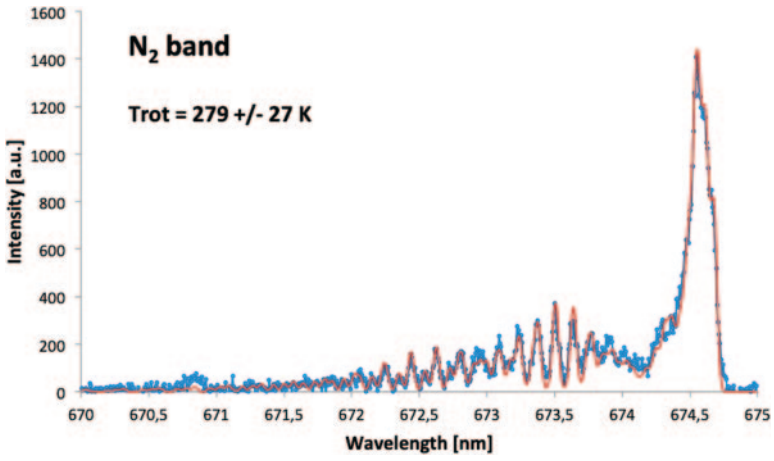

Fig. 6. – High resolution emission spectrum of the N_2 band: measured (red line) and simulated (blue dash-dotted line).

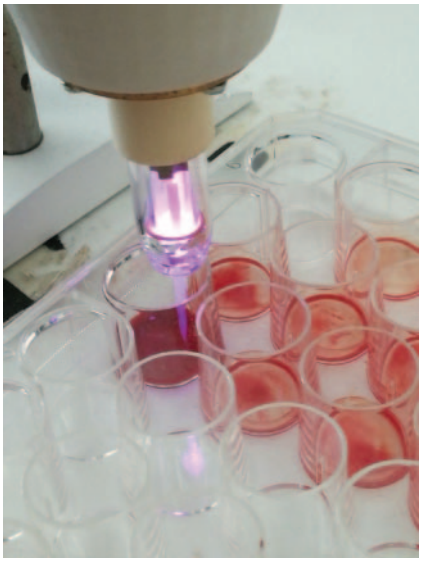

Fig. 7. – PCC treatment of blood samples in a 24-well plate.

3˙3. *In vitro tests on blood samples*. – The effect on blood coagulation of PCC was tested on different *in vitro* experiments; here, we present the most straightforward experimental evidence.

The PCC device has been designed with the idea of locally stimulating blood coagulation in patients whose normal coagulation is hampered by systemic diseases or due to pharmacological therapy (following, for instance, electrophysiological devices

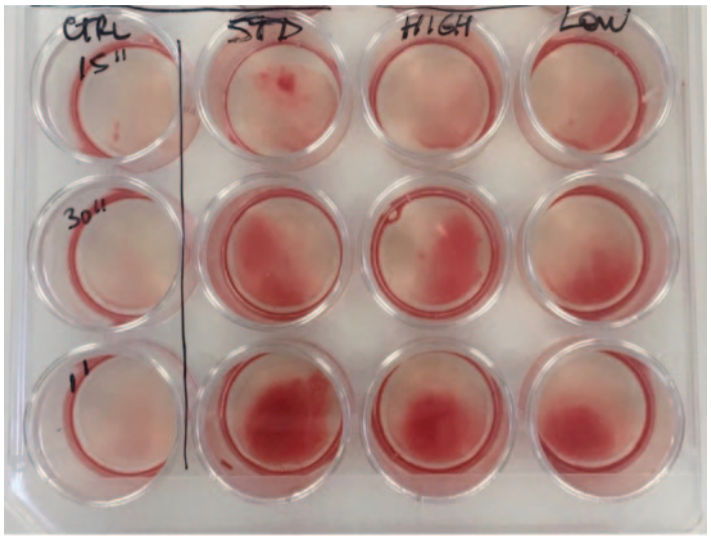

Fig. 8. – Well plate after PCC treatments: on the left control sample exposed to air for 15 s, 30 s and 1 min; on the other three columns the result for different PCC setup (standard, high and low as previously defined).

implantation). For this reason, blood samples were withdrawn from human patients assuming anticoagulant drugs and stored in Vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). Then, the blood was transferred to a 24-well plate (200 μl of blood per well) and mixed to a $CaCl_2$ solution to cancel the anticoagulant effect of the EDTA. Different PCC treatments were performed directly on the plate (fig. 7) and, after the treatments, the liquid blood was removed, while the clots remained at the well bottom. Figure 8 shows the result for different PCC setups (standard, high and low) and different treatment duration (15 s, 30 s and 1 min), compared to blood samples exposed to air (control). While control samples (left column) do not show any coagulation footprint, blood clots are present for all PCC setups after 30 s and 1 min treatments (second and third rows). A more quantitative and statistical analysis of the blood samples treatment can be found in [10].

4. – Discussion and future work

In this contribution, we presented a review of the applications of the plasma medicine, an emerging plasma science field. Starting from the first treatments of biomaterials, plasma medicine is constantly gaining attention and interest due to its ability to deliver therapeutic actions, minimizing side effects.

We focused on the application to blood coagulation, on which many international research groups are working with the ambitious goal to develop a tool able to promote locally blood coagulation and disinfection without interfering with any pharmacological therapy. Among these, the PCC is a very promising prototype, delivering a stable cold and low power plasma plume and promoting blood coagulation *in vitro* after a few tens seconds treatment. After successful applications on *in vivo* experiments on male Wistar rats [31, 32], two different pilot clinical trials on humans are in preparation (on chronic wounds in diabetics and on dermatological diseases). Next to that, an important effort is being devoted to the a deeper comprehension on the mechanisms behind the plasma actions. Once achieved, this step will probably boost the development of plasma medicine tools to be routinely used in hospital departments.

* * *

This work has been conducted as a part of the three-year research project named "A novel plasma medicine tool for accelerated haemostasis", which has received funding from the "Fondazione Con Il Sud" within the call "Brains2South". The tools, the experiments and the analyses presented have been performed together with the whole Brains2South research group. In particular, thanks to Dr. L. Cordaro and Dr. A. Fassina for the development and testing of the PCC prototype, to Dr. B. Zaniol for the spectroscopic measurements, to Dr. C. Gareri for the biomedical analyses and to Dr. E. Martines for inspiring and supervising the whole research project.

REFERENCES

[1] Kong M. G. *et al.*, *New J. Phys.*, **11** (2009) 115012.
[2] Tanaka H. *et al.*, *Rev. Mod. Plasma Phys.*, **1** (2017) 3.
[3] Ginsberg G. G. *et al.*, *Gastrointest. Endosc.*, **55** (2002) 807.
[4] Stoffels E. *et al.*, *Plasma Sources Sci. Technol.*, **11** (2002) 383.
[5] Gherardi M. *et al.*, *Trends Biotechnol.*, **36** (2018) 6.
[6] Martines E. *et al.*, *New J. Phys.*, **11** (2009) 115014.

[7] Brun P. *et al.*, *PLoS One*, **7** (2013) 3.
[8] Haertel B. *et al.*, *Biomol. Ther.*, **22** (2014) 477.
[9] Sakakita H. and Ikehara Y., *Plasma Fus. Res.*, **5** (2010) 2117.
[10] De Masi G. *et al.*, *Plasma Med.*, **8** (2018) 3.
[11] Fridman G. *et al.*, *Plasma Chem. Plasma Process.*, **26** (2006) 425.
[12] Kuo S. P. *et al.*, *IEEE Trans. Plasma Sci.*, **38** (2010) 1908.
[13] Metelmann H. R. *et al.*, *Clin. Plasma Med.*, **1** (2013) 30.
[14] Graves D. B., *Plasma Process. Polym.*, **11** (2014) 1120.
[15] Martines E. *et al.*, *Role of intracellular RONS in plasma-based cancer treatment*, in *Proceedings of the 23rd International Conference on Phenomena in Ionized Gases, July 9–14, 2017, Estoril (Portugal)* (Instituto de Plasmas e Fusão Nuclear, Instituto Superior Técnico, Universidade de Lisboa) 2017, p. 368.
[16] Weltmann K. D. *et al.*, *Contrib. Plasma Phys.*, **49** (2009) 631.
[17] Golda G. *et al.*, *J. Phys. D Appl. Phys.*, **49** (2016) 084003.
[18] Graves D. B., *J. Phys. D Appl. Phys.*, **45** (2012) 263001.
[19] Thannickal V. J. and Fanburg B. L., *Am. J. Physiol.-Lung Cell. Mol. Physiol.*, **279** (2000) L1005.
[20] Nisticò *et al.*, *Br. J. Dermatol.*, **151** (2004) 877.
[21] Dawe R. S., *Br. J. Dermatol.*, **149** (2003) 669.
[22] Durant S. T. *et al.*, *Mol. Cell. Biol.*, **26** (2006) 6047.
[23] Laroussi M. *et al.*, *New J. Phys.*, **5** (2003) 41.
[24] Norris L. A. *et al.*, *Best Pract. Res. Clin. Obstet. Gynaecol.*, **17** (2003) 3.
[25] Ikehara Y. *et al.*, *J. Photopolym. Sci. Technol.*, **26** (2013) 55.
[26] Kalghatagi S. U. *et al.*, *IEEE Trans. Plasma Sci.*, **35** (2007) 1159.
[27] Kuo S. P. *et al.*, *IEEE Trans. Plasma Sci.*, **40** (2012) 1117.
[28] Heslin C. *et al.*, *Plasma Med.*, **4** (2014) 153.
[29] De Masi G., Gareri C., Cordaro L., Fassina A., Cavazzana R., Martines E., Zuin M. and Indolfi C., *Dispositivo biomedicale al plasma per la coagulazione del sangue*, Patent Application No. 102018000007505 (July, 2018).
[30] de Izarra C., *Phys. D Appl. Phys.*, **33** (2000) 1697.
[31] De Masi G. *et al.*, *A low power atmospheric plasma source for accelerated blood coagulation*, in *7th International Conference on Plasma Medicine, June 17–22, 2018, Philadelphia (PA)* (Drexel University, Nyheim Plasma Institute) 2018.
[32] Gareri C. *et al.*, *Accelerated blood coagulation through the stimulation with a plasma jet*, in *7th International Conference on Plasma Medicine, June 17–22, 2018, Philadelphia (PA)* (Drexel University, Nyheim Plasma Institute) 2018.