Plasma Bullets Propagation Inside of Agarose Tissue Model

Dayonna Park, Gregory Fridman, Alexander Fridman, and Danil Dobrynin

***Abstract*— This paper demonstrates plasma bullets generated by microsecond pulses in He flow propagation inside of conductive agarose gel tubes mimicking tissue. The objective of this paper is to understand the possibility of internal diseases' treatment (e.g., lung or intestinal cancer) using plasma jets. The propagation dynamics is studied using fast imaging technique, and production of reactive species is demonstrated both in gas phase (using optical emission spectroscopy) and inside of the agarose gel (using fluorescent dye). In addition, it is demonstrated that plasma bullets may propagate not only in a straight tubes, but also in L-shaped tubes, as well as be split in T-shaped tubes. All these facts offer an indication of possible successful application of plasma bullets for treatment of internal diseases, for example, lung cancers or intestinal diseases.**

***Index Terms*— Fast imaging, plasma bullets, plasma jet, plasma medicine, reactive oxygen species, tissue model.**

I. Introduction

A NUMBER of studies performed by many groups in recent years show that so-called plasma bullets generated in noble gases may potentially be used for a number of biomedical applications, including, for example, cancer treatment [1]–[6]. This is because of production of a number of reactive oxygen species (e.g., hydrogen peroxide and OH radicals) that may trigger apoptotic mechanisms in cells [2], [6]–[8]. However, these antitumor effects are shown only *in vitro* studies–and never *in vivo*. This is partially because of the discharge–plasma bullets–generation methods, which is done inside of dielectric tubes (e.g., glass, quartz, plastic, and so on.) [1], [3], [5], [6]–[13]. Mechanisms of plasma bullets propagation inside of such tubes are still not clearly understood, and until now bullet propagation inside of tubes made of conductive material is not documented. We present a study that is focused on plasma jet propagating inside of an artificial tissue tubes based on agarose gel model. Previously, it was shown that agarose gel tissue model may be successfully used to mimic real tissues from the point of plasma-produced reactive species penetration [14], [15].

Manuscript received February 6, 2013; revised April 9, 2013; accepted May 16, 2013. Date of publication June 21, 2013; date of current version July 3, 2013.

The authors are with the A. J. Drexel Plasma Institute, Drexel University, Camden, NJ 08103 USA (e-mail: eunju.drexel@gmail.com; greg.fridman@drexel.edu; fridman@drexel.edu; danil@drexel.edu).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TPS.2013.2265373

II. Materials and Methods

In our experiments, we used a dielectric barrier discharge-based reactor with a glass dielectric [Fig. 1(a)] powered with 20-kV pulses with the duration of $\sim$8 μs at a frequency of 1 kHz [Fig. 1(b)]. Plasma bullets are generated in He atmosphere (99%, Airgas) supplied at 10 L/min into a 15-cm glass capillary with 5-mm external diameter and 1-mm thick walls. The internal powered needle electrode (0.5-mm diameter, stainless steel) and external grounded electrode (1-mm diameter copper ring) are placed 3 cm apart.

To monitor the discharge parameters we use P6015A high-voltage probe (75-MHz bandwidth, Tektronix) and CM-10-L current monitor (10 ns usable rise time, Ion Physics Corporation) connected to a 1-GHz DPO-4104B oscilloscope (Tektronix).

The discharge visualization measurements are performed using 4Picos intensified charge-coupled device (ICCD) camera from Stanford Computer Optics. The camera has an 18-mm diameter multialkaline photocathode with a spectral response from 180 to 750 nm. The camera's spectral response is 250–750 nm. Discharge optical emission spectrum is obtained using a fiber optic bundle (Princeton Instruments-Acton, 10 fibers–200-μm core) connected to the spectrometer (Princeton Instruments–Acton Research, TriVista TR555 spectrometer system with PIMAX digital ICCD camera, Trenton, NJ).

III. Results and Discussion

Plasma bullets traveling both in air and agarose gel tube structures are studied [Fig. 1(c)]. Agarose gel is traditionally used to mimic the biological substrates, such as tissues, skin, cell layers, and so on. Although it does not represent real tissue we show that one is able to alter the gel's buffering ability, density and fluidity to closely resemble tissue. Agarose gels of 1.5% wt are prepared using standard procedure with pure agar powder (Fisher) in either distilled H_2O or phosphate buffered saline (PBS, Fisher). Measurements of H_2O_2 penetration into agarose gels are done using AmplexUltraRed reagent (Invitrogen, ex/em: 530/590 nm) fluorescent dye. 75 μL of PBS containing 100-μM AmplexUltraRed with 200-U/μL horseradish peroxidase (MP Biomedicals) are placed on the top surface of $\sim$7-mm thick 4.5 $\times$ 4-cm agar slice, spread uniformly over the agar surface and incubated for $\sim$15 min before the treatment to provide presence of the dye in the agar volume. Two 2 $\times$ 4-mm slices of agar with thickness of 5 mm are then placed on top of the preincubated agar piece

0093-3813/$31.00 © 2013 IEEE

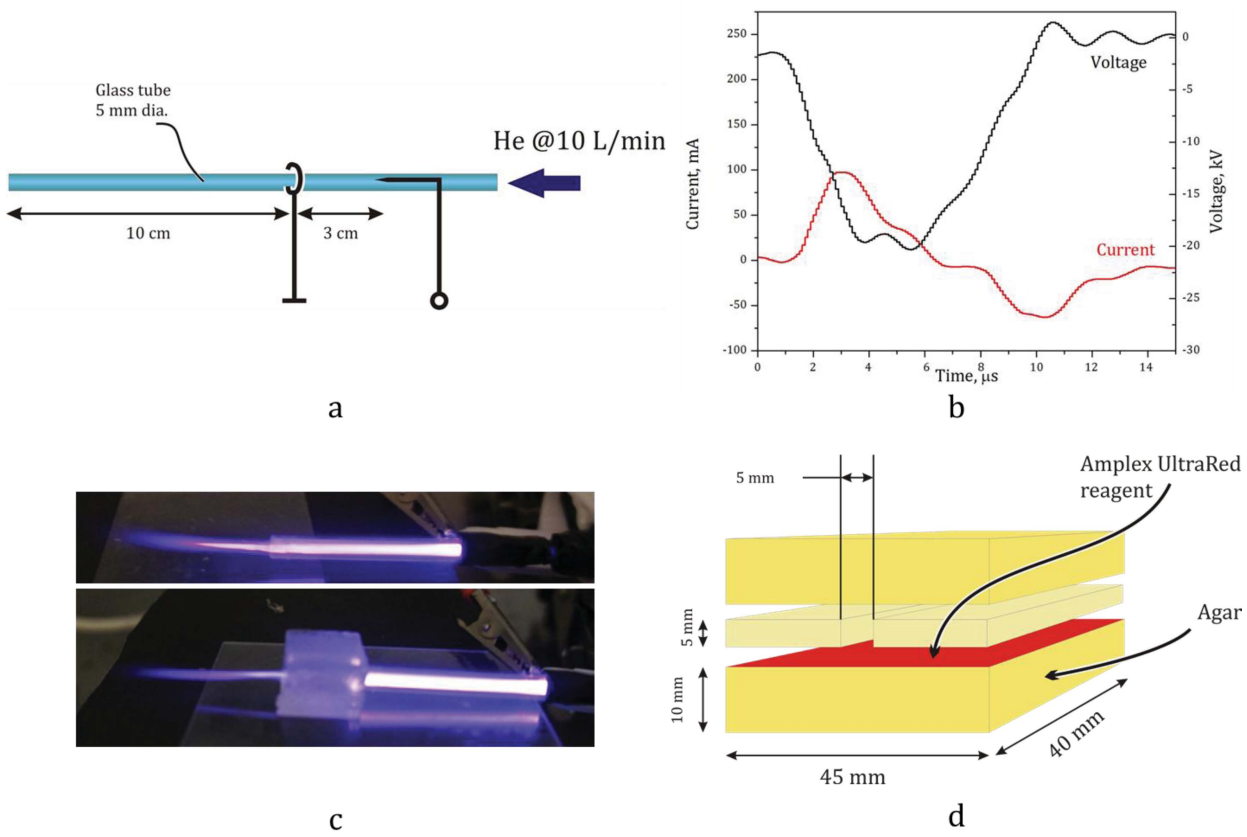


Fig. 1. (a) Experimental setup schematic. (b) Typical current and voltage waveforms. (c) Photographs of plasma bullets propagation in air and agarose gel tube photographs. (d) Schematic of agarose gel structure for detection of H_2O_2 produced by plasma bullets in agarose gel.

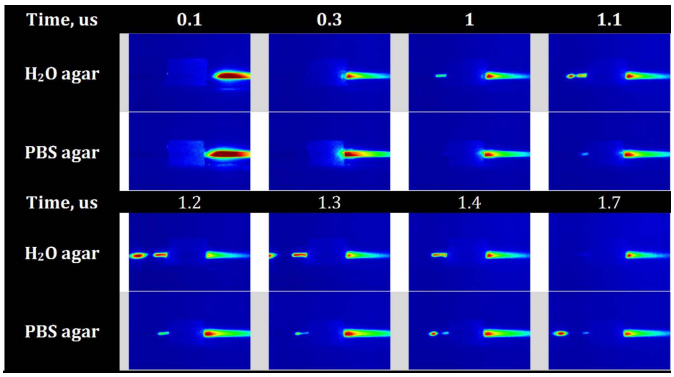


Fig. 2. Plasma bullets propagation inside of distilled water and PBS agarose tubes: fast imaging (100 ns exposure time).

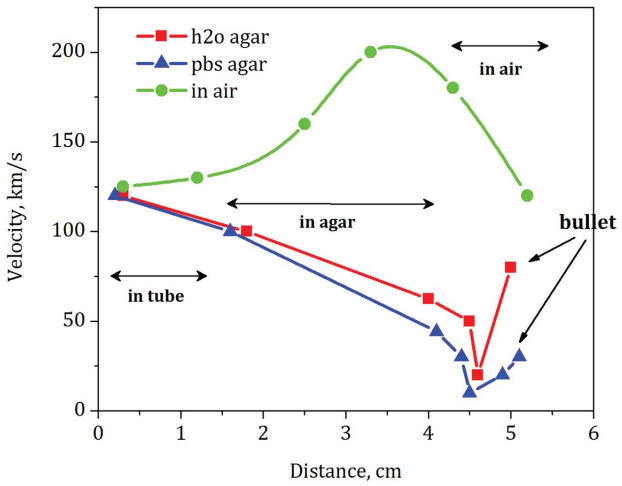


Fig. 3. Plasma bullets propagation velocity in air and inside of agarose tubes as a function of distance (after 1 mm long glass tube).

and covered with another agar slice identical to the first one to create a 5 × 5-mm hole [Fig. 1(d)]. Fluorescence from the treated samples is measured using an LS55 (Perkin Elmer) fluorescent spectrometer equipped with XY reader accessory. No treatment condition is used as a control.

Imaging of the plasma propagation in a straight or curved (L-shape) agarose tube is performed using nanosecond ICCD camera. Fig. 2 shows plasma bullet propagation in distilled water and PBS-based agarose tubes captured at different delays with respect to the high voltage pulse front. Plasma bullet appears as a well defined and homogeneous volume both at the entrance and exit of the agar. It is interesting, that at the exit of the agarose tube, there is a long-living tail or afterglow connected to the agar, from which a bullet is formed. One may notice that there is an effect of agar conductivity on plasma propagation: higher conductivity results in lower velocity of bullet. Fig. 3 shows plasma bullet propagation velocity as a function of distance for the cases of air (no agar), water, and PBS agar.

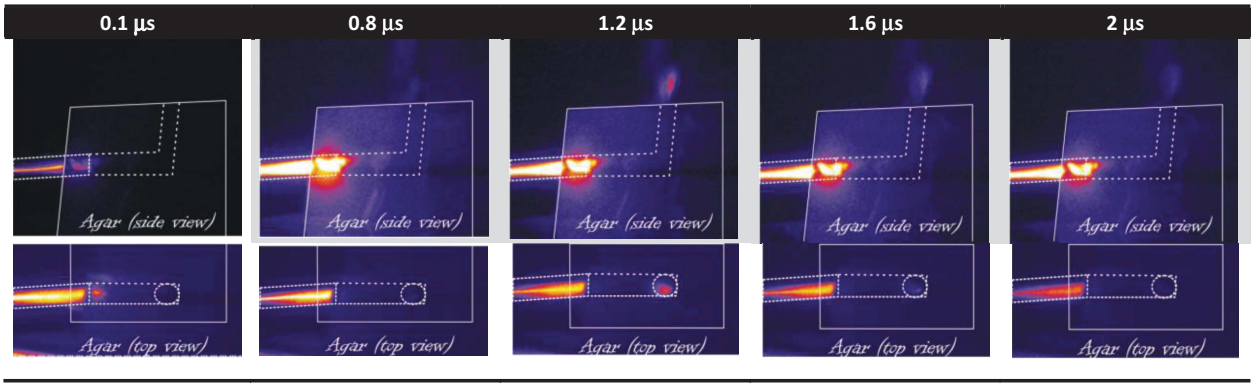


Fig. 4. Fast imaging of plasma bullet propagation inside of an L-shape agarose tube (100 ns exposure time).

Fig. 3 shows that over the few centimeters in air, the plasma front velocity increases, followed by almost linear decrease. On the contrary, the presence of agar results in bullet deceleration, which is more noticeable in the case of more conductive PBS agar. However, as soon as bullet is formed at the exit of the agarose tube, its velocity increases again. These different velocity evolutions and the corresponding plasma appearance modifications suggest that different mechanisms may be responsible for plasma propagation in the different zones–air versus agarose tube. One may argue that the effect of agarose tube wall charge accumulation creates screening effect that results in deceleration of the bullet. The tail portion of a bullet that is still attached to the agar (see Fig. 2, 1.1, and 1.2-μs time points) as it exits the tube, creates an accelerating electric field that causes increase of bullet's velocity. Simultaneously, as it was argued in [16], air molecules started playing an important role in the plasma bullet propagation mechanism.

We have made a number of attempts to image bullet propagation inside of agarose tube, however we could never record any light emission until the ionization front reached the agar exit. Fig. 4 shows plasma bullet imaging propagating in an L-shaped agarose tube captured from side and top at different time delays. Interestingly, plasma bullet at the exit opening of a conductive tube appears as a more or less uniform homogeneous structure that is not connected to the walls (not a donut-type shape). Again, in this case we also did not notice any light emission until the moment when ionization reached the tube exit.

Fig. 5 shows time-resolved imaging results of the bullet propagation inside of an L-shaped agarose tube as a function of time compared with the voltage pulse. It can be clearly seen that the bullet is generated first at the front of the voltage pulse, followed by a dark phase and the second bullet at the trailing edge of the pulse. This phenomenon may also be interpreted by the screening effect due to agarose tube wall charging–deposited charge prevents further generation of the bullet inside of the tube, and as soon as the voltage starts to decrease, electric field increases and the second bullet is produced.

To examine if plasma bullets, indeed, may split in a conductive agarose gel tube (as it would be important in the case of segmental bronchi treatment, for example), plasma bullets are allowed to propagate inside of a T-shaped agarose tube

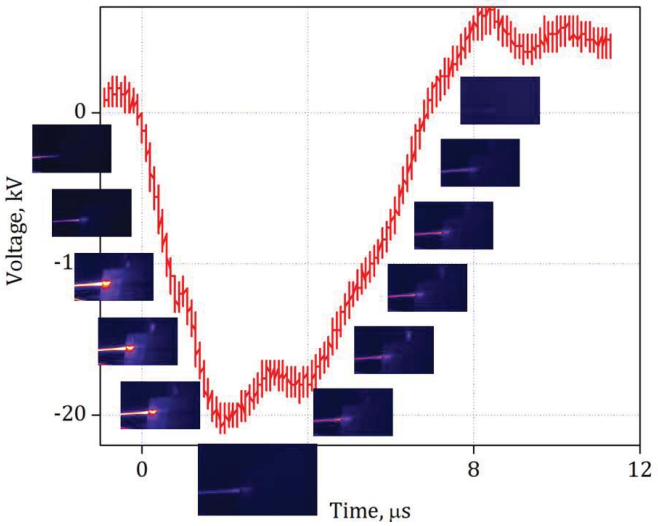


Fig. 5. Time resolved imaging of plasma bullet propagation inside of an L-shaped agarose tube (100 ns exposure time).

(Fig. 6). This result shows that plasma bullet type of plasma may be used for treatment of lungs and other complicated structures.

Optical emission spectra of plasma bullets are shown in Fig. 7. Although there are no qualitative differences between the emission from the bullets that are traveling only in a glass tube or also in an agarose tube, intensity of the emission lines changes. Comparison of the emission intensities of helium, oxygen, and OH lines (Fig. 7) suggests more effective production of OH radicals in the case of bullets that are traveling through agar (perhaps due to higher water content in agar compared with atmospheric air) at the expense of excited helium. Simultaneously, no difference is observed in the intensity of O atom emission.

To check qualitatively whether plasma bullets induce any chemical changes in the agar, a simple experiment on hydrogen peroxide detection using fluorescent dye is conducted. Fig. 8 shows detection of hydrogen peroxide production in agar by plasma bullet (left) using fluorescent dye compared with control where no plasma is generated in He flow (right). Intensities in arbitrary units show the results of fluorescence imaging of a bottom part of an agarose structure used in the experiment

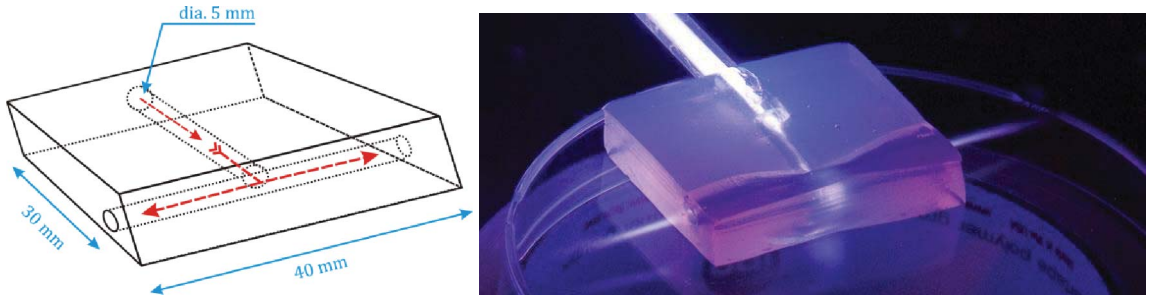


Fig. 6. Plasma bullet propagation inside of a T-shaped agarose tube. Tube schematic (left). Plasma photograph (right).

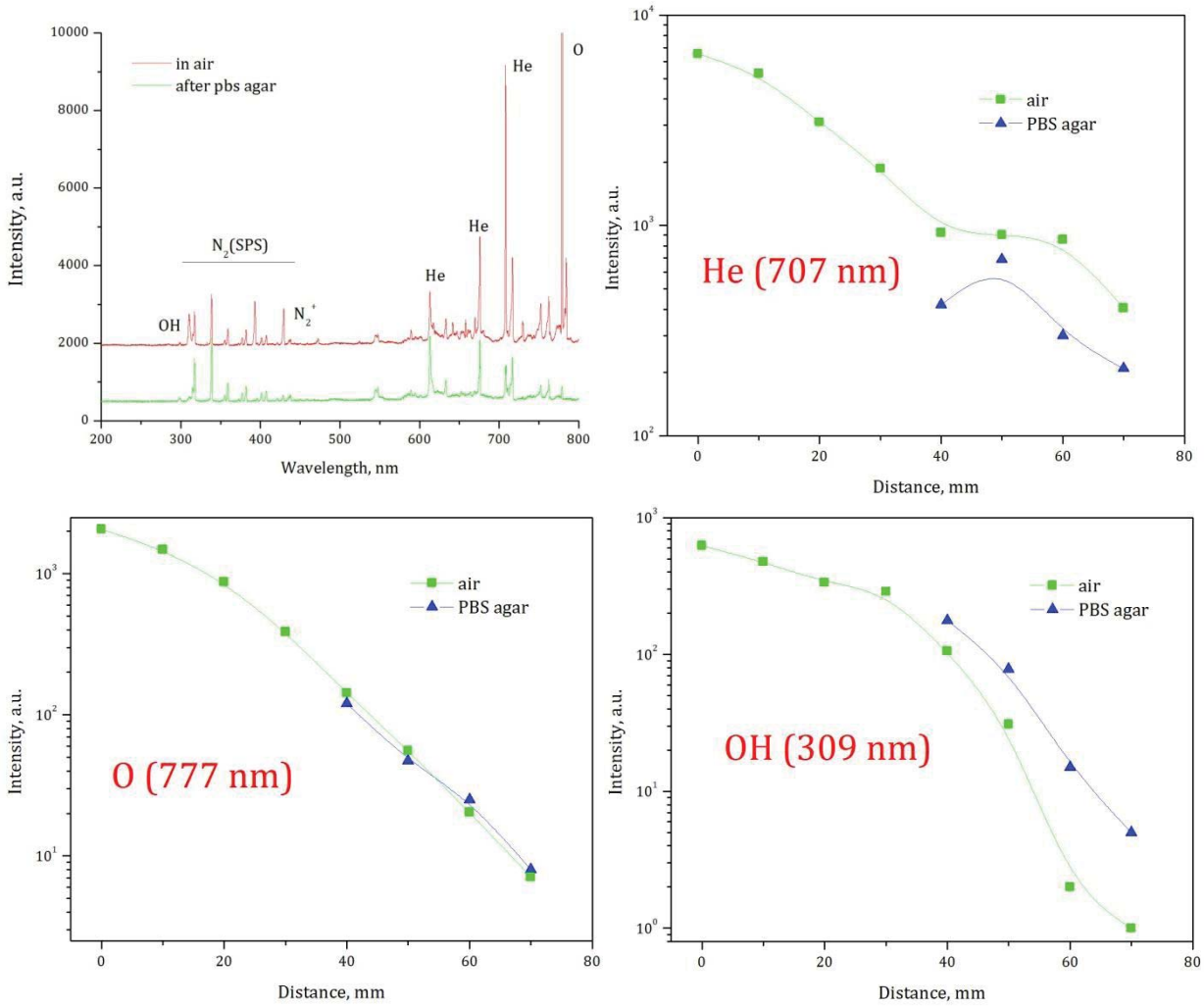


Fig. 7. Optical emission spectra of the plasma bullet in air with and without 4-cm-long agarose tube. Full spectra and emision intensities of He, O, and OH lines as a function of distance from the end opening of the glass tube.

(Fig. 1): hydrogen peroxide detected by AmplexUltraRed reagent is shown to be produced in agar due to plasma bullet propagation with diffusion length deep into the structure on the order of few millimeters. These findings suggest that plasma bullet production of reactive oxygen species inside of tissues (for, e.g., trachea and bronchi) may be possible, leading to development of new treatment or therapy methods of lung diseases and other similar applications.

In summary, by means of artificial agarose gel mimicking real tissues this paper shows that plasma bullets may propagate inside of conductive tubes. It is reported, that such propagation may result in production of reactive species (using H_2O_2 detection as an example) in the tissues as well as generation of short-living OH radicals in the gas phase. In addition, it is demonstrated that these bullets may propagate not only in straight tubes, but also in L-shaped tubes, as well as be split in

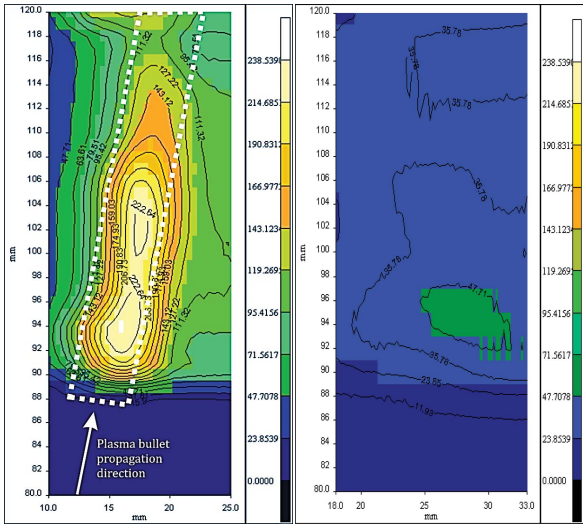


Fig. 8. Detection of hydrogen peroxide production in agar by plasma bullet (left) using fluorescent dye compared to control where no plasma was generated in He flow (right). Intensities are in arbitrary units.

T-shaped tubes. All these facts offer an indication of possible successful application of plasma bullets for treatment of internal diseases, for example, lung cancers or intestinal diseases. However, despite these purely experimental indications, more fundamental work is still needed to understand the mechanisms of plasma bullets generation and propagation, effects of tube walls and their characteristics (e.g., conductivity, material, water content, and so on.), as well as chemistry of plasma, its products that are delivered into the tissues, and their effects on cells.

REFERENCES

[1] E. Robert, E. Barbosa, S. Dozias, M. Vandamme, C. Cachoncinlle, R. Viladrosa, and J. M. Pouvesle, "Experimental study of a compact nanosecond plasma gun," *Plasma Process. Polymer*, vol. 6, no. 12, pp. 795–802, 2009.

[2] M. Vandamme, E. Robert, J. Sobilo, V. Sarron, D. Ries, S. Dozias, B. Legrain, S. Lerondel, A. Le Pape, and J. M. Pouvesle, "In situ application of non-thermal plasma: Preliminary investigations for colorectal and lung tolerance," in *Proc. 20th Int. Symp. Plasma Chem.*, Philadelphia, PA, USA, 2011, pp. 1–4.

[3] M. G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. van Dijk, and J. L. Zimmermann, "Plasma medicine: An introductory review," *New J. Phys.*, vol. 11 p. 115012, Nov. 2009.

[4] G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, and A. Fridman, "Applied plasma medicine," *Plasma Process. Polymer*, vol. 5, no. 6, pp. 503–533, 2008.

[5] K. D. Weltmann, E. Kindel, T. von Woedtke, M. Häahnel, M. Stieber, and R. Brandenburg, "Atmospheric-pressure plasma sources: Prospective tools for plasma medicine," *Pure Appl. Chem.*, vol. 82, no. 6, pp. 1223–1237, 2010.

[6] J. Y. Kim, S. O. Kim, Y. Wei, and J. Li, "A flexible cold microplasma jet using biocompatible dielectric tubes for cancer therapy," *Appl. Phys. Lett.*, vol. 96, no. 20, p. 203701, 2010.

[7] E. Stoffels, Y. Sakiyama, and D. B. Graves, "Cold atmospheric plasma: Charged species and their interactions with cells and tissues," *IEEE Trans. Plasma Sci.*, vol. 36, no. 4, pp. 1441–1457, Aug. 2008.

[8] G. Fridman, A. Shereshevsky, M. M. Jost, A. D. Brooks, A. Fridman, A. Gutsol, V. Vasilets, and G. Friedman, "Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines," *Plasma Chem. Plasma Process.*, vol. 27, no. 2, pp. 163–176, 2007.

[9] M. Laroussi and X. Lu, "Room-temperature atmospheric pressure plasma plume for biomedical applications," *Appl. Phys. Lett.*, vol. 87, no. 11, pp. 113902-1–113902-3, 2005.

[10] N. Mericam-Bourdet, M. Laroussi, A. Begum, and E. Karakas, "Experimental investigations of plasma bullets," *J. Phys. D, Appl. Phys.*, vol. 42, no. 5, p. 055207, 2009.

[11] Q. Xiong, X. Lu, Y. Xian, J. Liu, C. Zou, Z. Xiong, W. Gong, K. Chen, X. Pei, F. Zou, J. Hu, Z. Jiang, and Y. Pan, "Experimental investigations on the propagation of the plasma jet in the open air," *J. Appl. Phys.*, vol. 107, no. 7, pp. 3302–3307, 2010.

[12] Q. Xiong, X. Lu, K. Ostrikov, Z. Xiong, Y. Xian, F. Zhou, C. Zou, J. Hu, W. Gong, and Z. Jiang, "Length control of He atmospheric plasma jet plumes: Effects of discharge parameters and ambient air," *Phys. Plasmas*, vol. 16, no. 4, p. 043505, 2009.

[13] E. Robert, V. Sarron, D. Riès, S. Dozias, M. Vandamme, and J.-M. Pouvesle, "Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun," *Plasma Sources Sci. Technol.*, vol. 21, no. 3, pp. 034017-1–034017-12, 2012.

[14] D. Dobrynin, G. Fridman, G. Friedman, and A. Fridman, "Cold microsecond spark discharge plasma production of active species and their delivery into tissue," in *Plasma for Bio-Decontamination, Medicine and Food Security* (NATO Science for Peace and Security Series A: Chemistry and Biology). New York, NY, USA: Springer-Verlag, 2012, pp. 293–299.

[15] D. Dobrynin, G. Fridman, G. Friedman, and A. Fridman, "Penetration deep into tissues of reactive oxygen species generated in floating-electrode dielectric barrier discha," to be published.

[16] N. Mericam-Bourdet, M. Laroussi, A. Begum, and E. Karakas, "Experimental investigations of plasma bullets," *J. Phys. D, Appl. Phys.*, vol. 42, no. 5, p. 055207, 2009.



Dayonna Park received the B.A. degree in psychology from the West Chester University of Pennsylvania, PA, USA, in 2008. She is currently pursuing the Graduate degree with Drexel University, Philadelphia, PA, USA.

Her current research interests include biomedical applications, biological psychology, and cognitive and biological neuroscience.



Gregory Fridman received the Ph.D. degree in biomedical engineering from Drexel University, Philadelphia, PA, USA.

He is currently an Assistant Research Professor with the A. J. Drexel Plasma Institute, Philadelphia. He is a PI and CoPI on multiple grants, including NIH R01 on plasma-assisted stem cell differentiation and bone tissue regeneration. He has authored over 30 peer-reviewed papers and five book chapters with total citation index >1600 (h-index 17). His current research interests include integration of nonequilibrium air plasma discharges into biology and medicine, especially in pre-operative, post-operative, and intra-operative sterilization of living human tissue, blood coagulation, wound healing and tissue regeneration, and treatment of diseases (both on skin, i.e. melanoma; under the skin, i.e. leishmaniasis; and deep in the body, i.e. deep vein thrombophlebitis).

Dr. Fridman received the IEEE Nuclear and Plasma Sciences Society Outstanding Student in Plasma Science Award from the IEEE International Conference on Plasma Science.



Alexander Fridman received the B.S., M.S., and Ph.D. degrees in physics and mathematics from the Moscow Institute of Physics and Technology, Moscow, Russia, in 1976 and 1979, respectively, and the Doctor of Science degree in mathematics from the Kurchatov Institute of Atomic Energy, Moscow, in 1987.

He is the Nyheim Chair Professor with Drexel University, Philadelphia, PA, USA, and the Director of the Drexel Plasma Institute, working on plasma approaches to material treatment, fuel conversion, and environmental control. He has more than 30 years of plasma research experience in national laboratories and universities of Russia, France, and the U.S. He has published five books and 350 papers.

Prof. Fridman has received numerous awards, including the Stanley Kaplan Distinguished Professorship in Chemical Kinetics and Energy Systems, the George Soros Distinguished Professorship in Physics, and the State Price of the U.S.S.R. for discovery of selective stimulation of chemical processes in nonthermal plasma.



Danil Dobrynin received the M.S. degree in physical electronics from Petrozavodsk State University, Petrozavodsk, Russia, in 2008, and the Ph.D. degree from Drexel University, Philadelphia, PA, USA, in 2011.

He joined the A. J. Drexel Plasma Institute, as a Research Faculty Member and the Director of Applied Physics Laboratory in 2011. His current research interests include experimental plasma and gas discharge diagnostics, discharges in liquid phase, plasma applications in medicine and biology, and plasma engineering.