Biomedical Application of Non-Thermal Atmospheric Pressure Plasma and Its Usefulness
Guest Editor: Tetsuo Adachi

Medical applications of non-thermal atmospheric pressure plasma

Hiromasa Tanaka* and Masaru Hori

Institute of Innovation for Future Society, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

(Received 30 July, 2016; Accepted 3 September, 2016; Published online 17 December, 2016)

An innovative approach for producing reactive oxygen and nitrogen species is the use of non-thermal atmospheric pressure plasma. The technique has been applied in a wide variety of fields ranging from the micro-fabrication of electric devices to the treatment of disease. Although non-thermal atmospheric pressure plasmas have been shown to be clinically beneficial for wound healing, blood coagulation, and cancer treatment, the underlying molecular mechanisms are poorly understood. In this review, we describe the current progress in plasma medicine, with a particular emphasis on plasma-activated medium (PAM), which is a solution that is irradiated with a plasma and has broadened the applications of plasmas in medicine.

Key Words: plasma medicine, plasma cancer therapy, plasma-activated medium

Plasmas Generate Reactive Oxygen Species and Reactive Nitrogen Species

Plasma is the fourth state of matter, in addition to solid, liquid, and gas. Thermal plasmas, such as arc discharges, and low-pressure plasmas for surface treatments, have been used in industry. Innovative technologies for generating non-thermal plasmas at atmospheric pressure have recently been developed and applied in several industries, as well as in medicine and biology. In the life sciences, plasmas are a novel tool for producing oxidative stress. Understanding the interactions between a plasma and tissues/cells is currently an important issue in plasma medicine. Applications of non-thermal plasmas in blood coagulation, cancer treatment, and gene transfection have been extensively studied over the last four years as part of the Japanese government’s national “Plasma Medical Innovation” project.

Plasmas Generate Reactive Oxygen Species and Reactive Nitrogen Species

The major components of a plasma are electrons, ions, radicals, and light. Radicals are especially important to induce physiological outputs in cells/tissues. Indeed, plasmas induce reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells, both of which have significant impacts on cellular physiology, and many diseases have been associated with increased levels of oxidative stress. Many antioxidants induce anti-tumor, anti-inflammatory, and antibacterial activities, and the intake of natural antioxidants reduces the risk of cancer, diabetes, and other diseases. Cancer cells generate increased levels of ROS, and this property of cancer cells could be exploited for therapeutic benefit. Excessive ROS damages cancer cells and leads to cell death, while normal cells tolerate the same levels of ROS. Thus, pro-oxidants that induce oxidative stress, such as non-thermal plasmas, may have chemotherapeutic potential.

A device that generates a non-thermal atmospheric pressure plasma with high electron density has been invented, and the effects of direct non-thermal plasma exposure on lipids, proteins, and nucleic acids have been evaluated. Although L-ascorbate is a potent dietary antioxidant, high concentrations of L-ascorbate have pro-oxidant activities. Recently, a novel combinatorial therapy of a non-thermal plasma and L-ascorbate for the treatment of malignant mesothelioma was proposed. A brief pre-treatment with a pharmacological dose of L-ascorbate immediately prior to non-thermal plasma exposure dose-dependently sensitized malignant mesothelial cells to a non-thermal plasma. However, the authors also found that prolonged incubation with L-ascorbate protects malignant mesothelial cells from the cytotoxicity of non-thermal plasma exposure. These results suggest that therapeutic strategies should be considered based on the biphasic effects of L-ascorbate.

Plasma-Activated Medium for Cancer Therapy

Non-thermal atmospheric pressure plasmas have been widely used for medical purposes such as wound healing, blood coagulation, and cancer therapy. Most treatments involve the direct application of plasmas to lesions. However, recently, it was discovered that plasma-irradiated solutions induce physiological outputs in cells and tissues, and such indirect treatments could be a novel approach to chemotherapy. For example, plasma-irradiated medium (referred to here as plasma-activated medium or PAM) kills glioblastoma, ovarian, and gastric cancer cells, and PAM could be a potential anti-tumor drug for the treatment of peritoneal dissemination of cancers by intrathecal or intraperitoneal injections.

Intracellular molecular mechanisms of PAM-triggered cell death have been extensively studied since PAM was proposed as a novel plasma chemotherapy. PAM treatments as well as direct non-thermal plasma treatments generally induce ROS and apoptosis in cancer cells. PAM inhibits activation of survival and proliferation signaling networks in U251SP glioblastoma cells.

*To whom correspondence should be addressed.
E-mail: htanaka@plasma.engg.nagoya-u.ac.jp
which leads to apoptosis.\textsuperscript{(34,41)} In A549 lung adenocarcinoma cells, PAM inhibits the mitochondrial-nuclear network through a caspase-independent cell death pathway,\textsuperscript{(42)} and elevates intracellular \textit{Fe}(II) and hydroxyl radicals.\textsuperscript{(43)} PAM triggers intracellular zinc liberation in SH-SYSY neuroblastoma cells, which leads to zinc-dependent cell death.\textsuperscript{(44)}

Other Applications of Non-Thermal Plasmas in Medicine

The facilitation of blood coagulation by a non-thermal plasma is a novel method that is especially effective for stopping oozing blood in surgery.\textsuperscript{(14)} Indeed, a non-thermal plasma for blood coagulation was demonstrated to stop bleeding faster than natural coagulation (Fig. 2).\textsuperscript{(15)} Eosinophilic fibrous membrane-like structures were induced by the plasma, while the natural coagulation process usually contains erythrocytes.\textsuperscript{(16)} The inflammation recovery process after treatment with the non-thermal plasma or thermal coagulator was visualized using the radiopharmaceutical, 2-deoxy-2-\textsuperscript{18}F fluoro-D-glucopyranose (\textsuperscript{18}F-FDG), and it was shown that the former is less inflammatory.\textsuperscript{(17)} Electron microscopic analyses revealed that fragmented fibroblasts were seen in the electrocoagulation-treated skin and not in the plasma-treated skin.\textsuperscript{(18)}

Non-thermal plasmas have been applied in regenerative medicine. A low-dose plasma can promote cell growth while a high-dose plasma induces apoptosis or necrosis, which might reflect the dose dependence of oxidative stress.\textsuperscript{(11,45)}

Highly efficient and minimally invasive gene transfection has been achieved using non-thermal plasmas (Fig. 3).\textsuperscript{(24,26)} Electrical, chemical, and biochemical factors that generally affect the efficiency of gene transfection were investigated, and it was shown that non-thermal plasmas predominantly influences endocytosis and electroporation.\textsuperscript{(27)}

In the context of cardiac disease, inhalation of a non-thermal plasma resulted in lowered blood pressure and an increase in nitrous oxide concentration in the abdominal aorta in rats.\textsuperscript{(46)}

Concluding Remarks

Non-thermal plasmas are receiving increasing attention in medicine as a promising tool, and various applications have been proposed. Direct and indirect plasma treatments induce physiological outputs in cells and tissues ranging from cell death to cell growth. Despite extensive study, the molecular mechanisms that underlie these effects on cellular physiology remain poorly understood, and further work is required to address the exciting potential of non-thermal plasmas for clinical applications.
Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research on Innovative Areas “Plasma Medical Innovation” Grant No. 24108002 and 24108008, a Grant-in-Aid for Young Scientists (A) Grant No. 15H05430, and a Grant-in-Aid for Challenging Exploratory Research Grant No. 15K13390 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

1. Laroussi M. Low temperature plasma-based sterilization: overview and state-of-the-art. Plasma Process Polym 2005; 2: 391–400.
2. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. Plasma Process Polym 2008; 5: 503–533.
3. Kong MG, Kroesen G, Morfill G, et al. Plasma medicine: an introductory review. New J Phys 2009; 11: 115012.
4. Weltmann KD, Kindel E, von Woedtke T, Hähnel M, Stieber M, Brandenburg R. Atmospheric-pressure plasma sources: prospective tools for plasma medicine. Pure Appl Chem 2010; 82: 1223–1237.
5. Morfill GE, Kong MG, Zimmermann JL. Focus on Plasma Medicine. New J Phys 2009; 11: 115011.
6. Laroussi M. Low-temperature plasmas for medicine? IEEE Trans Plasma Sci 2009; 37: 714–725.
7. von Woedtke, Reuter S, Masar K, Weltmann KD. Plasmas for medicine. Phys Rep 2013; 530: 291–320.
8. Weltmann KD, von Woedtke T. Campus PlasmaMed—from basic research to clinical proof. IEEE Trans Plasma Sci 2011; 39: 1015–1025.
9. Weltmann KD, von Woedtke T. Basic requirements for plasma sources in medicine. Eur Phys J Appl Phys 2011; 55: 13807.
10. Yousfi M, Merbah N, Pathak A, Eichwald O. Low-temperature plasmas at atmospheric pressure: toward new pharmaceutical treatments in medicine. Fundam Clin Pharmacol 2014; 28: 123–135.
11. Toyokuni S. The origin and future of oxidative stress pathology: from the recognition of carcinogenesis as an iron addiction with ferroptosis-resistance to non-thermal plasma therapy. Pathol Int 2016; 66: 245–259.
12. Kalghatgi SU, Fridman G, Cooper M, et al. Mechanism of blood coagulation by nonthermal atmospheric pressure dielectric barrier discharge plasma. IEEE Trans Plasma Sci 2007; 35: 1559–1566.
13. Ikehara S, Sakakita H, Ishikawa K, et al. Plasma blood coagulation without involving the activation of platelets and coagulation factors. Plasma Process Polym 2015; 12: 1348–1353.
14. Miyamoto K, Ikehara S, Takei H, et al. Red blood cell coagulation induced by low-temperature plasma treatment. Arch Biochem Biophys 2016; 605: 95–101.
15. Sakakita H, Ikehara S. Irradiation experiments on a mouse using a mild-plasma generator for medical applications. Plasma Fusion Res 2010; 5: S2117.
16. Ikehara Y, Sakakita H, Shimizu N, Ikehara S, Nakanish H. Formation of membrane-like structures in clotted blood by mild plasma treatment during hemostasis. J Photopolym Sci Technol 2013; 26: 555–557.
17. Ueda M, Yamagami D, Watanabe K, et al. Histological and nuclear medical comparison of inflammation after hemostasis with non-thermal plasma and thermal coagulation. Plasma Process Polym 2015; 12: 1338–1342.
18. Akimoto Y, Ikehara S, Yamaguchi T, et al. Galectin expression in healing wounded skin treated with low-temperature plasma: comparison with treatment by electronical coagulation. Arch Biochem Biophys 2016; 605: 86–94.
19. Schlegel J, Köritzler J, Bokhammer V. Plasma in cancer treatment. Clin Plasma Med 2013; 1: 2–7.
20. Tanaka H, Mizuno M, Ishikawa K, et al. Plasma medical science for cancer therapy: toward cancer therapy using nonthermal atmospheric pressure plasma. IEEE Trans Plasma Sci 2014; 42: 3760–3764.
21. Tanaka H, Mizuno M, Toyokuni S, et al. Cancer therapy using non-thermal atmospheric pressure plasma with ultra-high electron density. Phys Plasmas 2015; 22: 122004.
22. Tanaka H, Mizuno M, Ishikawa K, et al. Plasma with high electron density and plasma-activated medium for cancer treatment. Clin Plasma Med 2015; 3: 72–76.
23. Tanaka H, Mizuno M, Kikkawa F, Hori M. Interactions between a plasma-activated medium and cancer cells. Plasma Med 2016; 6: 101–106.
24. Sasaki S, Kanzaki M, Kaneko T. Highly efficient and minimally invasive transfection using time-controlled irradiation of atmospheric-pressure plasma. Appl Phys Express 2014; 7: 026202.
25. Sasaki S, Kanzaki M, Kaneko T. Calcium influx through TRP channels induced by short-lived reactive species in plasma-irradiated solution. Sci Rep 2016; 6: 25728.
26. Jinno M, Ikeda Y, Motomura H, Kido Y, Tachibana K, Satoh S. The necessity of radicals for gene transfection by discharge plasma irradiation. J Phys-
27 Jinno M, Ikeda Y, Motomura H, Kido Y, Satoh S. Investigation of plasma induced electrical and chemical factors and their contribution processes to plasma gene transfection. *Arch Biochem Biophys* 2016; 605: 59–66.

28 Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol Aspects Med* 2011; 32: 234–246.

29 Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 2009; 7: 65–74.

30 Iwasaki M, Imai H, Matsudaira Y, *et al.* Nonequilibrium atmospheric pressure plasma with ultrahigh electron density and high performance for glass surface cleaning. *Appl Phys Lett* 2008; 92: 081503.

31 Okazaki Y, Wang Y, Tanaka H, *et al.* Direct exposure of non-equilibrium atmospheric pressure plasma confers simultaneous oxidative and ultraviolet modifications in biomolecules. *J Clin Biochem Nutr* 2014; 55: 207–215.

32 Putchala MC, Ramani P, Sherlin HJ, Premkumar P, Natesan A. Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity—a systematic review. *Arch Oral Biol* 2013; 58: 563–574.

33 Shi L, Wang Y, Ito F, *et al.* Biphasic effects of L-ascorbate on the tumoricidal activity of non-thermal plasma against malignant mesothelioma cells. *Arch Biochem Biophys* 2016; 605: 109–116.

34 Tanaka H, Mizuno M, Ishikawa K, *et al.* Plasma-activated medium selectively kills glioblastoma brain tumor cells by down-regulating a survival signaling molecule, AKT kinase. *Plasma Med* 2013; 1: 265–277.

35 Utsunomiya H, Nakamura K, *et al.* Effect of indirect nonequilibrium atmospheric pressure plasma on anti-proliferative activity against chronic chemo-resistant ovarian cancer cells in vitro and in vivo. *PLoS One* 2013; 8: e81576.

36 Torii K, Yamada S, Nakamura K, *et al.* Effectiveness of plasma treatment on gastric cancer cells. *Gastric Cancer* 2014; 18: 635–643.

37 Hattori N, Yamada S, Torii K, *et al.* Effectiveness of plasma treatment on pancreatic cancer cells. *Int J Oncol* 2015; 47: 1655–1662.

38 Utsunomiya H, Nakamura K, Tanaka H, Hori M, Kikkawa F. Selective cytotoxicity of indirect nonequilibrium atmospheric pressure plasma against ovarian clear-cell carcinoma. *Springerplus* 2014; 3: 398.

39 Utsunomiya H, Kajiyama H, Nakamura K, *et al.* Variable susceptibility of ovarian cancer cells to non-thermal plasma-activated medium. *Oncol Rep* 2016; 35: 3169–3177.

40 Iseki S, Nakamura K, Hayashi M, *et al.* Selective killing of ovarian cancer cells through induction of apoptosis by nonequilibrium atmospheric pressure plasma. *Appl Phys Lett* 2012; 100: 113702.

41 Tanaka H, Mizuno M, Ishikawa K, *et al.* Cell survival and proliferation signaling pathways are downregulated by plasma-activated medium in glioblastoma brain tumor cells. *Plasma Med* 2014; 2: 207–220.

42 Adachi T, Tanaka H, Nonomura S, Hara H, Kondo S, Hori M. Plasma-activated medium induces A549 cell injury via a spiral apoptotic cascade involving the mitochondrial-nuclear network. *Free Radic Biol Med* 2015; 79: 28–44.

43 Adachi T, Nonomura S, Horiba M, *et al.* Iron stimulates plasma-activated medium-induced A549 cell injury. *Sci Rep* 2016; 6: 20928.

44 Hara H, Taniguchi M, Kobayashi M, Kamiya T, Adachi T. Plasma-activated medium-induced intracellular zinc liberation causes death of SH-SYSY cells. *Arch Biochem Biophys* 2015; 584: 51–60.

45 Kalghatgi S, Friedman G, Fridman A, Clyne AM. Endothelial cell proliferation is enhanced by low dose non-thermal plasma through fibroblast growth factor-2 release. *Ann Biomed Eng* 2010; 38: 748–757.

46 Tsutsui C, Lee M, Takahashi G, *et al.* Treatment of cardiac disease by inhalation of atmospheric pressure plasma. *Jpn J Appl Phys* 2014; 53: 060309.