Serial Review

Biomedical Application of Non-Thermal Atmospheric Pressure Plasma and Its Usefulness

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Medical applications of non-thermal atmospheric pressure plasma

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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**

Plasma is the fourth state of matter, in addition to solid, liquid, and gas. Thermal plasmas, such as arch 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.[1–10] In the life sciences, plasmas are a novel tool for producing oxidative stress.[11] 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,[12–18] cancer treatment,[19–23] and gene transfection have been extensively studied over the last four years as part of the Japanese government's national "Plasma Medical Innovation" project.[24–27]

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.[11,28,29] 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,[11] 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,[30] and the effects of direct non-thermal plasma exposure on lipids, proteins, and nucleic acids have been evaluated.[31]

Although L-ascorbate is a potent dietary antioxidant, high concentrations of L-ascorbate have pro-oxidant activities.[32] Recently, a novel combinatorial therapy of a non-thermal plasma and L-ascorbate for the treatment of malignant mesothelioma was proposed.[33] A brief pre-treatment with a pharmacological dose (250–750 μM) 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 (Fig. 1).[20–23,34] 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.[34–39]

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.[21,22,35–37] PAM inhibits activation of survival and proliferation signaling networks in U251SP glioblastoma cells,

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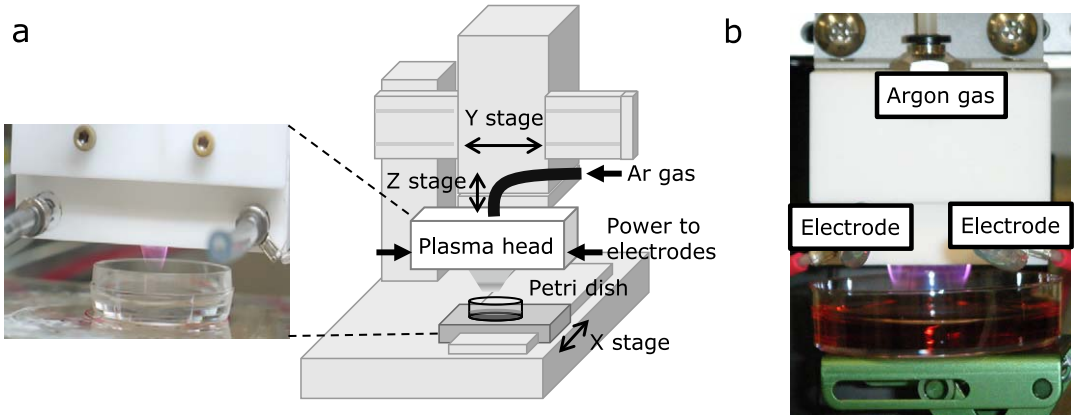

Fig. 1. (a) Non-thermal plasma for cancer treatment (reproduced from Iseki *et al.*[40]) (b) Plasma-activated medium for cancer treatment. Reprinted with permission from Ref (21).

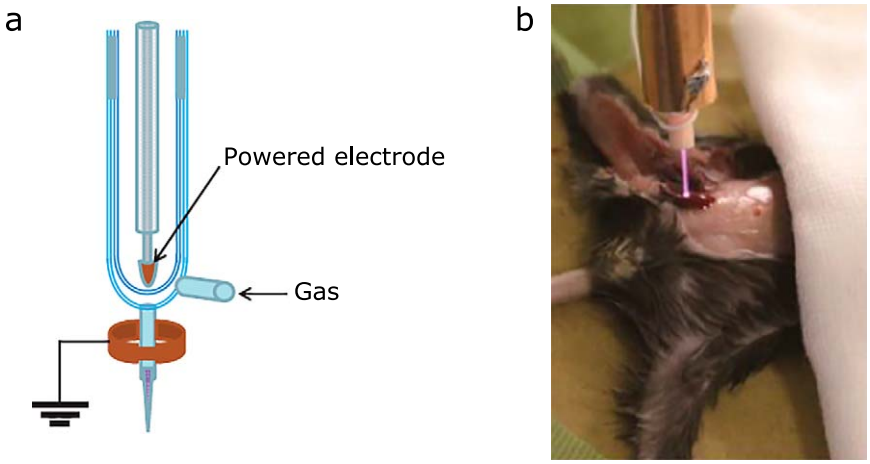

Fig. 2. Non-thermal plasma for blood coagulation. (a) Reprinted with permission from Ref (15), (b) Reprinted with permission from Ref (16).

which leads to apoptosis.[34,41] In A549 lung adenocarcinoma cells, PAM inhibits the mitochondrial-nuclear network through a caspase-independent cell death pathway,[42] and elevates intracellular Fe(II) and hydroxyl radicals.[43] PAM triggers intracellular zinc liberation in SH-SY5Y neuroblastoma cells, which leads to zinc-dependent cell death.[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.[14] Indeed, a non-thermal plasma for blood coagulation was demonstrated to stop bleeding faster than natural coagulation (Fig. 2).[15] Eosinophilic fibrous membrane-like structures were induced by the plasma, while the natural coagulation process usually contains erythrocytes.[16] The inflammation recovery process after treatment with the non-thermal plasma or thermal coagulator was visualized using the radiopharmaceutical, 2-deoxy-2-[^{18}F] fluoro-D-glucopyranose (^{18}F-FDG), and it was shown that the former is less inflammatory.[17] Electron microscopic analyses revealed that fragmented fibroblasts were seen in the electrocoagulation-treated skin and not in the plasma-treated skin.[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.[11,45]

Highly efficient and minimally invasive gene transfection has been achieved using non-thermal plasmas (Fig. 3).[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.[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.[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.

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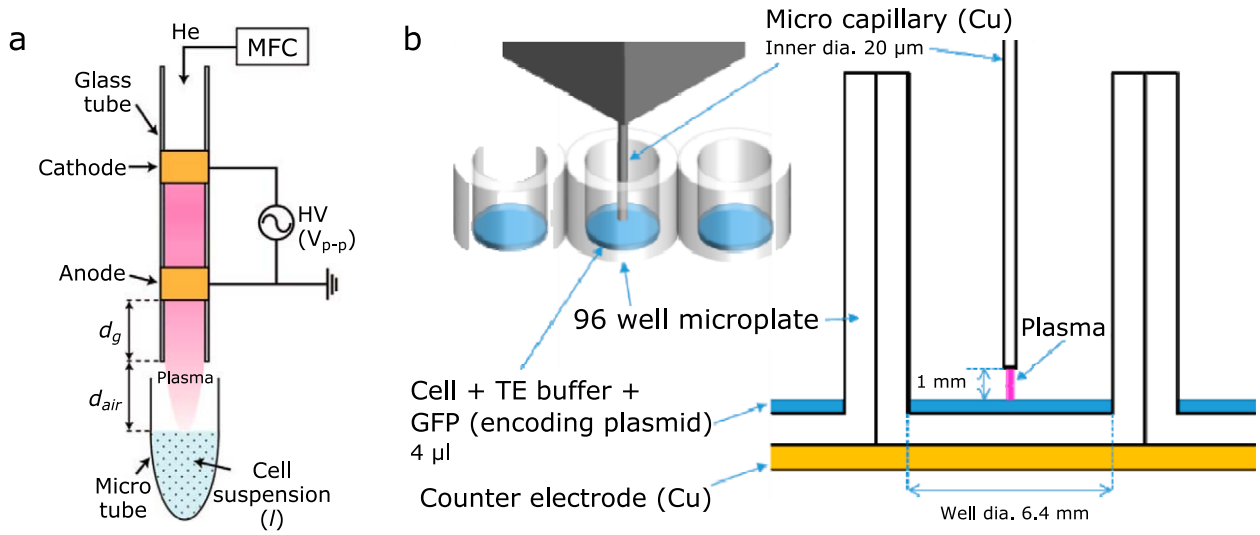

Fig. 3. Schematic of plasma gene transfection experiments. (a) Reprinted with permission from Ref (24), (b) Reprinted with permission from Ref (26).

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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.

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