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15-μm-sized single-cellular-level and cell-manipulatable microplasma jet in cancer therapies

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ABSTRACT

The authors describe a proposed 15-μm-sized, single-cellular-level, and cell-manipulatable microplasma jet device with a microcapillary glass tip and its potential in the development of cancer treatment therapies. The electrical and optical properties of the plasma jets and preliminary apoptosis results of cultured murine tumor cells and non-tumor fibroblast cells treated with the plasma jets are presented. The generated plasma jet was stable and enabled the treatment of cultured cells in cell culture plates regardless of the small inner diameter and low gas flow rate. The microplasma jet was observed inducing apoptosis in cultured murine melanoma tumor cells in a dose-dependent manner. Furthermore, the percentage of apoptotic cells of murine melanoma tumor cells induced by this plasma device was approximately 2.5 times bigger than that of murine fibroblast cells as indicated by an Annex V apoptosis assay. The apoptosis in cultured murine tumor cells by the 15-μm-sized single-cellular-level and cell-manipulatable microplasma jet device was also observed using an *in situ* apoptosis assay. We report on a novel microplasma jet device with the advantages of single-cellular-level and single cell-manipulatable plasma treatment with precise and solid stimuli. This highly precise plasma medicine, which enables new directed cancer therapies can be combined with current cell manipulation and cell culturing technologies without much difficulty.

1. Introduction

Atmospheric pressure plasmas are gaseous collections containing ionized charged particles with free electrons and strongly reactive, but short-lived radical species (Becker et al., 2006; Cooper et al., 2009; Hong et al., 2007; Kim et al., 2007; Kushner, 2005; Laroussi and Lu, 2005; Nie et al., 2008; Somekawa et al., 2005). Since atmospheric pressure plasmas have non-thermal plasmas behaviors, even if they contain many reactive species for effective treatments of materials, the temperature of these plasmas is low enough to avoid harming the region to be treated (Lee et al., 2009; Pompl et al., 2009). Moreover, the short lifespan of contained reactive species results in their complete disappearance after treatment. Atmospheric pressure plasmas also have a distinct advantage in that no expensive and large-size vacuum and add-on equipment is required for their fabrication.

These features allow atmospheric pressure plasmas to interact with biological and medical applications (Kong et al., 2009; Lloyd et al., 2010; Stoffels et al., 2008). In particular, atmospheric pressure plasmas have been worthy of note for use as promising cancer therapies, and there are some reports of their use in tumor cell treatment (Fridman et al., 2007; Kim et al., 2009; Lee et al., 2009; Stoffels et al., 2008; Zhang et al., 2008). Studies on the potential use of atmospheric pressure plasmas to treat cancer show that when exposed to the radicals, tumor cells undergo apoptosis quite rapidly (Fridman et al., 2007; Stoffels et al., 2008). Since there is no direct contact with the tumor tissues, plasma treatment significantly decreases the chance of complications as compared with either surgical excisions or laser treatment.

In order to define the mechanism of plasma-induced tumor cell apoptosis, it is preferable to create a plasma device that can treat tumor cells at the single cell level. Thus, the challenge is to generate and deliver plasmas to a single cell. Because it has a very simple structure consisting of a tube with electrodes, a microplasma jet device has been demonstrated as one of the easiest sources for generating such atmospheric pressure plasmas (Hong et al., 2007; Jiang et al., 2009; Kim et al., 2007, 2008; Laroussi and Lu, 2005; Lu et al., 2008; Nie et al., 2008; Sands et al., 2008; Shashurin et al., 2008; Walsh and Kong, 2008) with dimensions on the order of several hundred micrometers (Sakiyama et al., 2009; Stoffels et al., 2003; Yonson et al., 2006). Current plasma jet devices, however, are inadequate for cancer therapies in that their large size prevents any

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observation of the interaction between the jet and the tumor cell. They are also limited in their ability to define cell death pathways.

In this paper, we describe a single-cellular-level sized microplasma jet device with a microcapillary tip and investigate its potential for use in cancer treatment therapies. The discharge characteristics of the microplasma jets were investigated by monitoring the discharge current and optical emission as a function of time. Preliminary apoptotic characteristics of cultured murine tumor cells and non-tumor fibroblast cells treated with this microplasma jet were examined via Annexin V-FITC analysis. Furthermore, the induction of apoptosis in cultured murine tumor cells was also confirmed using an *in situ* apoptosis assay, the Invitrogen's Click-iT® TUNEL Alexa Fluor® 488 Imaging Assay.

2. Experimental

2.1. Fabrication of microcapillary tip-based plasma jet devices

We developed the single-cellular-level sized microplasma jet device using a microcapillary tip to deliver plasmas to tumor cells as shown in Fig. 1A. The microcapillary tip is sold under the trade name of TransferTip® (ES) from Eppendorf. The TransferTip® (ES) is originally used in cell manipulation for researches of embryonic stem cells in bio-medical fields. This microcapillary tip is a funnel-shaped glass tube with an inner diameter of 700 μm and an outer diameter of 1 mm gradually decreased to sub-hundred micrometers. The nozzle of the tip has an inner diameter of 15 μm and an outer diameter of 20 μm with 20° capillary angle. The image provided in Fig. 1B shows a close comparison of the dimensions of murine B16F0 melanoma tumor cells (ATCC CRL-6322) and the microcapillary tip without plasma. The figure shows that the inner diameter of the microcapillary tip is as small as a single B16F0 tumor cell. Thus, the plasma jet device with this microcapillary tip permits the treatment of small collections of tumor cells. Moreover, we expect that this microplasma jet device is applicable for treating cell cultures, specifically in developing accurate stimuli for cell manipulation and cell injection.

Because the inner diameter of the microcapillary tip was only 15 μm, the gas flow rate through the tip was extremely low, with a limit of approximately 10 standard cubic centimeters per minute (sccm). The gas flow rate was 100–3000 times lower compared to other plasma jet devices (Hong et al., 2007; Jiang et al., 2009; Kim et al., 2007, 2008; Laroussi and Lu, 2005; Lu et al., 2008; Nie et al., 2008; Sands et al., 2008; Shashurin et al., 2008; Walsh and Kong, 2008). In order to successfully generate the microplasma jet regardless of the low gas flow rate, high purity helium gas was used, which can reduce the applied voltage. In addition, a single electrode was placed 9.5 mm from the end of the microcapillary tip to secure a larger discharge space (I.D. 700 μm). A copper tape, 6 mm wide, was used as a single electrode. Despite these efforts, the discharge voltages were substantially higher as compared to other plasma jet devices (Hong et al., 2007; Jiang et al., 2009; Kim et al., 2007; Laroussi and Lu, 2005; Lu et al., 2008; Nie et al., 2008; Shashurin et al., 2008). When a sinusoidal voltage with a peak value of 10 kV and a frequency of 36 kHz was applied to the electrode, the microplasma jets were generated. Even though the gas flow volume rate is very low and the applied voltage was very high, the linear velocity of the generated charged particles and neutral helium gas abruptly increased to 943 m/s as they passed through the narrow nozzle of the tip with a 15-μm-inner diameter. Thusly, the solid plasma treatment by ionized charged particles is sufficient, regardless of the small inner diameter and low gas flow rate.

2.2. Optical and electrical measurement systems for microplasma jet device

A schematic diagram of the optical and electrical measurement system used to observe the properties of microplasma is shown in Fig. 1C. The voltage and current waveforms from the single electrode were measured using a high voltage probe (Tektronix P6015A) and a current probe (Tektronix P6021) to monitor the input electrical energy. In the driving circuit, an inverter was used to amplify the low primary voltage to a high secondary voltage. The driving circuit generated a sinusoidal voltage of several tens of kilovolts with a frequency of several tens of kilohertzs, and the photo-sensor amplifier (Hamamatsu C6386-01) was used to investigate the plasma emission. The wavelength-unresolved optical

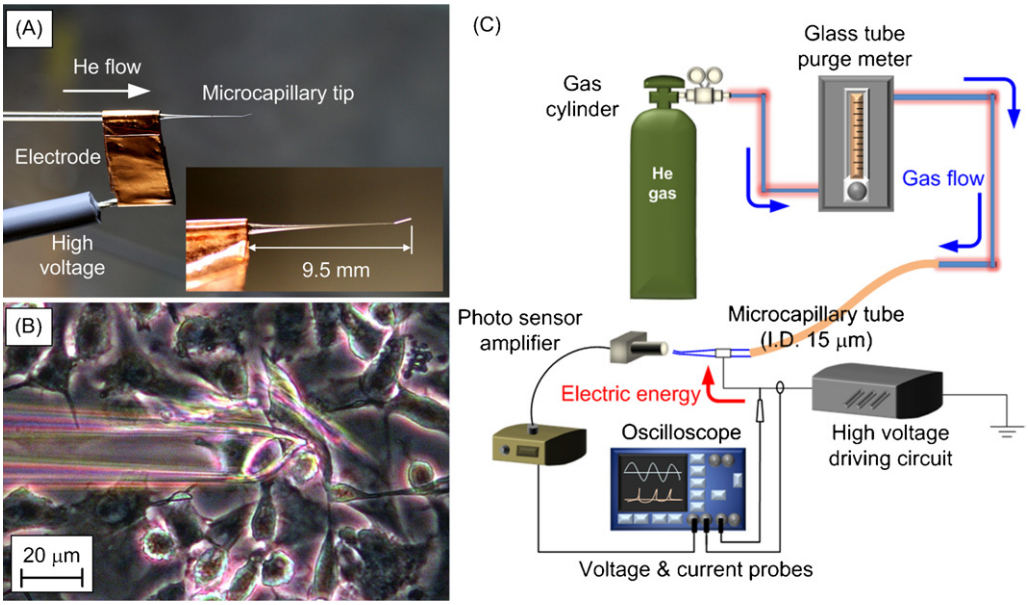

Fig. 1. Microplasma jet device with microcapillary tip. (A) Photograph of microplasma jet device with a microcapillary tip. The microcapillary tip is a funnel-shaped glass tube. The inner diameter of 700 μm gradually decreases to sub-millimeters with the nozzle of the tip having an inner diameter of 15 μm with 20° capillary angle. The copper tape, 6 mm wide, used as a single electrode, is wrapped around the tip 9.5 mm from its end. (B) Dimensional comparison between murine B16F0 melanoma tumor cells (ATCC CRL-6322) and microcapillary tip. (C) Schematic diagram of optical and electrical measurement system of microplasma jet devices.