Minimising plasma irradiation area by micronozzle device towards single-cell treatment

R. Shimane¹, S. Kumagai¹, M. Hori¹, and M. Sasaki¹

¹Department of Advanced Science and Technology, Toyota Technological Institute, 2-12-1, Hisakata, Tenpaku-ku, Nagoya 468-8511, Japan
²Department of Electrical Engineering and Computer Science, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

E-mail: mnr-sasaki@toyota-ri.ac.jp

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A micro plasma-nozzle device has been fabricated for plasma treatment for a single cell. The micro plasma-nozzle device consists of through-hole and trench structures. The nozzle device was attached to a guide tube (O.D.: φ1.5 mm, I.D.: φ1 mm) of an atmospheric pressure microplasma. Once plasma gas was supplied into the guide tube, most of the plasma gas was exhausted through trench structures, whereas the remaining plasma was excited through the nozzle hole. The nozzle device with φ5 μm holes achieved minimum plasma irradiation onto a PDMS film. The plasma-modified area was φ5.4–5.6 μm, which was much smaller than the area size modified by direct plasma irradiation from the guide tube (φ3–4 mm). The plasma irradiation caused protrusions on the PDMS surface. The minimised plasma irradiation formed 170 nm-height-protrusion-structures on the PDMS surface. The plasma irradiation through the nozzle device was applied to an onion tissue using a nozzle device with φ10 μm holes. The plasma irradiation formed φ10–15 μm holes in the onion cell membrane.

1. Introduction: Plasma has been used in material processing because of high reactivity. One of the representative applications is semiconductor microfabrication for large-scale integrated circuits (LSIs) and micro/nano electromechanical systems (MEMS/NEMS). Recently, with the development of plasma sources, plasma was applied in biological and medical studies. Disinfection and sterilisation has been carried out by irradiating plasma to a tissue [1, 2]. Plasma irradiation has caused cell activation or apoptosis. Some kinds of cancer cells have been selectively killed by irradiating plasma [3]. Plasma coagulation has been used in surgery [4]. In these treatments, plasma irradiates a broad area in a tissue, and all cells exposed to plasma are affected. Once the plasma treatment can be applied to a single cell, it will be possible to treat individual cells. The small hole made by plasma should be useful for drug administration. Selective activation or apoptosis of a single cell can be achieved. For this plasma-single-cell interaction, the plasma irradiation area should be smaller than the size of a cell (10–100 μm) [5, 6]. Compared with the injection treatment for a single cell with a microneedle/pipette [7] and the optical method using a laser [8], plasma irradiation is expected to achieve equal or lower invasive treatments.

Atmospheric pressure plasma is promising for irradiation under ambient conditions [1–4, 9–12], because sample cells are easily damaged under the low-pressure condition required for process plasmas. Atmospheric pressure microplasma can achieve higher plasma density (10¹⁵–10¹⁶ cm⁻³) [12] than the conventional low-pressure process plasma (10¹³–10¹² cm⁻³) [13]. This is advantageous for supplying a sufficient amount of reactive plasma species for the treatment. The size of the plasma is governed by Paschen’s law. The breakdown voltage is characterised by the parameter of the product of the pressure P and characteristic length d. Typical plasma size is estimated to be of the order of 10–100 μm under an atmospheric pressure condition (P ~101 kPa) [14], whereas the size is several tens of centimetres under the low-pressure condition.

The atmosphere of atmospheric pressure microplasma irradiation was reduced by using a guide tube with a small outlet structure. Using commercially available capillary tubes, the atmospheric pressure plasma was easily confined in a few-millimetre-diameter area. Further confinement was achieved by using a tapered capillary tube. The diameter of the tapered tube could be reduced to sub-micron size [11]. However, there are a few problems in using the tapered capillary tube. Because the inner diameter of the capillary tube shrinks towards the outlet end, the reactive plasma species are deactivated by the collision with the inner tube surface before the irradiation. In addition, fabrication of the small outlet tube is difficult. The outlet diameter of the tapered tube should be exactly controlled for plasma treatments for individual cells.

In the present study, a micronozzle device was fabricated and attached to an atmospheric pressure micro-plasma source to define the minimised plasma irradiation area. Plasma treatment was conducted. The area-limited plasma irradiation was investigated.

2. Structure of micro plasma-nozzle device: A micro plasma-nozzle device consisted of a trench structure for plasma gas exhaust and a small through-hole structure for minimised plasma irradiation (Fig. 1a). The micro plasma-nozzle device is attached to the outlet of a straight tube for the plasma guide to define the plasma radiation area (Fig. 1b). For the nozzle device that has a 200 μm (width) × 170 μm (depth) gas exhaust trench and φ10 μm through-hole, cross-sectional areas of the trench and nozzle hole are calculated to be 136 000 and 19.6 μm², respectively. The cross-sectional area of the trench is 10⁵ times larger than that of the through-hole. Most of the plasma is exhausted through the trenches, whereas remaining plasma exits through the nozzle hole. Therefore a cell attached to the nozzle device is not blown away by the plasma gas flow. Furthermore, compared to using a tapered tube, deactivation of reactive plasma species by collision with the

Figure 1 Schematic drawing of a microplasma-nozzle device
a Device structure
b Plasma irradiation against a single cell
inner tube surface can be decreased. This is advantageous for supplying the activated plasma species.

3. Experimental: Plasma-nozzle devices were fabricated by semiconductor micro-fabrication techniques. Sizes of the through-holes were exactly determined by the resolution of semiconductor micro-fabrication technology. A Si substrate was etched from the front and the back side. Photoresist film was patterned on a Si substrate (thickness: 200 µm) with a SiO₂ layer (thickness: 0.2 µm). The SiO₂ layer was etched with buffered HF. Trench structures (depth: 170 µm) were fabricated by reactive ion etching using Bosch process (Deep-RIE). The backside of the substrate was patterned and etched to make through-holes (membrane thickness of through-hole: 30 µm). Sizes of the through-holes prepared were φ5, 10 and 20 µm.

The fabricated micro plasma-nozzle device was attached to our atmospheric pressure inductively coupled plasma source with glue (Three Bond, 3732) [15]. A quartz glass capillary tube (O.D.: φ1.5 mm, I.D.: φ1 mm) was surrounded by a coil antenna. One end of the capillary tube was connected to a Teflon tube for gas supply. A floating metal wire was set inside the capillary tube to enhance plasma ignition. For the plasma ignition, He gas was supplied in the capillary tube and VHF power was supplied to the coil antenna. After an atmospheric pressure inductively coupled plasma was generated, He gas was replaced with other gas chemistry for the plasma irradiation.

Samples for plasma irradiation were directly attached to the micro plasma-nozzle device, and plasma irradiation was carried out. After an atmospheric pressure microplasma was ignited, plasma gas was replaced with Ar to increase the plasma emission. After the plasma irradiation, the sample surfaces were observed with an optical microscope (Keyence, VHX-200). Surface profiles were investigated by white light interferometry (Zygo, NewView 7300).

4. Results and discussion: Before using the plasma-nozzle device, a preliminary experiment of plasma-irradiation was carried out. An atmospheric pressure plasma was generated, and a plasma jet was formed from the outlet of the capillary tube by increasing the gas flow rate (Fig. 2a). The plasma jet length reached to ~10 mm (VHF power: 45 W, gas flow rate of Ar: 1.0 slm). A polydimethylsiloxane (PDMS) film (thickness: 100 µm) was irradiated with the plasma jet. The distance between the PDMS sample and the outlet of the plasma jet was set to 1–1.5 mm. The surface of the PDMS film was monitored as shown in Fig. 2b. The plasma irradiation caused a bump on the PDMS film surface (Fig. 2c). The modified area (∆φ3–4 mm) was larger than the plasma jet diameter, which was determined by the inner diameter of the guide tube (φ1 mm). This was considered to be because of the diffusion of reactive plasma species.

The fabricated plasma-nozzle device is shown in Figs. 3a–c. On the gas inlet side, four trench structures for the gas exhaust are clearly shown (Fig. 3a). The through-hole structure was located in the centre of the nozzle device (Fig. 3b). On the gas outlet side, the through-hole was also observed in the centre part (Fig. 3c).

After attaching the micro plasma-nozzle device to the capillary tube, an atmospheric pressure microplasma was generated under the same conditions as the preliminary experiment. Plasma emissions were observed from the through-hole (Figs. 4a and 4b). On the gas inlet side (Fig. 4c), plasma gas was exhausted through the trench structures, which contributed to lower the damage against the through-hole membrane in the plasma-nozzle device (thickness: 30 µm). During 10 minutes continuous discharge, the plasma nozzle device was maintained without damage.

A PDMS film (thickness: 100 µm) was directly attached to the plasma-nozzle device with shower holes (φ5, 10, and 20 µm), and plasma treatment was conducted. After 5 min plasma irradiation, the PDMS film sample was observed. The pattern of the nozzle holes was successfully transferred onto the PDMS film (Fig. 5a). The effect of plasma diffusion was minimal and each irradiated area was between 5.4 and 5.6 µm in diameter. The plasma-irradiated PDMS film showed protruding structures on the surface (Fig. 5b). The protrusion height was increased with increasing plasma irradiation time (Fig. 5c). The increase in protrusion height was saturated after 5 min plasma irradiation. There are several ingredients for the atmospheric pressure plasma irradiation such as free radicals, charged particles, excited (metastable) species and energetic photons (e.g. UV) [1]. Especially, considering Ar gas was used for the atmospheric plasma, we should pay attention to the metastable Ar atoms, which are noted 1s₂ and 1s₁ (Paschen notation) and located at 11.5 and 11.7 eV from the ground state, respectively [16, 17].

Plasma irradiation was conducted against an actual tissue. An onion tissue was used to investigate the minimised plasma irradiation. The sizes of individual onion cells were about 200 µm (Fig. 6a). The onion tissue was cut into a block and

Figure 2 Atmospheric pressure microplasma jet (Fig. 2a); optical micrograph of PDMS film surface irradiated by atmospheric pressure microplasma jet (Fig. 2b); surface profile of plasma-irradiated PDMS film (Fig. 2c)

Figure 3 Fabricated micro plasma-nozzle device
a Optical micrograph of gas inlet side
b SEM image of a through-hole on the gas inlet side
c Optical micrograph of gas outlet side

Figure 4 Micro plasma-nozzle device is attached to outlet end of capillary tube (Fig. 4a); plasma emission defined by through-hole (Fig. 4b); plasma gas is exhausted through trench structures (Fig. 4c)

Figure 5 Plasma irradiation onto PDMS film using shower-hole-nozzle device (shower-hole device shown in inset diameter of shower structure is φ285 µm) (Fig. 5a); surface profile of plasma irradiated PDMS film (Fig. 5b); protrusion height against plasma irradiation time (Fig. 5c)
irradiated by using the plasma nozzle device with φ10 μm shower holes. O₂ gas of 5% was added to the main Ar gas flow (1 slm) to enhance chemical reactions. VHF power of 45 W was supplied, and the 5 min plasma irradiation was carried out. The shower-hole pattern was transferred onto the onion tissue (Fig. 6b). In the magnified image, the depressed holes of φ10–15 μm were observed in the cell membrane (Fig. 6c). The patterned holes were larger than the shower holes of the plasma-nozzle device. This might have been caused when peeling off the onion tissue from the nozzle device. The hole structure obtained was obviously different from the protrusion structure on the PDMS film. It is the physical and/or chemical reactions in the atomic and molecular scales that patterned holes in the onion cell membrane. This plasma irradiation method for making a hole is completely different from the conventional methods using a microneedle or a micro-pipette. The minimised plasma irradiation can be a promising tool in cell treatments.

5. Conclusion: A micro plasma-nozzle device was fabricated for plasma treatment for a cell. The micro plasma-nozzle device was fabricated and attached to an atmospheric pressure micro-plasma source. Thorough-holes of φ5, 10 and 20 μm were fabricated. The micro plasma-nozzle was attached to the atmospheric pressure microplasma source. The plasma emission area was defined by the through-hole. The microplasma-nozzle device was not damaged during the plasma irradiation, which was enabled by exhausting excessive plasma gas at the gas inlet side. Plasma irradiation through the nozzle device transferred hole patterns onto samples. For the PDMS film, φ5.4–5.6 μm protrusion structures were obtained. For the onion tissue, φ10–15 μm holes were obtained.

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7 References


