Cold atmospheric plasma stimulation at the single cell scale revealed by scanning ion conductance microscopy

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Non-thermal atmospheric pressure plasma (NTAPP, also known as cold atmospheric plasma (CAP), has been widely utilized in various fields, such as agriculture, materials, and medicine. In medical and biological applications, CAP has been used for sterilization **[1]**, selective killing of cancer cells **[2]**, gene transfections **[3]**, and more. Despite its multifunctional applications, the mechanism underlying how plasma interacts with cell surface to induce cancer cell death or enhance cell permeability remains poorly understood. To investigate these phenomena, we integrated conventional CAP with a custom-built scanning ion conductance microscopy (SICM) system **[4]**, a powerful technique for visualizing cell surfaces topography. Using this system, we observed pore patterns **[5]** and the apparent changes on the cell surface **[6]** following plasma exposure. These findings suggest that CAP effects contribute to enhanced cell permeability. However, it remains challenging for fully evaluating CAPs impact on distant cells submerged in liquid, as CAP undergoes spatiotemporal changes immediately after generation. Moreover, the instability of CAP leads to low reproducibility in its effects on cells.

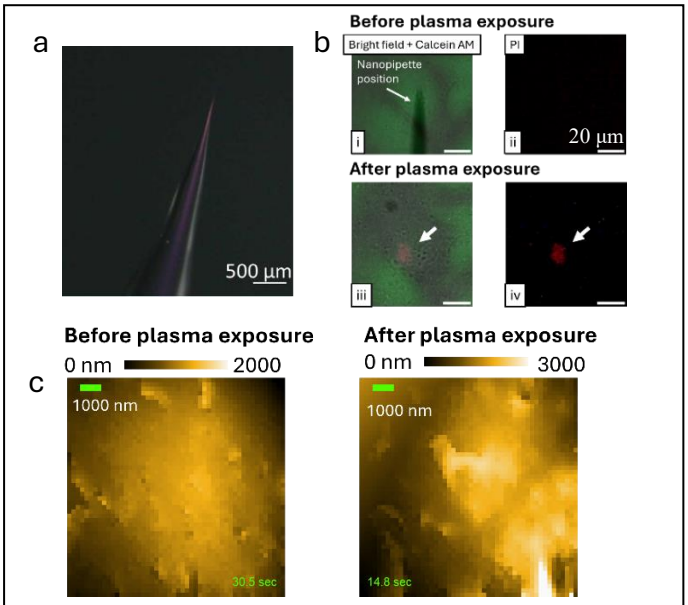

Fig. a, Plasma generation inside nanopipette probe. **b**, Fluorescent images of cell before (i and ii) and after (iii and iv) plasma exposure. **c**, SICM images of cell surface before and after plasma exposure.

To overcome these issues, we developed a novel CAP probe using a nanopipette (Fig. a), designed to directly target individual cells with plasma, thereby improving reproducibility of CAP's effects on cells. The effectiveness of this system was evaluated using LIVE/DEAD fluorescent dyes (Calcein AM as living cell indicator and Propidium Iodide, PI as the damaged/dead cell indicator) and SICM imaging. Preliminary results demonstrate that our system can successfully stimulate single cells (Fig. b, **[7]**) and induce surface modifications (Fig. c, **[7]**).

In the next steps, we will employ the nanopipette-based CAP – SICM system to investigate the delivery process of GFP-plasmid into cell, aiming to understand the entire process of DNA transfer across the cell surface via plasma. In addition, the role of nanopipette-based CAP-induced reactive oxidative species (ROS) generated by the nanopipette-based system will be explored using DCF-AM dye. The tuning and optimization of the nanopipette-based CAP system and the findings from this study will be presented.

References: **[1]** E. Takai et al., J. Phys. D. Appl. Phys. 46 (2013) 295402**. [2]** S. Iseki et al., Appl. Phys. Lett. 100 (2012)113702**. [3]** M. Jinno et al., J. Photopolym. Sci. Technol. 27 (2014) 399e404**. [4]** Watanabe S et al., Rev. Sci. Instrum. 90, 123704 (2019)**. [5]** N. G. Han et al., The 69th JSAP Spring Meeting 2022, 25p-E105-4, (2022)**.. [6]** N. G. Han et al., The 83rd JSAP Autumn Meeting 2022, 20a-A106-4, (2022)**. [7]** N. G. Han et al., The 85th JSAP Autumn Meeting 2024, 20a-P03-16, (2024).