doi:10.1017/S1431927620023442
Microsc. Microanal. 26 (Suppl 2), 2020

High-resolution Label-free 3D Mapping of metabolites of Single Living Cells

Alexander Erofeev[1], Petr Gorelkin[2], Pavel Novak[3], Christopher Edwards[4], Andrew Shevchuk[5], Alexander Vaneev[3], Alexander Majouga[3] and Yuri Korchev[5]

[1]National University of Science and Technology ‘‘MISIS"; Lomonosov Moscow State University, Moscow, Moskva, Russia, [2]Medical Nanotechnology; National University of Science and Technology MISiS, Moscow, Moskva, Russia, [3]National University of Science and Technology “MISiS”, Moscow, Moskva, Russia, [4]ICAPPIC Ltd, London, England, United Kingdom, [5]Imperial College London, London, England, United Kingdom

Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells.

Recently, we reported on the development of a label-free pH-sensitive nanoprobe consisting of a self-assembled zwitterion-like nanomembrane at the tip of a nanopipette (Zhang et al., 2019). This platform allows for SICM feedback-controlled precise positioning of the nanoprobe to the cell surface to monitor the local pHe with high spatiotemporal resolution and high sensitivity (Figure 1). Dynamic mapping of extracellular pH (pHe) at the single cell level is critical for understanding the role of H+ in cellular and subcellular processes, with particular importance in cancer. However, while several pHe sensing techniques have been developed, accessing this information at the single cell level requires significant improvement in terms of sensitivity, spatial and temporal resolution. In this study, we report on a novel zwitterionic label-free pH nanoprobe that addresses these long-standing challenges. The probe was developed by cross-linking glucose oxidase and poly-l-lysine at the tip of a glass nanopipette, resulting in drying-mediated self-assembly of a pH-selective nanomembrane with a sensitivity higher than 0.01 units, capable of fast response times (down to ~2 ms), and a high spatial resolution (~50 nm) .

Additionally, nanopipette probes still hold great promises as intracellular biosensors.

We describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5-200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of melanoma cell (Actis et al., 2014). These novel platinum nanoelectrodes are useful for understanding cell oxygen metabolism and can be employed to study the redox biochemistry and biology of cells, tissues and organisms. We showed that microinjury of Chara corallina internodal cells with the tip of a glass micropipette is associated with a drastic decrease in oxygen concentration at the vicinity of the stimulation site (Alova et al., 2019).

We applied the nanoelectrode to perform intracellular reactive oxygen species (ROS) measurement in cultured melanoma cells, HEK293 and LNCap cancer cell. Upon penetration of the cells the anodic current quickly increases followed by equilibration to a level above the one measured in the cell media (Actis et al., 2014). A cell can withstand multiple penetrations and we measured a substantial difference between the electrochemical signal measured inside and outside the cell. We believe these results show the potential of functional nanoelectrode to probe endogenous species into cells and with further improvements they

may allow the study of oxidative stress under influence of different drugs and nanoparticles (Erofeev et al., 2018). The efficiency of ROS generation under flavin mononucleotide blue light irradiation was measured in single melanoma cells by a label-free technique using an electrochemical nanoprobe in a real-time control manner (Akasov et al., 2019).

Copper is an essential element for life, but alterations in its cellular homeostasis can lead to serious neurodegenerative diseases, including Menkes and Wilson diseases, familial amyotropic lateral sclerosis, Alzheimer's disease, and prion diseases. Furthermore, it has been suggested that altered cellular copper concentration is also associated with development of cancer. Therefore, it is important to develop devices and methods for precise monitoring of cellular copper concentration. Highly sensitive selective nanocapillary sensor, which allows to measure copper inside single cells and organoids in real time was developed. The formation of reactive oxygen species in breast carcinoma cells under the action of copper-containing anticancer drugs was studied.

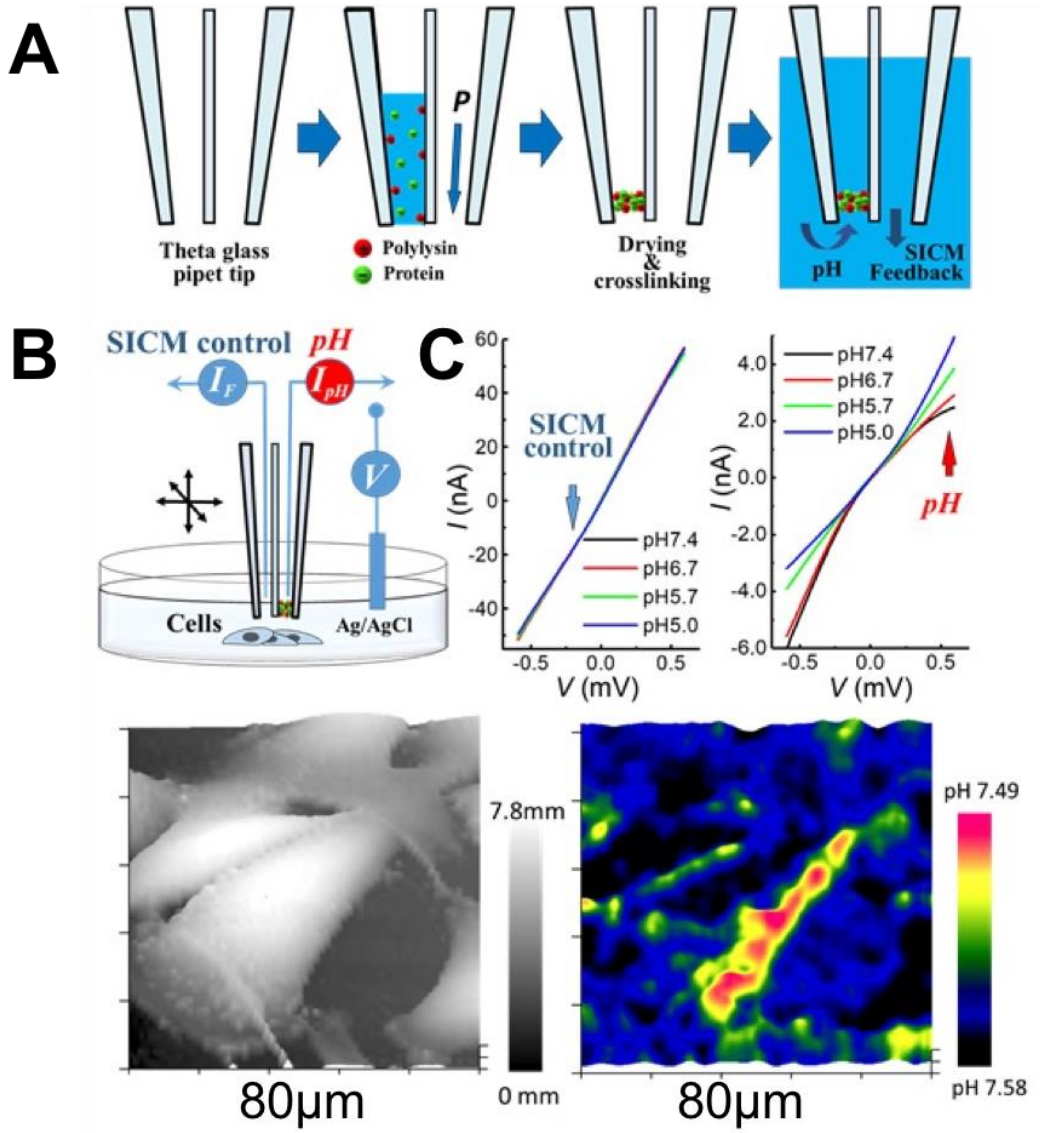

Figure 1. Fabrication of Scanning Ion Conductance Microscopy (SICM) feedback-controlled nanoprobe pH sensor to perform high-resolution 3D pHe mapping of living cells. (A) Cartoon of the fabrication

https://doi.org/10.1017/S1431927620023442 Published online by Cambridge University Press

procedures for pH-sensitive nanomembrane inside one barrel of a dual-barrel θ quartz glass nanopipette and keep another barrel opening with pressure for SICM feedback controlling. (B) A schematic diagram shows the operation of dual-barrel nanoprobe to perform both SICM controlling and pH measuring simultaneously. (C) The ion-currents flowing into two separated barrels of the generated dual-barrel nanoprobe have shown very different I-V responses to pH. One barrel on the right-side shows pH-sensitive and will be used for pH detecting at positive 0.6 V, at which voltage the nanoprobe has a great pH sensitivity. Another barrel on the left-side demonstrates a pH-insensitive and will be used as SICM contorl at negative – 0.2 V, where the nanoprobe sensor has a more stable ion-curents at varies pH. (D) 3D pHe mapping of living melanoma cells. The SICM topographical images (left) and pHe distributions (right) of same group of living melanoma cells can be obtained simultaneously by one SICM scanning.

References

Actis, P., Tokar, S., Clausmeyer, J., Babakinejad, B., Mikhaleva, S., Cornut, R., Novak, P., Shevchuck, A. I., Dougan, J. A., Schuhmann, W., Klenerman, D., Rusakov, O. D. A., Sviderskaya, E. V & Korchev, Y. E. (2014). Electrochemical Nanoprobes for Single-Cell Analysis. 875–884.

Akasov, R. A., Sholina, N. V, Khochenkov, D. A., Alova, A. V & Gorelkin, P. V (2019). Photodynamic therapy of melanoma by blue-light photoactivation of flavin mononucleotide. 1–11.

Alova, A., Erofeev, A., Gorelkin, P., Bibikova, T., Korchev, Y. & Majouga, A. (2019). Prolonged oxygen depletion in microwounded cells of Chara corallina detected with novel oxygen nanosensors. 1–13.

Erofeev, A., Gorelkin, P., Garanina, A., Alova, A., Efremova, M., Vorobyeva, N., Edwards, C., Korchev, Y. & Majouga, A. (2018). Novel method for rapid toxicity screening of magnetic nanoparticles. *Scientific Reports* **8**, 1–11. http://dx.doi.org/10.1038/s41598-018-25852-4.

Zhang, Y., Takahashi, Y., Hong, S. P., Liu, F., Bednarska, J., Goff, P. S., Novak, P., Shevchuk, A., Gopal, S., Barozzi, I., Magnani, L., Sakai, H., Suguru, Y., Fujii, T., Erofeev, A., Gorelkin, P., Majouga, A., Weiss, D. J., Edwards, C., Ivanov, A. P., Klenerman, D., Sviderskaya, E. V, Edel, J. B. & Korchev, Y. (2019). extracellular pH of single living cells. *Nature Communications* 1–9. http://dx.doi.org/10.1038/s41467-019-13535-1.

Acknowledgements. Electrochemical measurements of ROS level were carried out with financial support from the Ministry of Education and Science of the Russian Federation in the framework of increase Competitiveness Program of NUST ‘‘MISIS”, implemented by a governmental decree dated 16th of March 2013, No 211. Intracellular copper measurements were supported by the Russian Russian Science Foundation 19-74-10059.