Nanotechnology improved whole cell patch clamp

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PROJECT DESCRIPTION:

Patch clamp is an important way to acquire information from cells to monitor their conditions and response to chemicals. The key to a successful patch clamping experiment is to form a tight seal between the glass pipette and cell membrane, known as Giga-seal. In this project, the effect of physical and chemical properties of glass µ-pipettes on seal formation has been studied and improved with the use of micro and nanotechnology. SEM stereoscopic technique, focused ion beam milling and chemical treatments have been adopted for measuring, modifying and changing the surface properties of pipettes. The effect of each property has been tested by carrying out patch clamp experiments.

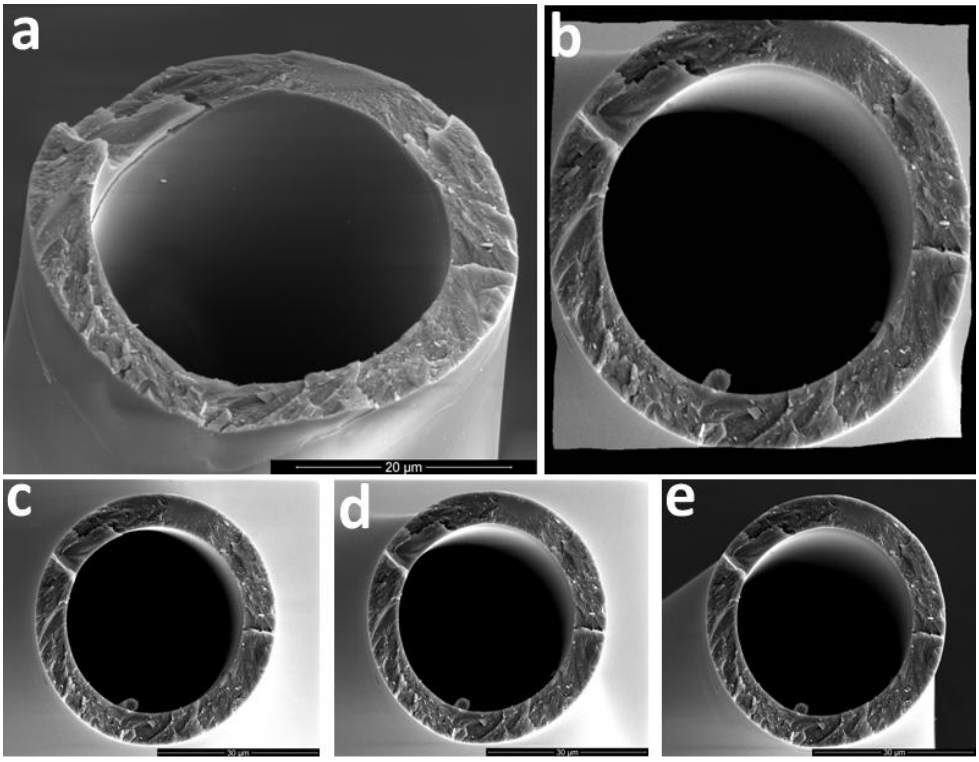

Figure 1. The high magnification SEM images have revealed the rough and uneven surface nature of the tips to be in contact with cells (a). Surface properties of pipette tip can be measured from the Digital Elevation Model; (b) A digital model of the pipette created using 3D reconstruction based on SEM stereoscopic technique; (c-d) West, middle and east stereo pair SEM images each taken from different prospective are used in 3D reconstruction.

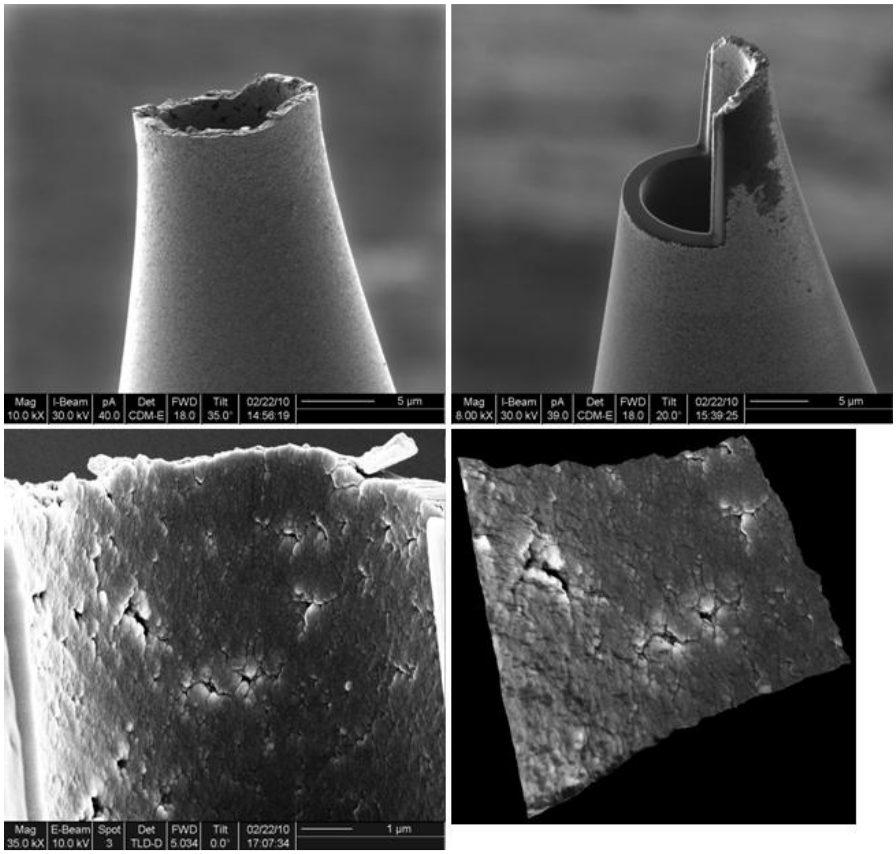

Figure 2. Half of the pipette was cut using focused ion beam milling to enable access to inner wall of pipette. The 3D-reconstruction results show that the inner of pipette is rough and could compromise the seal by increasing the chance of ions escape [1] .

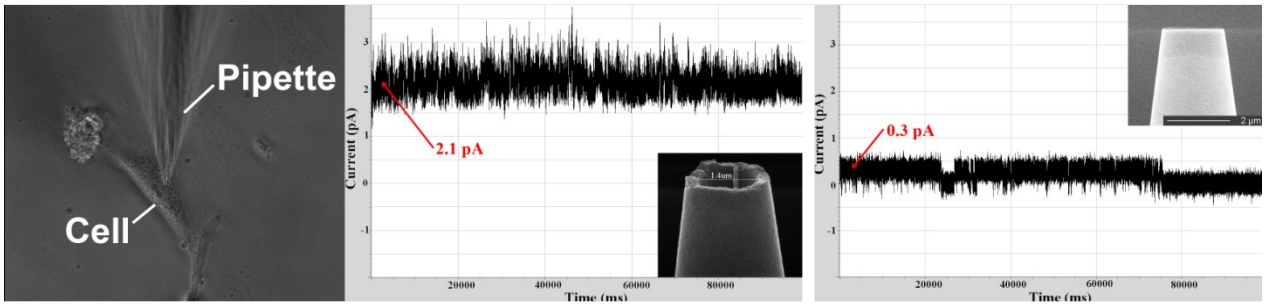

Figure 3. Patch clamp experiment carried out by polished and unpolished pipettes show very smaller leakage current for polished pipettes. A polished pipette with smoother surface gives a better contact condition. Higher seal resistance made it possible to measure the activity of a single ion channel which is the highest possible resolution in patch clamping [2].

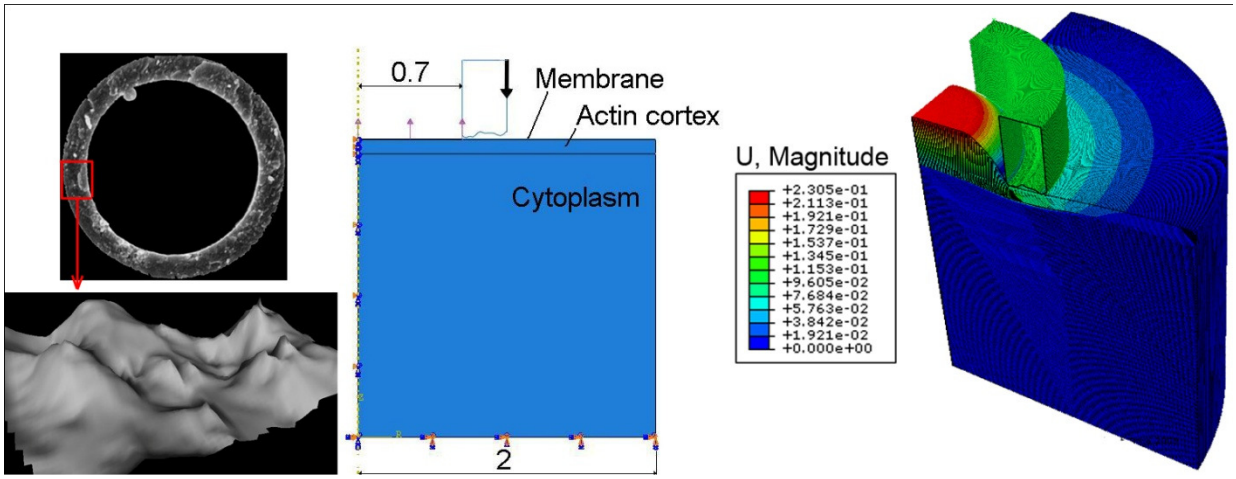

Figure 4. FEA simulation was carried out to find out how a cell deforms under the rough tip of a pipette. The modelled pipette is modelled based on the real tip profile of the glass micropipette. The result of FEA shows that the membrane cannot cover all of the cavities of the tip. As a result current can leak from the space between the cell and the pipette and compromise the seal [3].

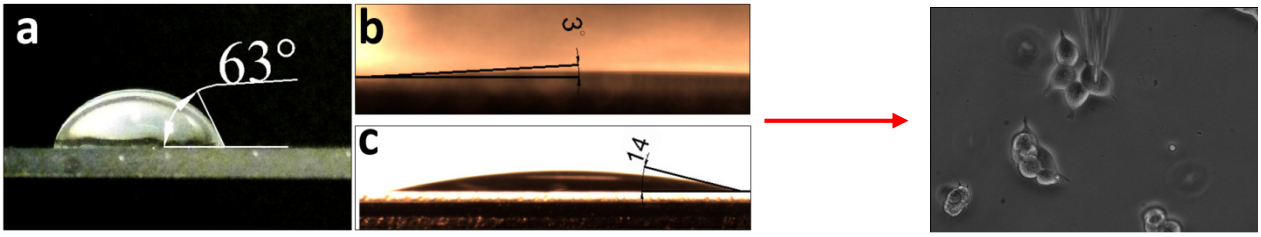

Figure 5. To test the effect of hydrophilicity, glass µ-pipettes were treated by Piranha Solution. The surface of pipette surface has contact angle of 63 degrees before treatment, (a). Glass surface became highly hydrophilic after treatment (b) and was able to keep its property after 24 hr (c).

Selected publications:

[1] M Malboubi M, Y Gu and K Jiang, Surface property characterization of glass µ-pipettes using SEM stereoscopic technique, Micro Nano Engineering, 36th International Conference, Genoa, 19–22 September 2010.

[2] M. Malboubi, H. Ostadi, S. Wang, Y. Gu and K. Jiang, Effects of the surface morphology of pipette tip on Giga-seal formation, Engineering Letters, 17 (4) (2009) 281-285.

[3] M. Malboubi, Y. Gu and K. Jiang, Experimental and simulation study of the effect of pipette roughness on giga-seal formation in patch clamping, Microelectronic Engineering, Volume 87 , Issue 5-8 (2010) 778-781.