Planar patch clamp combined with atomic force microscope (AFM)

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Atomic force microscopy and patch clamp are two incompatible techniques as long as patch clamp will be routinely done using glass pipettes. This project focuses on the simultaneous measurements of ion-channels using ligand tethered AFM tips combined with a planar patch clamp device.

Traditionally, single channel patch clamp measurements are done using glass pipettes that are made by a heat-and-pulling technique. AFM force spectroscopy allows the determination of chemical reaction constants of single ligand-receptor pairs. However, it is impossible to sense single ligand-gated ion channels using agonists tethered to an AFM tip with pipette probes due to mechanical instabilities. Sub-micrometer pores have been successfully fabricated on planar substrate and whole cell patch clamp measurements have been demonstrated. Nevertheless, yield is not very high and gigaohm seals, necessary for single channel recording, are not routinely obtained. In this project, one aim is to understand the parameters controlling seal resistance, which will enable the development of systems capable of performing single channel electrophysiology measurements. This could have major applications in parallel drug screening as well as sensor applications.

The project is split in two main parts: (1) developing a micromachining process to produce pipette-like shapes on a planar surface with glass-like material and (2) setting up a customized experimental environment to perform simultaneous patch clamp and AFM measurements.

![Microfabrication process flow](image1.png)

1. Pattern a silicon oxide disk on a Si wafer
2. Isotropic silicon etch and oxide strip
3. Silicon oxide coating
4. Pore pattern with a FIB and backside silicon etch
5. Final oxidation step

Figure 1: microfabrication process

It is believed that several parameters related to surface morphology and chemistry could play a significant role in the cell membrane adhesion to the substrate and thus in the formation of gigaohm seals. A first obvious difference between a pipette and a pore made on a planar sample is shape. We address this issue by first replicating a pipette tip shape on a planar substrate with the aim of systematically exploring different shapes and chemistries to investigate their role in forming a gigaohm seal.

Figure 1 describes the microfabrication process flow to manufacture custom shaped planar patch clamp substrates. Silicon domes are produced and pores drilled by using a focused-ion-beam. Figure 2 represents a scanning electron micrograph of a silicon dome.

![SEM image of a 6µm-high silicon dome](image2.png)

Figure 2: SEM image of a 6µm-high silicon dome

For the second part of the project, a profusion cell has been designed and machined so that it meets all the necessary requirements to perform patch clamp and AFM measurements. Peek™ was chosen as the bulk material for this cell because of its remarkable properties in terms of electrical isolation and ease of machining. The figure shows the profusion cell installed on the AFM stage.

![Profusion cell installed on the AFM stage](image3.png)

Fig. 3: profusion cell installed on the Bioscope AFM stage.