Testing Silicon Planar Patch-Clamp Devices

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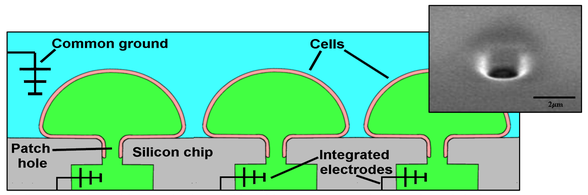
Background

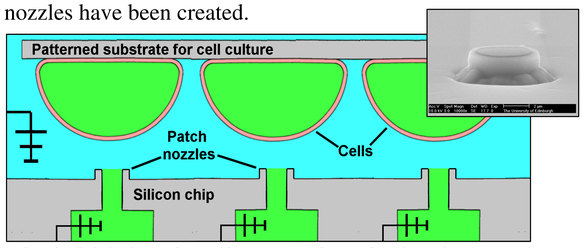
An ongoing project at Edinburgh University aims to create an array of planar patch-clamps in a single planar substrate (a silicon chip) for multichannel whole-cell recordings from cultured neural networks. Advancement of the project depends on successfully achieving high-resistance seals with neurons by using a chip with a single aperture, a goal which has already been achieved in commercial systems for pharmaceutical testing. Chips with various surface and aperture characteristics have been created.

Neurons will be cultured directly on the surface of the chip, with their growth guided by a patterned surface to position them over patch-holes. Then suction will be applied to achieve a >GΩ seal and whole-cell breakthrough with each of the cells. The patch apertures will be individually addressed by integrated electrodes for multichannel recording.



Cells cultured on chip, patch-clamped by chip. **Inset :** SEM image of patch-hole.

Alternatively, neurons may be cultured on a separate patterned surface, and this surface subsequently lowered onto the chip. For this application patch apertures with protruding nozzles have been created.



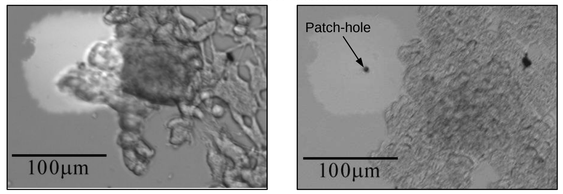
Cultured cells being lowered onto chip. **Inset:** SEM image of patch-nozzle.

Chips were compared to standard glass electrodes for their ability to form >GΩ seals. Additionally the above paradigms were compared in terms of their practicality by testing chips with a range of methods.

Different silicon dioxide surface coatings were tested: deposited (PECVD) vs thermally grown (TO) and boron doped (+B) or not. Furthermore, different hole diameters were tested, in the range 1.5 - 4µm.

Culturing cells on devices

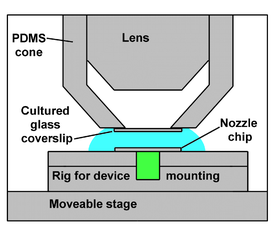
AtT20 cells (a mouse pituitary tumour line with some neuron-like properties) were cultured on the surface of devices in standard growth medium and standard incubator conditions for periods of 6-13 days. UV sterilisation failed, so antibiotics were applied to prevent infection. Where cells grew over holes, poor adhesion to the PECVD(+B) surfaces contributed to the cells becoming dislodged in preparation for patch-clamping. Separate tests of surfaces (contact angle, surface roughness and cell culturing) suggested that TO+B would be better for cell adhesion.



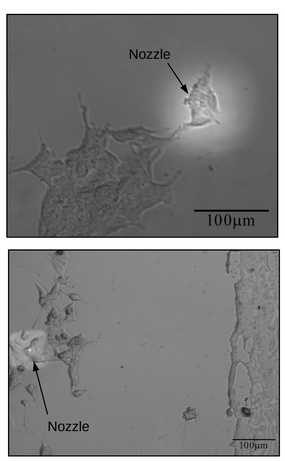
AtT20 cells growing on chip. **Left:** Before preparation for patch-clamping. **Right:** After.

Lowering cultured cells onto nozzle chips – method I

AtT20 cells were cultured on glass coverslips. A cone was made of silicone rubber (PDMS) which would hold a coverslip upside down over the end of a microscope lens in focus. The lens could then be lowered towards the chip, and the chip could be manoeuvred horizontally to line up the nozzle with a cell.



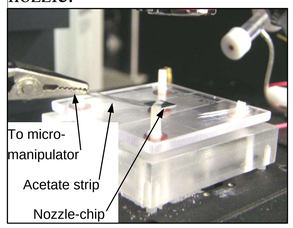
It was frequently difficult to lower coverslips far enough to contact the nozzles. The nozzles are ≃2µm high and the cells ≃10µm high, so small amounts of debris or surface irregularities can easily prevent contact. Culturing cells on a layer of glia may raise cells at the same time as cushioning them.

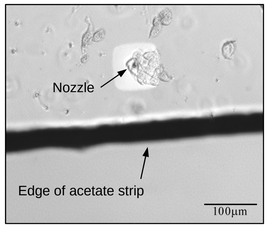


Cultured cells in focus. **Above:** The surface of the chip is out of focus as closer proximity is prevented. **Below:** Contact is achieved.

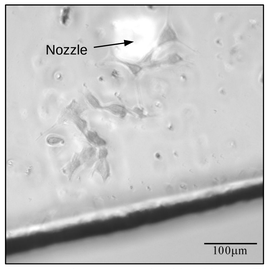
Lowering cultured cells onto nozzle chips – method II

AtT20 cells were cultured on strips of acetate. The strips could then be attached to a micro-manipulator and flexed against the chip to manoeuvre the cells down onto the nozzle.





Cells could be more easily brought in contact with the nozzle, however surface irregularities in the acetate could still prevent contact. Cells did not generally adhere well to the acetate. Investigation of different types of acetate and coating with Poly-D-Lysine (PDL) may improve this method.



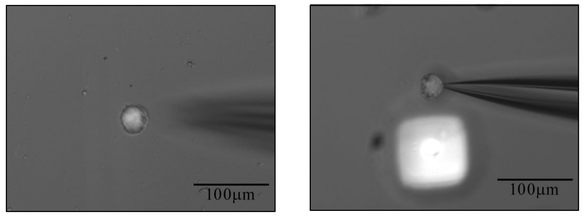
Acetate strip over nozzle-chip. **Above:** The cells are not well stuck down. **Below:** Exceptionally, these cells appear well stuck down and are extending neurites.

Furthering both lowering methods will require research into variation in cell morphology and the physiological effects of compression and distortion on cells.

Lowering dissociated cells onto nozzle chips

This alternative method was used only for testing the ability of the chips to form seals. Neurons were dissociated from snail ganglia, labelled with Cell Tracker Green and allowed to settle on the surface of the chip. They were gripped by suction from a glass pipette, lifted over the nozzle and lowered until contact was achieved. Thereafter suction was applied through the nozzle and the seal resistance (voltage response to current pulses) was recorded. Neurons were also placed in a PDL-coated Petri dish and whole-cell recordings were attempted by the regular patch clamping method, with fire-polished pipettes matched by hole-diameter to the chips.

Cells adhered to the surface of the TO(+B) chips. They lost fluorescence after being lifted, which may indicate damage.



Snail neuron labelled with Cell Tracker Green on surface of chip. **Left:** Before lifting. **Right:** After lifting and prior to patch-clamping with nozzle chip. Notice the loss of fluorescence.

Patch-clamping results

The difficulties described reduced experimental yield so that only a small number of attempts at patch-clamping were achieved by any method. All seals achieved with AtT-20 cells were of low resistance, max. 50MΩ.

For experiments with snail neurons, all seals achieved were of low resistance, max. 50MΩ. Data from planar patch-clamping attempts (mean 4MΩ s.d. 5MΩ) vs standard patch-clamping controls (mean 51MΩ s.d. 28MΩ) was ranked according to maximum seal resistance achieved. A Mann-Whitney Rank Sum test confirmed the significance of this difference ($U_{standard}$=6.5, n_{planar}=7, $n_{standard}$=13, two-tailed, P<0.01).

Conclusions

The methods for testing these chips have been refined. All methods have practical difficulties, however no method can yet be eliminated. Many of the earlier patching attempts may have failed due to the use of chips with large hole diameters. Control experiments indicated that AtT-20 cells should be patch-clamped with holes of <1.5µm.

Notwithstanding this, the chips consistently perform worse than glass electrodes in seal formation and no gigaseals or whole-cell recordings have yet been achieved. Surface roughness may be the problem however debris on patch aperture preventing seal formation is a likely scenario. This is especially likely to be a problem where cells are cultured on chips. A further problem with culturing cells on chips is the need to fill the patch apertures with intracellularly compatible solution prior to patch-clamping.