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SU-8 Micropipettes for Gentle Single-cell Manipulation

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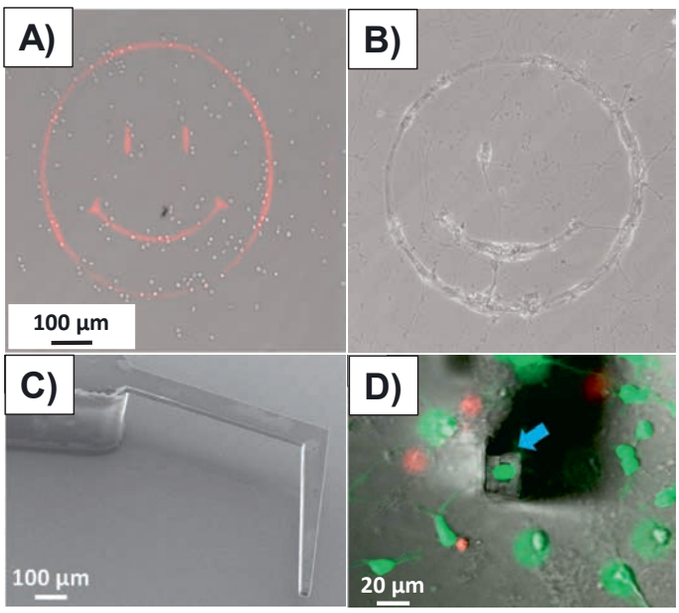
In 1904, Marshall Barber reported about the invention of glass micropipettes for bacterial isolation and inoculation. A century afterward, glass pipettes are a standard tool for single-cell manipulation. As they are rigid, the technical challenge is to implement a feedback to enable a controlled and thus gentle approach onto a cell.

We established a microfabrication process to produce atomic force microscopy (AFM) cantilevers with an embedded microchannel in order to take advantage of the force feedback. They are bendable because they are entirely made of the resin SU-8. With these devices, we are able to determine the adhesion force of hundreds of yeast cells on glass and chemically coated glass enlarging the statistical relevance of such experiments. The tough issue was to find the right material compatible with the SU-8 processing as sacrificial layer for the formation of the microchannel. We used an electrochemically deposited copper film which allows for a flexible choice of its thickness up to tens of micrometers. In this way, we can load the probe with a solution containing live cells and utilize it as a tool to pattern single-cells on a substrate with micrometric precision. We defined two main protocols: the direct deposition (additive patterning) and the selective removal of cells (subtractive patterning). Cells can be placed in elastomeric wells and on flat adhesive surfaces by physical confinement and mechanical squeezing upon application of a positive pressure. On the other hand, detachment and removal of chosen cells from a cell layer is achieved by exerting a negative pressure depending on the chemical functionalization of the substrate (adhesive or repulsive). In this way, we realized complex networks of neurons that could be tracked after several days. Such patterns can be eventually adjusted by subsequent *in situ* deposition or removal of mature cells. They represent the first milestone for the bottom-up approach of engineering neuronal circuits with controlled topology to investigate basic mechanisms in neuroscience such as signal transmission and neurocomputation.

Our sideways microfabrication scheme offers the possibility of designing various types of cantilevers with different shapes and lengths within a single wafer. We are now envisaging the fabrication of AFM SU-8 pipettes with embedded self-sensing aiming at a system with several micropipettes, each one having a different task and thus driven independently, for applications not only in sequential single-cell manipulation but in multimaterial 3D printing.



A) Subtractive patterning of primary hippocampal neurons on pre-patterned surfaces: Neurons before and B) after removing the unwanted cells from the pattern. By 12 days *in vitro*, neuronal processes originating from the cells on the left side of the smiley closed the loop by following the pattern. C) SEM image of a side-view SU-8 micropipette with a 500-µm long tip. D) Superposition of a bright field optical image and of the corresponding fluorescent one showing a pipette approached onto the selected neuron in matrigel for the isolation process (the blue arrow indicates the rectangular probe apex).

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