Supplemental Online Material

to

Growing neuronal islands on multi-electrode arrays using an accurate positioning-µCP device

by R. Samhaber, M. Schottdorf, A. El Hady, K. Bröking, A. Daus, C. Thielemann, W. Stühmer, and F. Wolf

Introduction

On this website, you can find detailed instructions for building a micro contact printing (µCP) machine and for using it to spatially structure neuronal cultures. Its purpose is to allow any lab without access to a lithography facility to structure cultures of neurons with the method developed in our group.
You can find a detailed description as to the preparation of neuronal cultures, the protocols used, and the construction of the µCP machine including all CAD files (for Autodesk Inventor) to build your own!
This project has been carried out at the Max Planck Institute for Experimental Medicine in the department of Molecular Biology of Neuronal Signals, headed by Prof. Dr. Walter Stühmer. On the following pages, one can find a detailed and illustrated protocol of the stamping process. In the downloads section, all construction sheets for the stamping machine and the molds are available.

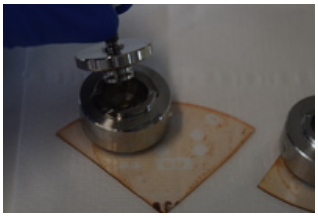

Fig. 1

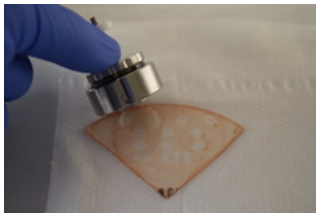

Fig. 2

Fig. 3

Fig. 4

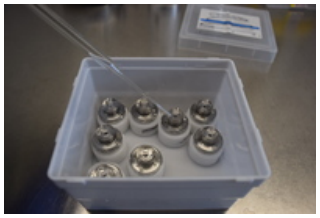

Fig. 5

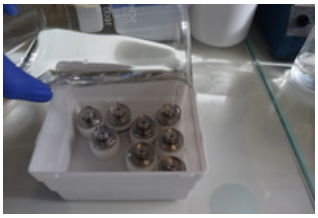

Fig. 6

Stamp and MEA preparation

Fabrication of the stamps

The stamps are fabricated by applying freshly prepared and degassed PDMS to molds which are placed on a photolithographically prepared mask. The molds are covered from the inside with a thin film of SDS. This is done by placing a drop of SDS solution in the inside and by spreading it evenly with the finger. The molds are then placed onto the masks and filled with freshly prepared and degassed PDMS. We add enough PDMs to fill the entire mold with the viscuous liquid. Then a stamp-holder is inserted into the liquid. This assembly is left for 48h to cure (see **Fig. 1**). After two days, the stamps are removed from the mold by pulling at the stamp holder which is now embedded into solid PDMS (see **Fig. 2**) and (see **Fig. 3**). In one run, we usually prepare 8 stamps. After removal of the stamps they are placed for 30s in 70% Ethanol for both removal of excess SDS and sterilization. Then, the pin is removed and they are placed in Teflon holders. Next, a single drop of SDS is placed on each mold (see **Fig. 4**). It is left there for 20min, sucked off by vacuum (see **Fig. 5**), the stamp is rinsed in ddH_2O (see **Fig. 6**) and thoroughly dried with dry nitrogen (see **Fig. 7**). This procedure should create a thin layer of SDS to facilitate later stamping. Then, a drop of

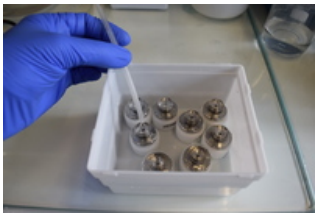

Fig. 7

Fig. 8

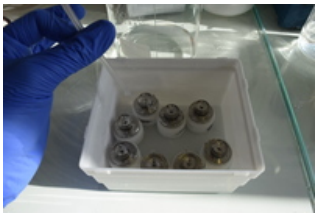

Fig. 9

FITC-PLL is placed on the stamps and left there for an hour (see **Fig. 8**). Subsequently, the drop of PLL is sucked off and the stamps are ready for use (see **Fig. 9**)

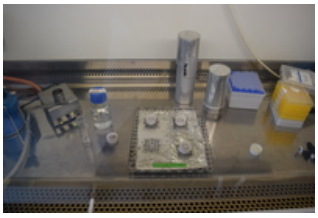

Fig. 10

Fig. 11

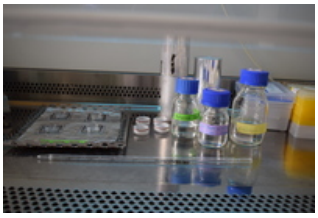

Fig. 12

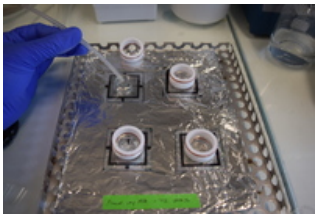

Fig. 13

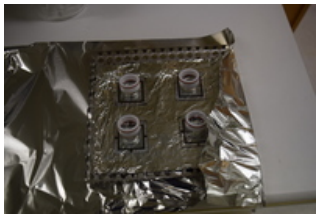

Fig. 14

Fig. 15

Preparation of the MEAs

First, used MEAs are cleaned for 12h to 24h in a 1% Terg-A-Zyme solution in ddH_2O (for instance as described by Multichannel Systems). They are then thoroughly cleaned from any left Terg-A-Zyme, filled with ddH_2O and sterilized in an autoclave. After the autoclaving cycle has completed, they are rinsed with sterile ddH_2O (see **Fig. 10**) and filled with fetal calf serum (see **Fig. 11**) to hydrophilize the surface. The FCS is left in there for at least 30 min. Then, the FCS is sucked off, the MEA rinsed in ddH_2O again and left to dry (see **Fig. 12**). MEAs are conveniently dried in the air stream of the bench under UV light to prevent contamination. After making sure that all water residues have evaporated, the MEAs are filled with a 3-GPS solution in Toluene. This solution is left in the MEAs for 20 min, removed and the MEA quickly rinsed 3 times witch pure toluene. After sucking off all excess toluene, the MEAs are dried with a dry nitrogen stream (see **Fig. 13**) and placed in an oven at 100°for 1 h to remove any remaining toluene (see **Fig. 14** and **Fig. 15**) After this procedure, the silanized surface is ready for the stamping. The prepared MEAs and the stamps are kept on the bench, in a sterile environment and the stamping process is immediately started.

Chemicals & Tools

The tools and molds are available as CAD sheets in the download section and to follow this protocol, you will need the following chemicals:

Chemical	**Supplier**
Tergazyme	Sigma-Aldrich
PDMS Sylgard 184	Dow Corning
10% w/v sodium dodecyl sulfate in ddH_2O	Sigma Aldrich
1% (3-glycidoxypropyl)trimethoxysilane	Sigma Aldrich
Toluene	Sigma Aldrich
Poly-L-lysine FITC labeled 1 mg/ml in PBS 70,000-150,000 MW	Sigma Aldrich
Fetal Calf Serum (FCS)	Biochrom
Trypsin/Ethylenediaminetetraacetic acid (EDTA) Trypsin: 0.05%; EDTA: 0.02%	Biochrom

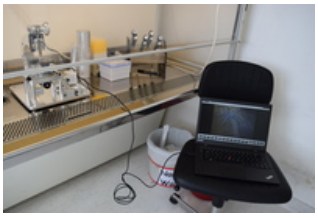

Fig. 16

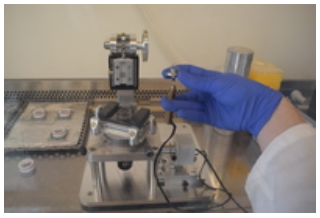

Fig. 17

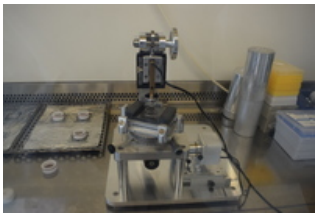

Fig. 18

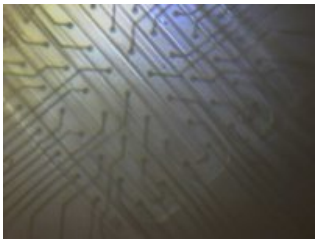

Fig. 19

Fig. 20

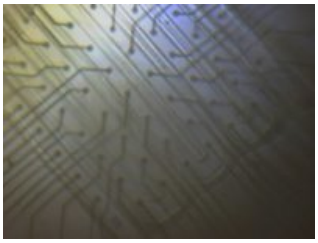

Fig. 21

Stamping

Stamping – How to?

For the stamping, we first spray the stamping apparatus with plenty of 70% ethanol and put it on the bench. We leave it to dry and connect it to a computer for readout of the images (**Fig. 16**). Next, we place one stamp into the stamp holder and place the stamp holder into its slot (**Fig. 17**). Then we put one of the prepared MEAs onto the MEA holder of the stamping machine (**Fig. 18**).

The build in inverted microscope with the webcam allows for inspection of the position of the MEA. Next, the stamp is carefully lowered in such a manner that it stops above the MEA without touching its surface. This can be monitored by observing how the stamp comes into focus of the microscope. After lowering the stamp, the stage is adjusted such that the stamp's pattern matches the adjusted orientation of the MEA (**Fig. 19**). After both, the stamp and the MEA have been aligned, the stamp is lowered until it touches the surface of the MEA (**Fig. 20**). Touching the MEA can easily be seen by a change of color (**Fig. 21**). The stamp is left on the surface of the MEA for 2min, removed, and the MEA is finished. Next, 50.000 E18 neurons in 100

µl NB medium are placed onto the electrode field. The neurons are allowed to settle for 4h and then the MEA is filled with 1ml of fresh medium. The medium needs to be changed once a week. Every MEA is stamped with its own PDMS stamp and every stamp is used only once.

CAD Sheets & Download

Comments

The machine has several main components. The base plate is connected to a column. On its top there is an eccentric which moves a linear guide with the stamp. With this linear guide, the stamp is lowered onto a MEA which is clamped into place on a table with x, y and C-axis manipulators. The C-Axis manipulator of our machine is taken from a microscope and therefore in the construction sheets, it is referred to as 'dummy'. The table assembly is held in place with four pillars. Under the table assembly, there is an inverted microscope consisting of a prism, an achromatic lens and a CCD Chip which allows monitoring the position. The achromatic lens with f=18.5mm focal length is roughly 2f away from the MEA and projects the MEA's image in 2f distance onto a 1/4″ CCD (Sony ICX098BQ) of a commercial webcam (Philips ToUCam Pro II). The type and size of the CCD chip is not important, but the focal length of the lens and the position of the CCD needs to be adjusted accordingly.

Downloads

We have prepared a collection of the CAD construction sheets, see **Fig. 8** of the stamping machine and its components as Autodesk Inventor Part and Assembly files, a few more pictures and a Microsoft Excel list of its components. You can find it for download online. Also, we have prepared a collection of construction sheets for the stamps. You can find them online, too.

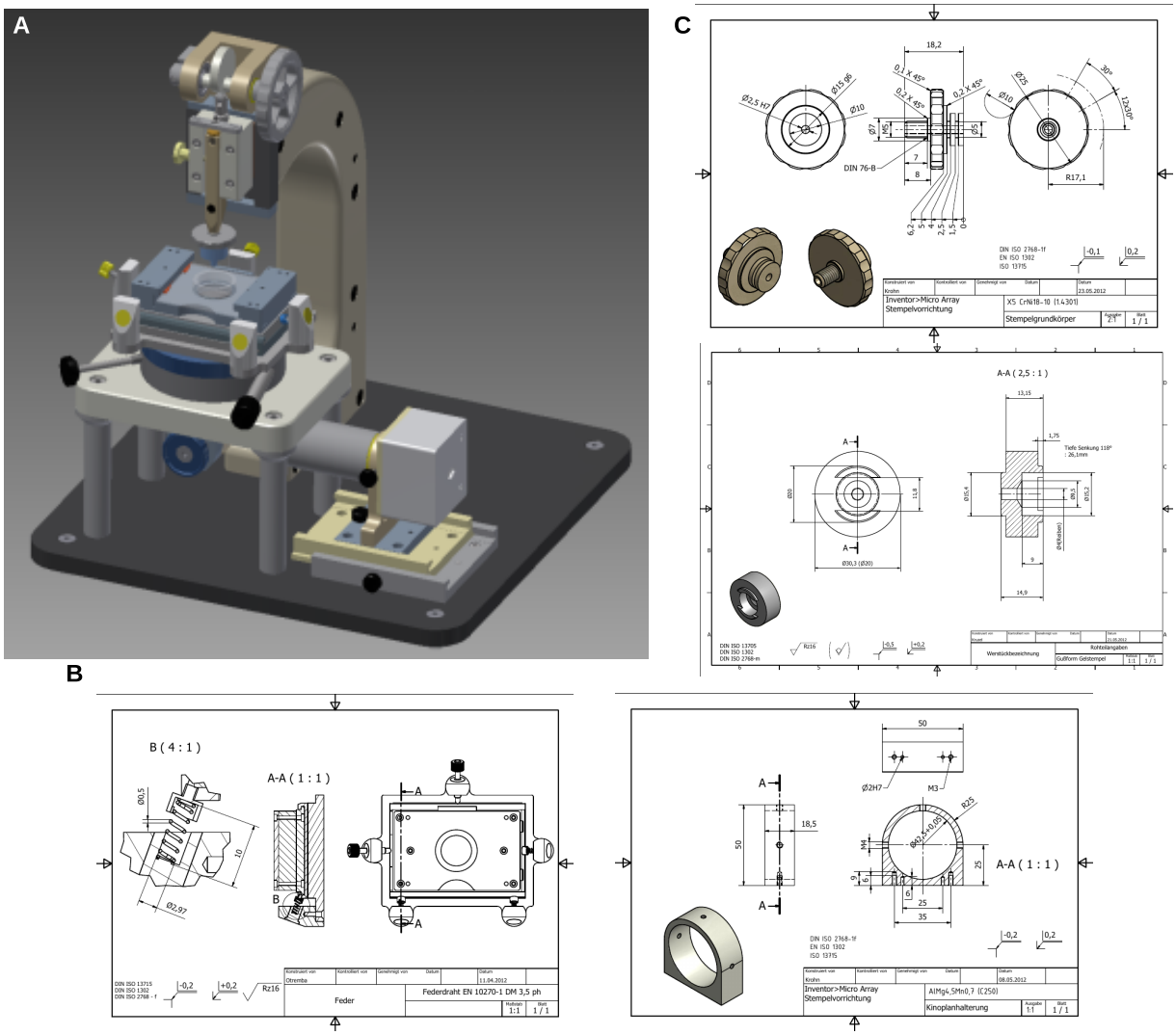

Fig. 8: Examples of the online supplemental material. **A** A rendering of the CAD construction sheets. **B** A few example construction sheets of the stamping machine. **C** A few example construction sheets of the stamp holders.