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Aligned Microcontact Printing of Biomolecules on Microelectronic Device Surfaces

Lars Lauer, Sven Ingebrandt, Martin Scholl, and Andreas Offenhäusser*

***Abstract*—Microcontact printing (μCP) of extracellular matrix proteins is a fascinating approach to control cell positioning and outgrowth, which is essential in the development of applications ranging from cellular biosensors to tissue engineering. Microelectronic devices can be used to detect the activity from a large number of recording sites over the long term. However, signals from cells can only be recorded at small sensitive spots. In this paper, we present an innovative setup to perform aligned μCP of extracellular matrix proteins on microelectronic devices in order to guide the growth of electrogenic cells specifically to these sensitive spots. Our system is based on the combination of a fine-placer with redesigned micro stamps having a rigid glass cylinder as backbone for attachment in the alignment tool. Alignment is performed moving the device with an optical table under microscopic control of the superimposed images from stamp and device surface. After successful alignment, the stamp is brought into contact with the device surface by means of a high-precision lever. With our setup, we were able to pattern up to 40 devices per hour. A lateral alignment accuracy of $< 2\mu$ m has been achieved. Aligned neuronal growth on patterned devices was demonstrated with dissociated hippocampal neurons.**

***Index Terms*—Alignment, extra cellular matrix, extracellular recording, field effect transistors, microcontact printing (μCP), microelectrodes, microelectronic devices, neuronal networks, 3D-BioMEMS.**

I. Introduction

Since the technique of microcontact printing has been established by the group of Whitesides in 1994 [1], it has been applied in various studies to influence cellular growth *in vitro*. When proteins of the extra cellular matrix, such as fibronectin or laminin, are transferred to a cell-repulsive substrate surface with a micro stamp, cells will preferentially adhere to the patterned areas. Shape and dimension of the transferred protein pattern will determine cellular shape and function [2]–[4]. Recent studies, evaluating the impact of geometrical parameters on neuronal growth, have shown that it is possible to precisely control the position of individual cell bodies and the outgrowth of the neurites [5]–[7]. Thus, microcontact printing allows to form artificially designed neuronal networks *in vitro*, when appropriate structural dimensions of the pattern geometry are chosen.

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Fig. 1. FET-based microelectronic device. The sensitive silicon chip is mounted on a zero-force socket. A glass ring glued to the socket forms a petri dish for cell-culture on the device surface.

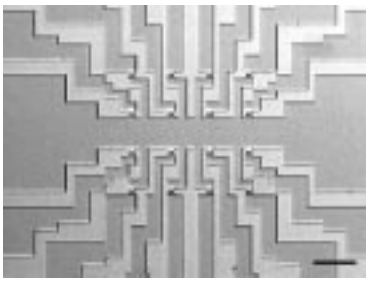

Fig. 2. Micrograph of the sensitive FET surface. The scale bar represents 200 μm.

This technique may be extremely useful in combination with planar microelectronic devices for extracellular signal recording. As those devices are designed for long-term simultaneous signal recordings from multiple cells, analysis of dynamic changes over time within the network is possible. Microelectronic recording devices based on arrays of field effect transistors (FETs) [8]–[11] and electrodes [12]–[14] have been developed and characterized in the recent years. Electrode-based systems with up to 64 recording channels are commercially available (e.g., Multi Channel Systems MCS GmbH, Germany) and a strong demand for these systems is raised in various fields of biomedical research.

A key challenge for the transfer of microcontact printed networks to such recording systems though, remains the alignment between cells and recording site. Only if the neurons are precisely placed on the sensitive spots of the device surface, recordings are possible.

There have been successful attempts to achieve alignment with mask aligner systems [15] and comparable custom-made setups [16]. However, these approaches are restricted to planar substrates and require special equipment, which usually is not available in most bio-labs.

In order to make the capabilities of aligned microcontact printing available for a wider set of applications and a larger group of scientists, we have designed an innovative solution for fast and precise alignment between microelectronic device and micro stamp. Our setup is based on inexpensive and commercially available equipment from the semiconductor industry. The whole system fits into a clean bench, thus, substrates can be kept sterile during the patterning process. A further application of the setup could be the aligned transfer of protein patterns to any three-dimensional microstructure such as BioMEMS.

II. Materials and Methods

The described alignment setup has been designed for application on two different types of microelectronic recording devices available in our lab. Type 1 is based on an array of FET (Fig. 1) with an accessible circular surface area of $\sim$3 mm in diameter. The sensitive gates of the FET on the sensor surface are arranged in a 200 $\times$ 200 μm^2 grid (Fig. 2). Type 2 utilizes gold micro electrodes (Fig. 3) with an acces-

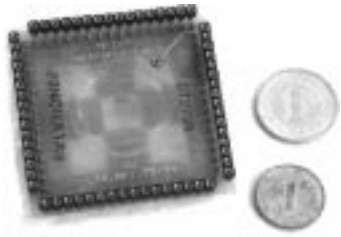

Fig. 3. Electrode-based microelectronic device. Microelectrodes on a transparent glass chip are used for signal recordings. The glass chip is attached to a printed circuit board by backside contacts. A glass ring forms a petri dish on the board.

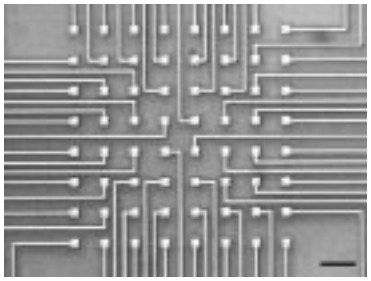

Fig. 4. Micrograph of a microelectrode chip surface. The scale bar represents 200 μm.

sible circular surface area of $\sim$5 mm in diameter. The electrodes are arranged in a 200 $\times$ 200 μm^2 grid (Fig. 4).

For the alignment of micro stamp and microelectronic device, we are using a modified positioning unit from the semiconductor industry[1] (Fineplacer-145 "PICO," Finetech electronics, Germany). Fig. 5 shows the general idea of the positioning process. The micro stamp S is attached to an arm A which can be moved from an upright position (first picture) to the surface of the microelectronic device D (second picture). Alignment is performed in the upright position of arm A under optical control with the microscope M. For this purpose, the images of the device surface D and the micro stamp surface S are superimposed by the beam dividing prism P. When substrate and stamp are aligned, patterning is performed by moving stamp S with arm A to the device D. After a few seconds of contact time, arm A is lifted up again. Both substrate and stamp are released and replaced by the next set of stamp and substrate.

Crucial for a high alignment accuracy in the μm range are the precision of the joint of arm A and a tight fit of micro stamp S to arm A. The joint of the applied system has a certified accuracy of $<$5-μm lateral deviation.[2] To be able to combine the requirement of a tight fit of micro stamp S into arm A, with the necessity to structure a substrate surface of only 2-mm diameter, a hybrid micro stamp design was created. This design, together with the tools required for stamp production and handling is described in the following sections.

A. Design of Micro Stamps and Alignment Setup

With micro stamps fabricated from pure polydimethylsiloxane (PDMS) material, alignment is not possible under the above described conditions. Due to the gravitational forces on the mounted micro stamp S, a long and flexible cylindrical stamp with small diameter is deformed between upright position and bent position of arm A. This, of course, will destroy the alignment. We have solved this problem

[1]This unit is usually used to position electronic devices to printed circuit boards.

[2]In our experiments, we found the deviation to be even smaller in the range of $<$2 μm.

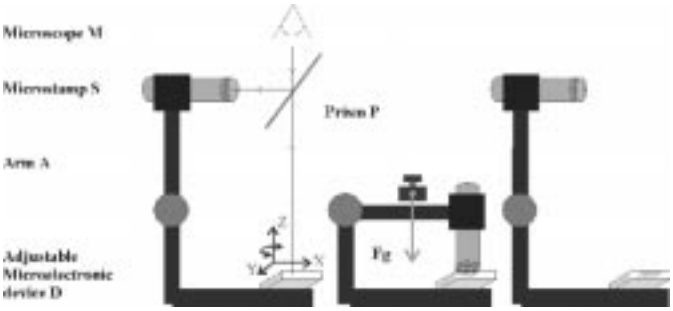

Fig. 5. Schematic sketch of our setup for aligned microcontact printing. Microstamp S and microeletronic device D are aligned under microscopic control through microscope M and prism P. For patterning, arm A is bent to the substrate surface. The patterning force Fg is controlled by a weight on the bendable arm A.

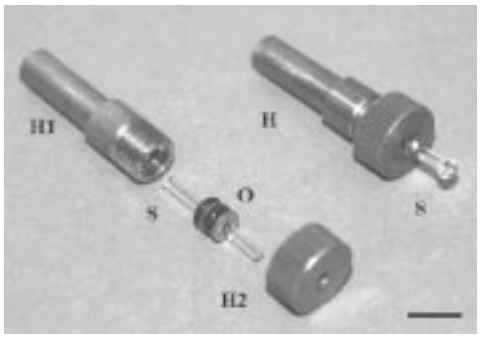

Fig. 6. Photograph of a hybridic microctamp and its holder. H: Holder (H1: socket, H2: cap), S: Microstamp, O: O-rings used to center the stamp in the holder. Scale bar equals 1 cm.

Fig. 7. Photograph of the complete setup in alignment position. S: Stamp in holder, A: bendable arm, P: Prism, D: Microelectronic device attached to positioning table. OT: Optical table for device positioning.

with a hybrid design of our micro stamps, combining the advantages of thin film micro stamps [17] with the easy handling of macroscopic stamps. Each stamp has a 2-cm-long and 2-mm-thick glass cylinder as a rigid backbone. On one tip of this backbone a drop of PDMS is applied which can be structured by curing on a master stamp mould. Fig. 6 shows, how such a hybrid micro stamp fits tightly into a special high-precision holder H. The glass cylinder of the micro stamp S is centered with two O-rings O into the holder socket H1. When cap H2 is screwed onto socket H1, both O-rings are squeezed between socket wall and glass cylinder. This results in a tight-fit of the glass cylinder in the holder.

Figs. 7 and 8 show photographs of the complete setup. The micro stamp S is mounted at the bendable arm A of the alignment tool. The microelectronic device D is attached to an adjustable optical table OT (MVT 40B and WVT 40, Owis, Germany) which can be moved in

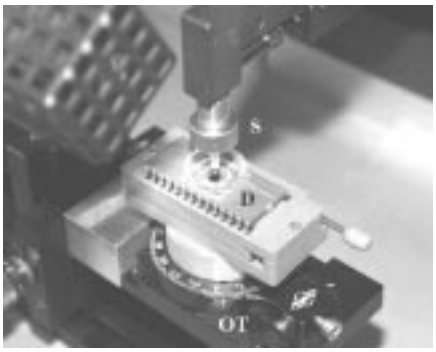

Fig. 8. Photograph of the stamp and substrate in patterning position. S: Stamp in holder, D: Microelectronic device attached to positioning table, OT: Optical table for device positioning.

x, y, and z direction and rotated around the z axis with micrometer screws. Alignment is achieved in the configuration shown in Fig. 7, by observation of stamp S and substrate D through prism P. Patterning is performed by bending arm A to the device as shown in Fig. 8.

B. Stamp Production

The structures designed for our experiments were transposed to a chrome mask by an electron beam writer. Applying UV-photolithography, master stamps were produced out of spin coated 12.5-μm-thick photo resist layers (AZ 4562, Clariant GmbH, Germany) on 0.6-mm-thick silicon wafers (MEMC Electronic Materials, Germany). Photo structuring was performed by UV-irradiation in contact mode. A subsequent hard bake at 110 °C for 90 min was applied to strengthen the photo resist structure and to smooth the edges.

Hybrid PDMS-glass micro stamps were then fabricated by curing drops of PDMS (Polydimethylsiloxane, Sylgard 182, Dow Corning, Germany) on the tips of glass cylinders in a special molding tool. The individual steps of this process are described in the following section:

First, a glass cylinder was sorted out for the molding process and cleaned with acetone. Depending on the surface area to be structured, glass cylinders with different surface areas were used. Glass cylinders for basic stamps with 2-mm stamp diameter were produced by cutting glass cylinders of 2-mm diameter to a length of 2 cm. Second, a master stamp with the desired structure was chosen. To prevent sticking of PDMS on the master stamp, its surface was treated for 1 h with 200 mM sodium dodecyl sulfate solution (SDS 71729, Fluka, Germany). Third, a drop of PDMS was picked up with the tip of the glass cylinder from a $\sim$0.25-mm-thick layer of PDMS provided on a glass cover slip. As shown in Fig. 9, the glass cylinder S was then placed with the uncoated end into a hole in the molding tool MT. The master stamp MS was placed upside down onto the molding tool T. By means of the adjustment screw AS on the backside of the molding tool, the glass cylinder was pushed up to the master stamp MS, until the PDMS drop was in contact with the mould on the full diameter of the glass cylinder tip. In this configuration, the whole setup was placed into an oven for curing during 24 h at 60 °C. A second curing step at higher temperature (110 °C, 1h) was performed after the removal of the master stamp.

C. Patterning

In order to increase the stamp hydrophilicity, PDMS stamps were stored for at least 24 h in deionized water. Just before patterning, stamps were taken out of the water and sterilized in a 70% ethanol bath for 1 min. Inking took place for 30 s in 25 μg/ml ($\sim$0.25 μM) Laminin[3] (1 243 217, Boehringer Mannheim GmbH, Germany) dissolved in PBS. The inked stamp was then dried in a soft nitrogen stream and

[3]Laminin from EHS-sarcoma (mouse), sterile.

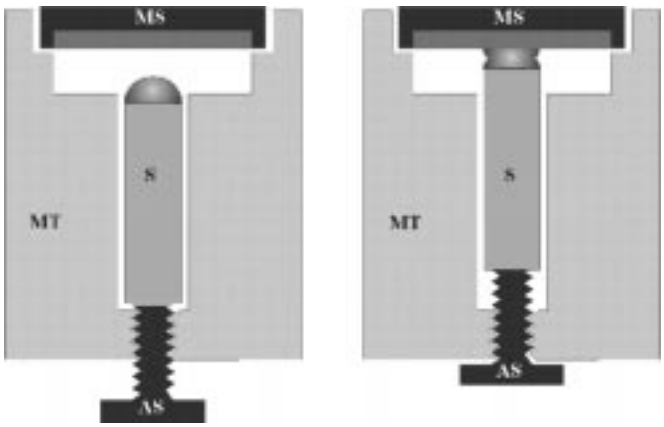

Fig. 9. Production of hybridic microstamps in a special moulding tool. MT: Moulding tool, S: hybridic microstamp, MS: Master stamp, AS: Adjustment screw.

mounted in the above described stamp holder with tweezers. After successful alignment, the stamp was pressed to the device with a force[4] of 50 g/cm^2 for 10 s [18], [19]. Initial calibration of the alignment setup was performed by aligning a visible pattern on the substrate surface to the shape of the relifted micro stamp with the adjustment screws of the beam dividing prism.

D. Primary Cell Culture

For experiments with living neuronal cells, dissociated primary hippocampal neurons have been cultured as described previously [6]. In brief, rat hippocampi were dissected from E18 sprague dawley rats. The isolated tissue was incubated in 0.25 % trypsin (Sigma) dissolved in $Ca^{2+}-$ and $Mg^{2+}-$ free Hank's balanced salt solution (HBSS, Gibco) for 8 min at 37 °C. After trypsination, the tissue was rinsed five times for 5 min in 2 ml plating medium (Neurobasal medium with B27 supplement, Gibco). Dissociation of cell aggregates into single cells was supported by pushing the cell dispersion though a fire polished pipette. Cells were plated at low densities between 15 000 and 30 000 cells/cm^2 and incubated at 37 °C in 5% CO_2 enriched atmosphere at saturated humidity. In an additional washing step after 60 min incubation time, unattached cells were removed from the substrate surface.

III. Results and Discussion

We were able to achieve a reproducible alignment accuracy of $<$2-μm lateral deviation between the stamp structure and the sensor spots on a microelectronic recording device. This accuracy is only achieved, when the setup is calibrated. Recalibration is required prior to each experiment to account for thermal deadjustment of the beam dividing prism. Fig. 10 shows an exemplary phase contrast micrograph of a micoelectrode device surface, structured with laminin in a grid structure of 20-μm-wide nodes and 2-μm-wide lines. One can clearly distinguish the protein structure on the electrode array of the sensor device.

After some practice, up to 40 devices could be patterned in only one hour. This method opens an efficient way to combine the method of microcontact printing with extracellular recording techniques. We believe, that the strength of our approach lies in the simplicity of the setup. It should be valuable especially for biological laboratories with only limited technical equipment. The entire setup can be assembled in one to two weeks. The costs of the required equipment in total is ca. 8000 EUR, including stereo microscope and light source.

[4]Force control was achieved with a calibrated counterweight on the bendable arm of the setup.

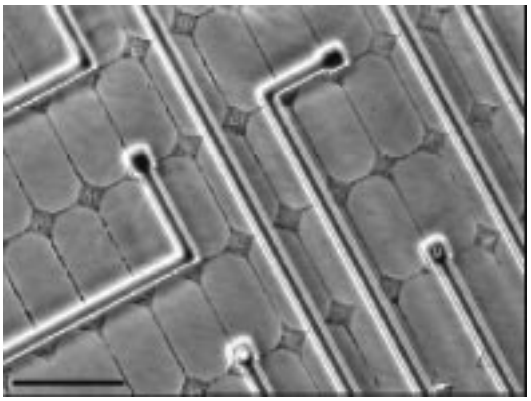

Fig. 10. Phase contrast micrograph of a laminin grid pattern (mesh width: 100 $\times$ 50 μm^2; line size: 2 μm; and node size: 20 μm) aligned to the microelectrodes of a microelectronic recording device. Scale bar equals 100 μm.

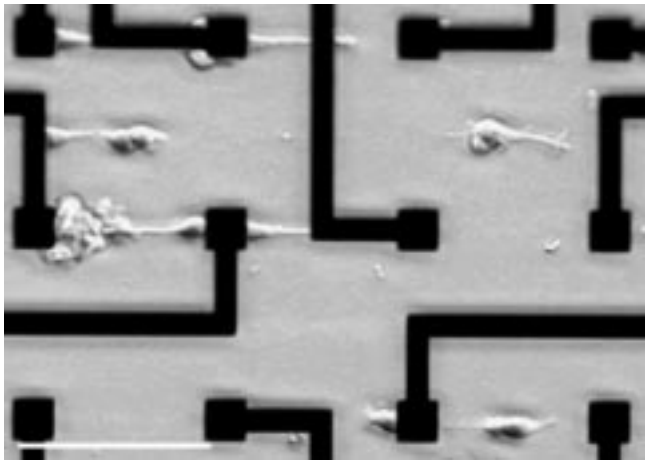

Fig. 11. Primary hippocampal neurons from E18 sprague dawley rats cultured on laminin patterned microelectronic recording devices. Micrographs taken at day 2 *in vitro* with an inverted microscope (Olympus IX 50). For maximal nodal focus, a pattern geometry of gap-interrupted lines (line size: 4 μm; node size: 20 μm; gap size: 10 μm; and gap distance: 100 μm) has been applied. Scale bars equal 100 μm.

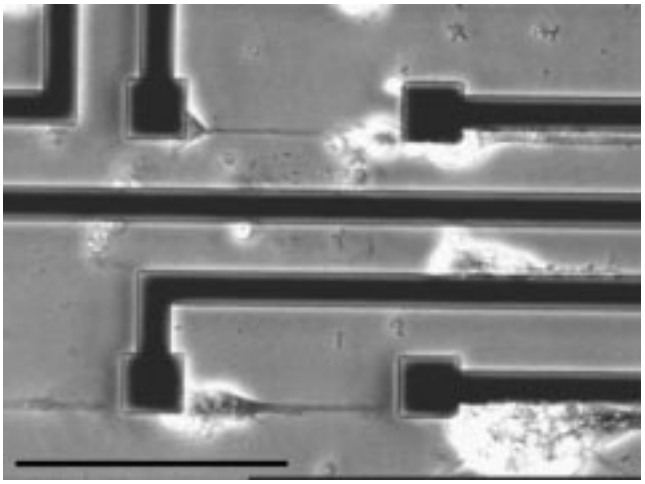

Fig. 12. Primary hippocampal neurons from E18 sprague dawley rats cultured on laminin patterned microelectronic recording devices. Micrographs taken at day 2 *in vitro* with an inverted microscope (Olympus IX 50). For maximal nodal focus, a pattern geometry of gap-interrupted lines (line size: 4 μm; node size: 20 μm; gap size: 10 μm; and gap distance: 100 μm) has been applied. Scale bars equal 100 μm.

In experiments with primary hippocampal neurons, prepared from E18 sprague dawley as described above, we were able to evoke aligned and structured neuronal growth on patterned device surfaces. A gap interrupted line structure (4-μm line width, 20-μm node size, 10-μm gap size; nodes and gaps in distances of 100 μm, lines separated by 50 μm) was applied in the experiments. This structure geometry had been chosen, according to previously published results [7], in order to achieve maximal compliance of cell bodies to the nodes of the pattern. In Figs. 11 and 12, phase contrast micrographs of the cultures at day 2 *in vitro* are shown. In Fig. 11, one can clearly distinguish individual neuronal cells growing along the patterned lines. Although a perfect observation through the substrate is blocked at the electrode spots (pictures taken with an inverted microscope), one can clearly distinguish the shape of neuronal cell bodies growing on top of the electrodes in Fig. 12. The presented technique of aligned microcontact printing, hence is a powerful approach for controlling neuronal growth on extracellular recording devices. It could be successfully shown, that neuronal cells can be effectively driven to grow preferentially on the sensitive spots of an electrode chip.

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