Molding Microchannel and Brain Implant Scaffolds from Microstructured Double Layer Photo Resin Master Casts
Concepts and Examples

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1 OBJECTIVES

Replica-casting finds wide application in soft lithography (Qin et al., 2010) and microfluidics (Bettinger et al., 2010). Most commonly, structures are molded with micro- to nano-patterned photoresists as master casts into polydimethylsiloxane (PDMS). PDMS features many favorable properties. It reproduces geometric details with nanometer fidelity, has low cytotoxicity, is transparent in the visible spectrum, biostable both *in vitro* and *in vivo*, can be plasma-bonded to itself, has low water permeability and is simple to handle and process. After curing, the PDMS can be easily peeled from the master and the latter usually be reused (Anderson et al., 2000) if patterns are not undercut (Yun et al., 2008). Here, we demonstrate a straightforward replica-molding process that can be exploited for the generation of perforated microchannel scaffolds for the *in vitro* use in axonal guidance and regeneration studies on microelectrode arrays (MEAs) or the production of tissue-conformal *in vivo* MEAs for neuroprosthetic applications, respectively.

2 METHODS

Bi-level casting patterns in high-aspect ratio negative photoresist *(e.g.*, SU-8) to generate microchannels (lower level) and perforating vias (second level) are made by standard photolithography. A first mask defines all features, a second mask just the through holes. Figure 1 summarizes the following fabrication steps: (1) A clean silicon wafer is spin-coated (2) with a 1st negative photo resin layer (<50 µm) and (3) UV photo-crosslinked through a first photomask to define both channels and sockets for vias. The procedure is repeated in (4) and (5) for a 2nd photoresist layer (< 150 µm) to define the via through-holes. After removing uncured photoresin (6), the bi-level microstructure (7) can be coated with PDMS pre-polymer (8), cured after its leveling, and peeled to result in a microchannel scaffold with via holes (9).

Such microchannels can either be used as physical guidance cues for axons and dendrites or be filled with conductive polymers (*e.g.*, PEDOT:PSS, carbon-polymer composites) (10) and backside-insulated (11) to yield MEAs with electrodes and contact pads at the via holes for *in vitro* and *in vivo* application (12).

3 RESULTS

Figure 2 depicts a conceptual cartoon of a microchannel tile with via holes for cell seeding (somata) and guidance channels for axonal elongation into target wells (axons). If the substrate is a MEA, electrodes can extracellularly record action potentials (Figure 3). This allows for studying neural development and axonal regeneration *e.g.*, after inflicting injury by laser microdissection (Difato et al., 2011).

Furthermore, micro-channels will help in gaining a better understanding of the electrode characteristics and underlying biophysics of signal generation and spread.

If instead microchannels and through-holes are filled with a biocompatible electrical conductor, MEAs can be inexpensively produced for both *in vitro* and *in vivo* application (Figure 4).

The flexibility of PDMS allows for the fabrication of highly tissue-conformal devices with softness that matches that of brain tissue. This promises the reduction of implant-inflicted tissue damage and the increase of MEA long-term stability.

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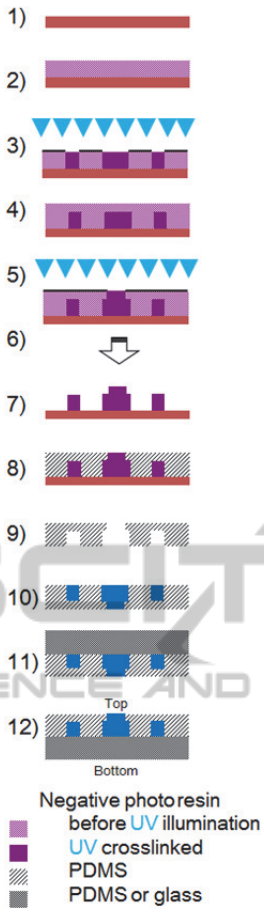

Figure 1: Process flow of master generation and microchannel replica molding in PDMS therefrom (9). The scaffold can either be used directly in neural guidance studies or be functionalized *e.g.,* with conductive polymers (10-12).

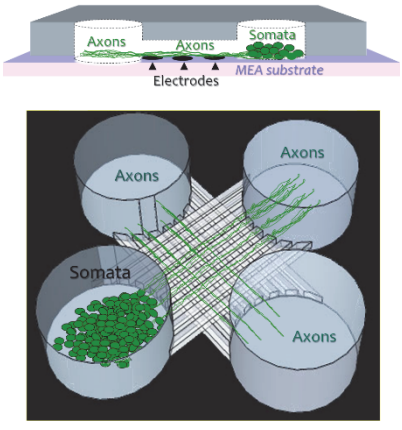

Figure 2: PDMS microstructure including 4 big reservoirs interconnected by an 8 × 8 matrix of channels. Channel crossing points match the electrodes grid.

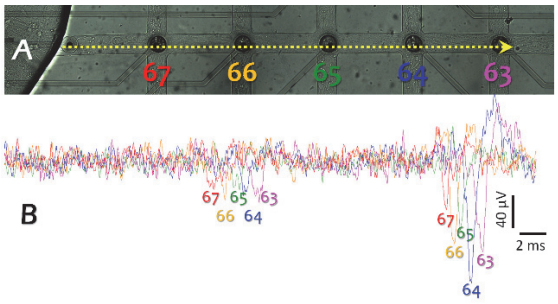

Figure 3: (A) Axons of rat cortical neurons in the left well grow through a 40 µm wide microchannel thereby passing over five Ø 30 µm electrodes with 200 µm pitch along their way (Multi Channel Systems, Germany). (B) Despite the slightly varying signal shapes, a signal delay of less than 2 ms suggests that activity was recorded from the very same axon.

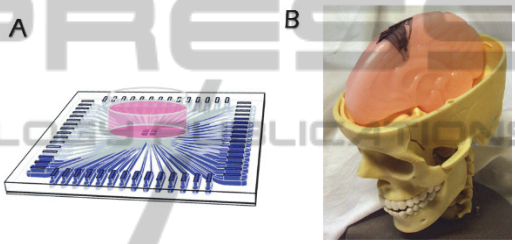

Figure 4: (A) Cartoon of an 5x5 cm^2 *in vitro* polymer MEA with conductive microchannels after their filling with conductive polymers. An 8x8 matrix of ~Ø 80-120 µm through-holes at the center allows for the extracellular recording and stimulation of action potentials from neurons or cardiomyocytes in *in vitro* studies. (B) Mockup of a 40 µm thin *in vivo* MEA with conductive polymer pads, tracks and electrodes to demonstrate high tissue conformity.

4 DISCUSSION

We exemplarily demonstrated that the replica-fabrication of bi-level PDMS microchannel devices allows for their use in different contexts. Besides its cost-efficiency and ease of handling, the main advantage of this strategy is the automatic formation of through-holes (perforations) without further post-processing steps (*e.g.,* manual punching). These through holes permit the localized placing of cells in microchannel tiles for *in vitro* neurite guidance studies. However, microchannel scaffolds can also be functionalized by filling channels and vias with electroconductive material, thereby turning them into MEAs. If such conductive material forms thin films only, a channel feature may still serve for the delivery of anti-inflammatory or neuroactive drugs. The concept furthermore leaves a wide degree of

freedom in choosing scaffold materials with tissue-matching stiffness.

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