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Polymer-Based Shaft Microelectrodes with Optical and Fluidic Capabilities as a Tool for Optogenetics

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Abstract**—In this work, we describe the fabrication of a polymer-based shaft electrode which can conduct light as well as fluids to a target brain region and record electrical neural signals from the same tissue volume simultaneously. This multifunctional neural probe is intended to facilitate optogenetic in vivo experiments.**

I. INTRODUCTION

BESIDES electrical recording and stimulation of brain tissue, in recent years, two modalities turned out to be particularly interesting. For the promising new research field of optogenetics [1], fluidic as well as optical contact to nervous tissue is necessary. With fluid, a gene delivery vector is applied to the brain area of interest. Thereby the neurons in this area are genetically modified in a way that they can be stimulated or inhibited via light with a wavelength of 473 nm and 593 nm, respectively. In this work, we describe a microimplant that combines these modalities with standard electrical recording, to facilitate optogenetic in vivo experiments. Combining these three modalities, optogenetic research can be conducted with a single electrode shaft which can be chronically implanted into freely moving mice. It comprises a microfluidic channel to apply the gene delivery vector, an optical waveguide to transmit light, and microelectrodes to record neural activity from the brain region of interest.

The implant concept is shown in Fig. 1. It comprises a polyimide-based shaft including nine platinum electrode sites with a diameter of 30 µm. The thin-film layer composition is identical to the foil described in [2] with a platinum thin-film sandwiched between two polyimide (PI) layers. Within the PI foil, the electrode sites are connected via conductor paths on a flexible cable to solder pads designed to contact SMD connectors (Omnetics Connector Corp., Minneapolis, MN, USA). A SU-8 (MicroChem Corp., Newton, MA, USA) waveguide is placed on top of the PI shaft separated from the PI by a 200 nm thick tungsten-titanium layer. This metal cladding is needed to prevent the light from coupling into the PI substrate which has a higher refractive index than the SU-8. The waveguide is covered with a sputtered gold layer, thus, it can be glued into the optical adapter without light coupling into the epoxy glue. The waveguide ends in front of the electrode sites. Thus, neurons which are stimulated by the light exiting the waveguide will be closest to the electrode sites for recording. The channel is implemented by attaching a U-profile with an outer cross-section of 190 µm by 65 µm, an inner cross-section of 50 µm by 45 µm and a length of 7 mm to the rear side of the PI shaft. The U-profile was made of SU-8 and was processed on a separate wafer. The channel outlet at the tip of the shaft is a

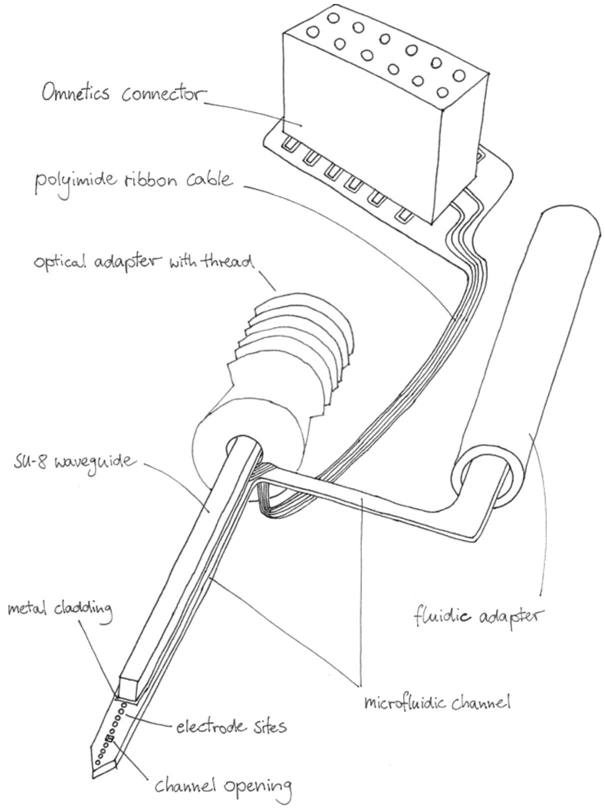

Fig. 1. Schematic of the concept of a multimodal polymer shaft electrode comprising electrical, optical and fluidic modalities.

hole in the PI between two electrode sites. This means that the fluid can be applied to the same tissue volume which is also electrically and optically contacted.

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II. MATERIALS AND METHODS

A. MEMS Processing

A 5 µm thick layer of PI (U-Varnish-S, UBE, Tokyo, Japan) was spin coated on a silicon wafer (Fig. 2). Platinum was sputter deposited in a 300 nm thick layer and structured by lift-off to define electrode sites, tracks, and solder pads. A second layer of PI was spin coated onto the structured metal. A 200 nm thick layer of tungsten-titanium alloy (10 % titanium) was sputtered and structured by lift-off to serve as the cladding layer between the SU-8 waveguide and the PI. The electrode and solder pad openings as well as the perimeters of the shaft were opened by an O_2-plasma etch (80 W, 30 mTorr; RIE Multiplex, STS, Newport, UK). The waveguides were formed by spin-coating SU-8 3050 obtaining a layer with a thickness of 150 µm which was structured by UV light in a mask-aligner to define the waveguides. To polymerize the material, a post exposure bake was conducted on a hotplate at 65 °C for 5 h. After developing the SU-8 structures, the complete wafer was covered with a 200 nm thick sputtered gold layer to complete the metal cladding of the waveguides.

SU-8 3025 was spin coated and structured by UV light to form a 20 µm thick channel cover, while the walls (height of 45 µm) were made from SU-8 3050.

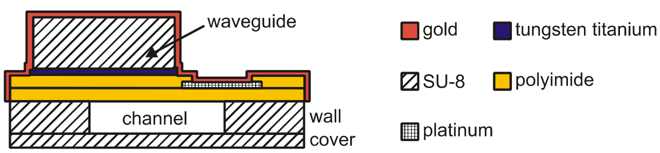

Fig. 2. Cross-section of the processed shaft with the SU-8 U-profile glued to the backside forming a channel structure.

B. Hybrid Assembly

The PI shafts as well as the SU-8 channels were pulled off the wafers using tweezers. Epoxy (UHU plus endfest 300, UHU GmbH, Bühl, Germany) was applied to the channel walls using a tapered tungsten wire. After attaching and aligning the channel to the rear side of the shaft, the epoxy was cured at 70 °C on a hotplate for 15 min. The channel inlet was glued to a metal tube with an inner diameter of 410 µm (blunt end stainless steel tips: I&J FISNAR, Fair Lawn, NJ, USA) which can be connected to the tubing of the pump during the experiment. The waveguide was glued into a custom made optical connector. This connector was designed to be chronically attached to the skull and can be connected to a standard optical multimode silica fiber (diameter 200 µm) during recording sessions.

The tip of the shafts with the electrode sites and the waveguide front end as well as the solder pads for the Omnetics connectors were opened by dipping these parts into potassium iodide/iodine solution for 2.5 min. Subsequently, a silver wire ground electrode and the connector were both SMD soldered to the platinum solder pads.

C. Fluidic and Optical Characterization

The microchannel was attached to a pressure sensor (RBIP-015DB, Sensortechnics GmbH, Puchheim, Germany) which was connected to a flow rate sensor (ASL 1430, Sensirion, Staefa, Switzerland). Water was supplied by connecting the flow rate sensor to a water reservoir. The fluid pressure was controlled by connecting the reservoir to an adjustable air pressure supply.

With the same process as described in paragraph IIA, test waveguides were fabricated on a PI substrate with a cross-section of 150 µm by 150 µm and a length of 6.5 cm. The cleaved side of an optical fiber (one cleaved end and one end with FC connector, 62.5 µm core diameter, graded index, multimode patch cable, Thorlabs, Newton, NJ, USA) and the waveguide were aligned in a way that the front ends face each other. UV light-curing optical glue (Norland Optical Adhesive 81, Norland Products Inc., Cranbury, NJ, USA) was applied at the interface to fix the interface in the position of optimal optic transmission.

Waveguides were characterized using the cutback method. Laser light with a wavelength of 473 nm and 593 nm respectively was coupled into the waveguide by connecting the optical fiber glued to it to the respective laser light source. Light leaving the waveguide was measured while the waveguide was cut by 2.5 mm at a time to obtain the waveguide's attenuation. Light power was measured with an optical multimeter.

III. RESULTS

The multimodal shaft electrode could be assembled as described. Although the application of the epoxy glue to the channel walls and the attachment to the electrode shaft requires very steady hands, the yield of functional channels was about 100 %. Fig. 3 shows an assembled device with an adapter for each modality connected to the MEMS-processed PI shaft. Fig. 4 depicts a close-up of the shaft's tip with the channel, channel opening, waveguide and electrode sites. Measured in phosphate buffered saline, the impedance

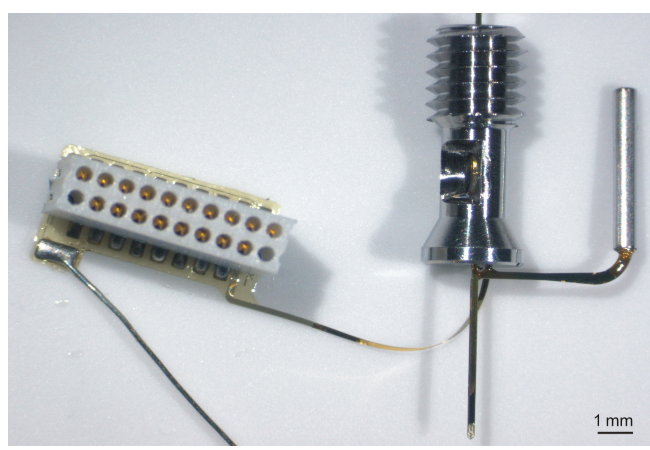

Fig. 3. Picture of a fully assembled device showing the soldered connector, the polyimide ribbon cable, the shaft with the optical adapter and the channel with the fluidic adapter

magnitude of the electrode sites at 1 kHz was about 280 kΩ to 350 kΩ with an access resistance of about 20 kΩ and a cutoff frequency of about 4 kHz.

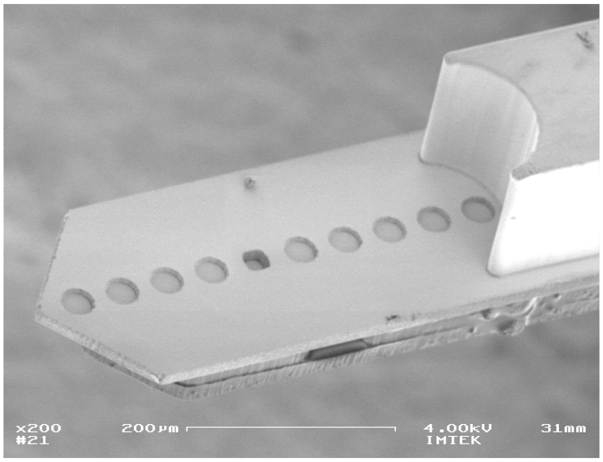

Fig. 4. Scanning electron micrograph depicting the shaft's tip with 9 electrode sites and one channel opening between them as well as a second channel opening in the SU-8 wall at the side of the channel and the waveguide at the right side of the picture

A. Fluidic Channel

The flow rate was measured as a function of the pressure over the length of the microchannel (Fig. 5, circles). The Hagen-Poiseuille equation was used to calculate the theoretical flow values (Fig. 5, straight line) for a rectangular channel cross-section. (dynamic viscosity of water at 25 °C: $\eta = 8.904 \cdot 10^{-4}$ Pa·s).

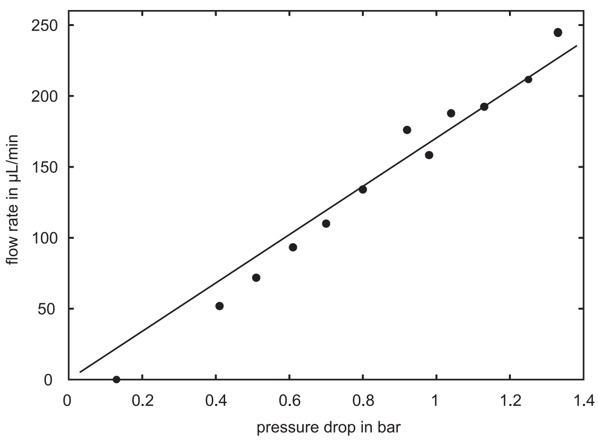

Fig. 5. The flow rate as a function of fluid pressure; the circles show the measured values, the straight line represents the theoretical values calculated with the Hagen-Poiseuille equation for rectangular cross-sections with a width of 50 µm, a height of 45 µm and a length of 7 mm.

For the application of the gene delivery vector in mice, a pressure of 1.38 bar is used which corresponds to a flow rate of about 240 µL/min (Fig. 5). Thus, it would be possible to deliver the required amount of fluid of 500 nL to the target tissue in 125 ms.

B. Optical Waveguide

Fig. 6 depicts the transmitted light power as a function of the waveguide length. The measurement for both wavelengths was conducted with the same waveguide. The input power of the waveguide was 21 mW (at 473 nm) and 14 mW (at 593 nm). The background power of the room's illumination was measured during every measurement step and was found to be about 45 µW at 593 nm and 98 µW at 473 nm. The values in Fig. 6 were adjusted by subtracting the respective background power from the measured values. By using a linear fit (Fig. 6, straight lines), the transmission loss could be obtained as the slope of the linear fit with -6.4 dB/cm (473 nm) and -1.5 dB/cm (593 nm). The coupling loss between the optical fiber and the waveguide was estimated by the ordinate intercept of the linear fit which was -3.4 dB (473 nm) and -3.8 dB (593 nm). From this data, the output power of a 15 mm long waveguide was 1.35 mW (-12 dB) at 473 nm and 3.6 mW (-6 dB) at 593 nm.

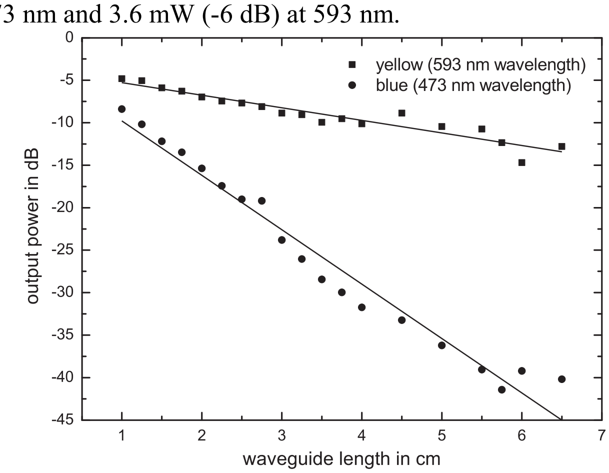

Fig. 6. The waveguide's output power as a function of waveguide length measured with yellow and blue light

The 15 mm long waveguides of fully assembled multimodal shafts were connected to an optical fiber via the optical adapter. For less coupling losses, the waveguide face at the optic adapter was polished (Aluminum Oxide Lapping Sheets, Thorlabs, Newton, NJ, USA) and oil was applied between the faces of the two light guiding structures. At the wavelength of 473 nm, the output power of the waveguides was 1 to 2 mW while the output power of the optical fiber was 35 mW. Thus, the loss of light power due to the coupling loss between optical fiber and waveguide and the attenuation within the waveguide accounted for between 15.4 dB to 12.4 dB.

C. In Vivo

Two mice (male C57BL6/J; 2-3 months old; Harlan Ltd) were anaesthetized with isoflurane (induction 5%, maintenance 2.5%) in O_2. Body temperature was maintained with a heating pad (CMA/150, CMA/Microdialysis). Mice were secured in a stereotaxic frame and unilaterally implanted in the amygdala with a multimodal shaft electrode aimed at the following coordinates: 1.7 mm posterior to bregma, ±3.3 mm lateral to midline, and 4.4 mm deep from the cortical surface. The electrodes were lowered with a hydraulic pump at a speed of 10 µm/s. The implant was secured using skull screws, cyanoacrylate adhesive gel and dental cement. Systemic (Metacam) and local (Naropin) analgesia was applied before and after the surgery. Animals were allowed to recover for 10 days after surgery. All animal procedures were executed in accordance with institutional guidelines and were approved by the Veterinary Department of the Canton of Basel-Stadt.

Initial testing of the device took place, wherein electrodes were connected to the recording and stimulation setup via the electrical connector, the optical and the fluidic adapter. Light and fluid could be applied to the brain tissue but no neural signals could be recorded.

IV. Discussion

The concept of processing shaft and U-profile on different wafers results in two independent microfabrication processes, allowing fabrication of the U-profile from a biodegradable material for example. However, the manual attachment of the U-profile to the PI shaft was very time consuming compared to a batch process on wafer level. Nonetheless, the fabrication was reproducible and resulted in a high yield of functional devices. Manual assembly is a particular problem for multimodal electrodes, which has yet to be fully addressed. Each modality had to be separately connected to the macroscopic world of recording systems, light sources and fluid pumps. Each new modality adds a further manual assembly step after the batch processing.

The SU-8 channels were found to be relatively brittle and tended to break during the connection to the tube of the pump system. Thus, in a future approach, it might be favorable to also use PI as the channel material which has a larger tensile strength and elongation at break than SU-8. SU-8 was chosen for its ability to be easily processed with thick layers and high aspect ratios. A highly viscous, photodefinable PI could be a good candidate for a new channel approach.

In the optical waveguides, the attenuation of blue light (-6.4 dB/cm) was higher than of yellow light (-1.5 dB/cm). This corresponds to the literature showing that the attenuation increases with decreasing wavelength of the light [3]. The measured attenuation of the presented waveguides was in the range of values which could be found in the literature (-1 dB to -5 dB for cross-sections of about 50 µm by 50 µm and wavelengths above 600 nm [3]).

Cho *et al.* [4] were the first to fabricate a device with an on wafer level integrated waveguide followed by Zorzos *et al.* [5]. All other publications described the hybrid integration of a standard optical fiber [6-8]. The hybrid integration of an optical fiber into the electrode shaft has the advantage of an implanted light guide with very low attenuation. The drawback of the hybrid assembly is the poor reproducibility of the hand-made approach. The MEMS-integrated polymer waveguide has a higher light attenuation but comprises very defined and reproducible dimensions e.g. the distance between the waveguide end face and the electrode sites. With MEMS-integration the size of the shaft can be reduced.

The device developed in this work had an integrated optical waveguide which was directly assembled to an optical adapter. This adapter was developed to be chronically implanted. Thus, the complete device fit on the mouse’s head and was lightweight enough to be chronically implanted.

V. Conclusion

A microelectrode with the ability to transfer fluids, light and electrical signals in a chronic implantation would be a major step towards the application of optogenetics in freely moving rodents. In this work, a microimplant was manufactured which could connect the target brain tissue via these three modalities. While the implementation of either fluidic channels [9-11] or optical waveguides [4,5,7,12] into neural implants was already described by other groups, the presented study is, to our knowledge, the first to combine all three modalities in a single polymer-based device.

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