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Directing neuronal cell growth on implant material surfaces by microstructuring

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Abstract: For best hearing sensation, electrodes of auditory prosthesis must have an optimal electrical contact to the respective neuronal cells. To improve the electrode–nerve interface, microstructuring of implant surfaces could guide neuronal cells toward the electrode contact. To this end, femto-second laser ablation was used to generate linear microgrooves on the two currently relevant cochlear implant materials, silicone elastomer and platinum. Silicone surfaces were structured by two different methods, either directly, by laser ablation or indirectly, by imprinting using laser-microstructured molds. The influence of surface structuring on neurite outgrowth was investigated utilizing a neuronal-like cell line and primary auditory neurons. The pheochromocytoma cell line PC-12 and primary spiral ganglion cells were cultured on microstructured auditory implant materials. The orientation of neurite outgrowth relative to the microgrooves was

determined. Both cell types showed a preferred orientation in parallel to the microstructures on both, platinum and on molded silicone elastomer. Interestingly, microstructures generated by direct laser ablation of silicone did not influence the orientation of either cell type. This shows that differences in the manufacturing procedures can affect the ability of microstructured implant surfaces to guide the growth of neurites. This is of particular importance for clinical applications, since the molding technique represents a reproducible, economic, and commercially feasible manufacturing procedure for the microstructured silicone surfaces of medical implants. © 2012 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 00B: 000–000, 2012.

Key Words: surface microstructure, PC-12 cells, spiral ganglion neurons, cochlear implant electrode, guided neuronal outgrowth

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INTRODUCTION

In the inner ear, sound waves are converted into fluid pressure oscillations that induce movement of the basilar membrane. This movement is transferred to the mechanosensory hair cells of the organ of Corti. It consists of approximately 16,000 hair cells¹ located on the basilar membrane that amplify (outer hair cells, OHC) and convert mechanical movement into electrical signals (inner hair cells, IHC). These signals are generated by deflection of the hair bundles, opening the mechanically gated ion channels leading to depolarization of IHC. Consecutively, roughly 30,000 afferent nerve fibers are responsible for transmitting the electrical signals to peripheral auditory neurons, the spiral ganglion cells (SGCs).² The spiral ganglion harbors the primary neurons of the auditory pathway and is responsible for signal transmission to the central nervous system (CNS). Cochlear implants (CI) are the treat-

ment of choice for severe to profound sensorineural hearing loss based on damages of the IHC. By contrast, for treatment of retrocochlear localized damage, that is, of the auditory nerve or more centrally, auditory brainstem implants (ABI) or auditory midbrain implants (AMI) are available.

The CI are an inner ear prosthesis for the direct electrical stimulation of the auditory nerve.^{3–5} It consists of an array of maximal 22 platinum electrode contacts embedded in insulating silicone. This limited number of electrode contacts takes over the task of the IHC and the frequency discrimination is accordingly poor compared to the natural hearing sensation.^{6–8} For example, hearing perception of CI-treated patients is hindered in the event of multiple speakers or in order to perceive complex sounds such as music.⁹ To improve frequency discrimination, the number of electrode contacts should be increased. However, the

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FIGURE 1. Schematic drawing of the (a) gradient microstructure, (b) constant 5 μm microstructure, (c) microscopic overview: left unstructured control, right: laser structured silicone surface.

current technology does not allow increasing of the number of the electrodes, since this is associated with disadvantages concerning the thickness and the flexibility of the electrode. The elasticity of the electrode is determined by the number of the platinum wires as each single electrode contact is connected to a separate wire.³ Currently, the CI are inserted into the scala tympani. A bony wall separates the electrode and the SGCs, necessitating high electrical stimuli for sufficient neuronal activation.³ Additionally, the spread of the electrical field limits the locally focused stimulation. Also, unspecific fibrous tissue formation around the electrode increases the transitory resistance and interferes with an optimal nerve–electrode interaction.^{10,11} Therefore, inhibition of connective tissue growth and minimizing of the distance between neuronal cells and electrode are major goals in auditory implant related research.

Surface properties influence the implant–tissue interactions.^{12–14} In previous experiments, a reduced fibroblast growth on microstructured platinum surfaces was demonstrated.¹⁵ Further optimization of the electrode–neuron interface could be achieved by directing the orientation of neurons, especially in central auditory prosthesis with close contact to the neurons, such as AMI^{16,17} and ABI.¹⁸ Parallel microgrooves on surfaces of various materials had been shown to promote the alignment of neuronal cells,^{19–25} astrocytes,^{26,27} as well as fibroblasts.^{28,29} The preferred orientation of neuronal cells is parallel to the microgrooves. However, a few studies found perpendicular orientations.^{23,25} Therefore we compared the neuronal-like cell line PC-12^{30–32} with the primary SGCs isolated from the inner ear. Thus, surface structures were manufactured that could guide neuronal cell growth in the direction of the platinum contact material and could be used to minimize the distance between the neuronal cells and the electrode contact.

MATERIALS AND METHODS

Femtosecond laser structuring

For investigation of the influence of surface topographies on neuronal cells, two microstructure designs were generated. The first design was an array of grooves with a gradual width [Figure 1(a)], that is, the groove width was continually increased from 1 μm to 10 μm over the entire length of 3 mm. The distance between grooves was 11 μm . The depth of the structure was = 1 μm . Each sample contained the structured area ($\sim 1 \times 3 \text{ mm}^2$) and the non-structured area around the structure ($\sim 2 \text{ mm}$ on each side), which served as control. The second design was an array of grooves with a constant width of 5 μm . The centre-to-centre groove distance was 10 μm , and the depth of the structure $\sim 1 \mu\text{m}$ [Figure 1(b)]. This design was manufactured on 25 mm² size samples, which were completely structured (without non-structured control area).

As a model for platinum electrodes, a thin platinum layer (50 nm) was sputtered on microstructured glass slides. The structuring of glass slides was preformed by femtosecond (fs-) laser ablation. For this purpose, we used a commercially available amplified Ti-Sapphire femtosecond laser system (Femtopower Compact Pro, Femtolasers Produktions GmbH, Austria). This system delivers sub-30-fs pulses at 800 nm central wavelengths with energy of up to 1 mJ, and a repetition rate of 1 kHz. The laser beam was focused by a Schwarzschild objective (36-fold magnification, numerical aperture NA = 0.5). The large area surface patterns were fabricated by translation of samples with constant velocity using an x-y motorized translation stages (PI Physik Instrumente, Karlsruhe, Germany). Airflow was applied to remove ablated particles during the manufacturing process.

Liquid silicone rubber (LSR 30, provided by Cochlear Ltd.) was used as a model for the silicone carrier. Silicone

elastomer samples were microstructured by two different procedures—molding and laser ablation. In the first procedure, the fs-laser fabricated microstructures were transferred into the silicone by molding. Liquid silicone was placed on the structure, degassed in an excicator, and cured at 110° for 90 min. Finally, silicone samples had a surface topography corresponding to a negative replica of the original microstructure in glass.

The alternative procedure was structuring silicone sheets by laser ablation. Therefore, a commercially available Ti-sapphire fs-laser system (THALES, France) was used. This system delivers 150 fs laser pulses with an energy of up to 0.1 mJ at a central wavelength of 780 nm. The system operates at a repetition rate of 5 kHz. As focusing optics, the microscope objective (Axiovert S100, Carl Zeiss AG, Germany) with 20-fold magnification and a numerical aperture of 0.6 was employed. The samples were manufactured using an x-y motorized translation stage (PI Physik Instrumente, Karlsruhe, Germany). Airflow was applied to remove ablated particles during the manufacturing process.

Characterization of the microstructure

In order to analyze the quality of the microstructured samples, the material surfaces were examined by scanning electron microscopy (SEM) as described and published earlier.¹⁵ In addition, the surfaces of the microstructured samples were obtained with atomic force microscopy (Nano Wizard II, JPK-Instruments, Germany). For the topographic images, cantilevers with a tip (pointprobe NCH-20 silicon SPM-sensor, Nanoworld Innovative Technologies, Switzerland) were used. The SPM pictures were taken in air in contact mode and at a line rate of 0.2 Hz.

PC-12 cell culture

Rat pheochromocytoma neuronal-like PC-12 cells (ATCC Number CRL-1721) were used to test the following types of materials and structures: silicone elastomer (unstructured control, molded, and ablated gradient structures) and platinum (unstructured control, gradient, and constant 5 μ m microstructuring). Prior to use, all substrates like cell culture flasks and material samples were coated for 1 h with calf skin collagen solution at 37°C (30 μ g/mL, Calbiochem, Germany). After removal of the collagen solution and rinsing with Hanks buffer the samples were used for the experiments.

PC-12 cells were maintained adherent in RPMI 1640 medium with stable glutamine supplemented with 10% horse serum, 5% fetal bovine serum (FBS), and 1% penicillin-streptomycin (all from Biochrom, Germany) and cultured at 37°C in humidified air containing 5% CO₂. The PC-12 cells expressed enhanced green fluorescent protein (eGFP) to facilitate examination of the cells by UV fluorescence microscopy.¹⁵

For the experiments, PC-12 cells were seeded at a density of 55 cells per mm² in 55 mm petri dishes (Nunc, Germany) containing the material samples. To induce neuronal cell differentiation the RPMI 1640 medium was supplemented only with 1% penicillin-streptomycin, 1% FBS, and

100 ng/mL nerve growth factor (NGF, PAN Biotech, Germany). After 6 days the neurite growth was analyzed.

Spiral ganglion cell culture

SGCs were seeded on the following types of materials and structures: silicone elastomer (unstructured control, molded, and ablated gradient structures) and platinum (unstructured control and constant 5 μ m microstructuring). SGCs were dissected from Sprague-Dawley rats at postnatal day 3–5 in accordance with the German law governing the care and use of animals (TSchG, §4(3)). The use of the animals for scientific purposes had been approved by the regional council (Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany). The SGC preparation and cell culture procedure was previously described in detail.³³ In short, after decapitation, the head was bisected midsagittally and the cochleae removed. The spiral ganglion was separated from the modiolus under microscopic view. After isolation of the spiral ganglion, an enzymatic and mechanical dissociation was performed.

Material samples were placed in 48-well plates (Nunc, Denmark) and were coated with ornithin and laminin (both from Invitrogen, Germany) in order to allow adhesion of SGCs on the surface of the cell culture dish. Cells were seeded at a density of 3×10^4 cells per mL and cultured in DMEM with stable glutamine supplemented with 10% FBS, 1% penicillin-streptomycin (all components from Biochrom AG), and 50 ng/mL brain-derived neurotrophic factor (BDNF Invitrogen, Germany) at 37°C and 5% CO₂ for 48 h. Fixation was performed with a 1:1 acetone/methanol solution.

In order to visualize SGCs and their neurites, cell cultures were stained immunochemically for the 200-kD neurofilament using the Vectastain Elite ABC-Kit (Vector Lab, USA) according to manufacturer's protocol. The staining was visualized using diaminobenzidine (Peroxidase Substrate Kit DAB, Vector Lab, USA).

Documentation of neuronal cell growth and analysis of the results

The neuronal outgrowth was determined on cultivation day 2 (SGCs) and on cultivation day 6 (PC-12). Images were taken of each cell exhibiting a neurite outgrowth of at least twice the length of the cell soma. The neurites were measured from the edge of the soma to the longest extension of the neurite. Neurite lengths of both types of cells were determined using imaging software (SIS, Germany).

The neurite angle α was measured between the termination of the neurite and the groove direction. The neurite orientation Θ was calculated using the following equation: $\Theta = \cos(2\alpha)$. For $\Theta > 0$ the neurite grew in a parallel direction to the microstructure, $\Theta < 0$ described a perpendicular orientation of the neurite. All data are presented as mean $\pm$ standard error of the mean (SEM). The Student's *t*-test was used for statistical assessment and statistical significance is defined as $p < 0.05$.

FIGURE 2. Comparison of the different microstructures on electrode materials with scanning electron microscopy SEM (left) and scanning probe microscopy SPM (right): (a,b) ablated silicone elastomer, (c,d) molded silicone elastomer, (e,f) platinum sputtered glass. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS

Generation of microstructures and their quality analysis

The influence of linear microgrooves on neurite orientation was investigated on the two CI materials: silicone elastomer and platinum. Silicone sheets (LSR-30) were structured directly by laser ablation. During the direct laser ablation process, silicone particles in the range of 100 nm up to 1 μm were deposited in and near the grooves. Such particles led to an increase in surface roughness and to a partially change of the groove topography, as visualized in the SEM and SPM images [Figure 2(a,b)]. Well-defined microstructured silicone samples were produced indirectly by molding. The molding process led to a homogeneous topography and a very high surface quality [Figure 2(c,d)]. As model for the platinum contact material, microstructured glass samples were sputtered with platinum. These platinum-coated microgrooves showed a well defined geometry and high quality [Figure 2(e,f)].

Effect of the microstructured surface on neurite orientation

Both types of cells (PC-12 and SGCs) adhered to the surfaces and developed neuronal extensions on the microstruc-

tured electrode materials. For silicone elastomers, representative pictures of both cell types are shown in Figure 3. On silicone surfaces, the microstructuring procedure itself had a strong influence on the neurite orientation. When grown on molded silicone elastomer, PC-12 cells and SGCs developed neurites significantly parallel to the direction of the grooves [Figure 4(a,b), Table I]. By contrast, the cells showed no significant orientation of their neurites on silicone elastomer microstructured by direct laser ablation when compared to non-structured control surfaces.

To investigate cell behavior on microstructured platinum surfaces, we used a platinum sputtered glass sample with linear microgrooves in a gradual as well as with a constant groove width. The non-structured platinum area served as control. Initial screening of neurite orientation on all platinum groove widths was performed utilizing PC-12 cells. For further investigation, we cultured SGCs only on 5 μm grooves known to attenuate fibroblast growth in previous studies¹⁵ [Figure 4(c)].

Neurites of the PC-12 cells were orientated in the direction of the linear grooves on both types of microstructured platinum: gradient and constant 5 μm structure. Grown on constant microstructured platinum the SGCs showed a

FIGURE 3. Microstructure guided neurite outgrowth of SGCs (left) and PC-12 cells (right) on cell culture dish as control (a,b) as well as on silicone elastomers: (c,d) unstructured silicone, (e,f) molded gradient microstructure, (g,h) ablated gradient microstructure. Bars: 50 μ m.

similar orientation as the PC-12 cells, that is, strong orientation parallel to the groove direction. No differences between orientation of PC-12 and SGCs on the constant structured platinum were observed. On unstructured platinum control surfaces, neurites grown from SGCs did not show any preferred orientation.

Effect of microstructured surfaces on neurite length

The influence of linear microgrooves on neurite length was also investigated. On molded silicone surface structures, the neurite length of PC-12 cells and SGCs seems to be independent from the microstructure when compared to the unstructured control [Figure 5(a), Table II], whereas direct ablation led to significantly shorter neurites of the PC-12

cells. By contrast, direct ablation did not influence the neurite length of SGCs when compared to the unstructured control surface [Figure 5(b)]. Interestingly, the neurite length of the SGCs grown on molded silicone microstructures as well as on the corresponding unstructured surface area was significantly increased when compared to directly ablated silicone or platinum. Neither gradient nor constant microstructures did affect neurite length of PC-12 cells cultured on platinum when compared to the unstructured surface [Figure 5(c)]. By contrast, SGCs seeded on platinum showed shorter neurites on constant microstructures when compared to the unstructured control surface.

DISCUSSION

Guidance of neurites toward the electrode contact material is important in order to minimize the geometrical distance between electrode contacts and the primary auditory neurons, the SGCs. Prerequisite for a reduction of this distance would be the growth of neurites into the scala tympani where the CI is located. In general, this can be considered as feasible.³⁴ When envisioning the fact that the biggest part of the surface area of CI electrodes consists of a carrier material (silicone) it is more likely for the neurites to attach to the silicone rather than the electrically stimulating platinum contacts.

Previously, it was shown that linear grooves generated on platinum led to a reduction of fibroblast growth.¹⁵ The objective of the present study was to investigate such structured materials for their ability to influence neuronal growth. Ideally, the fibroblast reducing surface of an auditory electrode should attract neurons and guide their neurites toward the platinum electrode contacts.

The neuronal cells PC-12 and SGCs were able to adhere and to develop neurites on the tested materials. Indirect patterning of the silicone surface as generated by molding guided neurites of both cell types in parallel to the grooves, as also observed earlier in various other studies on PC-12 cells^{30,31} and primary isolated neuronal cells such as CNS neuroblasts²⁵ and hippocampal cells.³⁵ However, in our study microstructures generated by direct laser ablation did not influence the direction of neurite outgrowth in either of the two cell types. When envisioning the manufacturing

FIGURE 4. Direction of the neuronal outgrowth of PC-12 cells and primary SGCs on (a) silicone elastomer with molded microstructure, (b) silicone elastomer with direct laser ablated microstructure, (c) platinum. Both cell types showed significant neuronal outgrowth parallel to the grooves on platinum and on molded silicone microstructures but not on the ablated silicone. Positive values refer to parallel orientations, negative values to perpendicular orientations of the neurites.

TABLE I. Average of the Direction of Growing Neurites on the Different Electrode Materials

Material	Procedure	Samples	<i>p</i>	<i>n</i>	Structured	<i>n</i>	Control
PC-12 neurite growth direction [cos(2α)]							
Silicone elastomer	Ablation	11	n.s.	689	0.06 ± 0.15	417	0.13 ± 0.19
	Mold	8	<0.005	364	0.38 ± 0.13	159	−0.02 ± 0.17
Platinum	Gradient	3	<0.005	137	0.75 ± 0.08	42	0.05 ± 0.14
	Constant 5 μm	5	<0.05	270	0.49 ± 0.08		
SGCs neurite growth direction [cos(2α)]							
Silicone elastomer	Ablation	13	n.s.	373	0.00 ± 0.16	326	0.02 ± 0.23
	Mold	9	<0.0001	254	0.57 ± 0.15	151	−0.12 ± 0.16
Platinum	Constant 5 μm	15	<0.05	452	0.51 ± 0.21	76	0.15 ± 0.15

n: number of cells, *p*: level of significance, n.s.: not significant.

process by direct laser ablation two major reasons may account for this finding: (i) the quality of the generated structure and (ii) the chemical properties. The quality of the direct laser ablated microstructure is inferior when compared to the molded structures. During laser ablation particles of ablated material may be deposited near the grooves. Thus, a local nanostructure consisting of debris generated by direct laser ablation can overlay the initially generated microstructure especially on small grooves [Figure 2(a)]. The resulting rough and inhomogeneous surface of the directly ablated silicone may be influencing and misleading the sensitive structure of the neurite growth cone. In addition, thermal damages of the silicone material during the ablation process needs to be taken into consideration. By direct laser ablation, chemical reactions may occur within the silicone polymer releasing possible cytotoxic products.^{36,37} Schlie et al. demonstrated that culturing of fibroblasts on silicone samples directly ablated by fs-laser resulted in a significant increase in DNA damage compared to molded silicone surfaces.³⁶ In contrast, indirect surface patterning like casting or imprinting did not influence the chemical composition of the surface, as also demonstrated by Li et al. (2007).³⁸ This result is more than favorable, considering the fact that the manufacturing technique of indirect patterning such as molding is more economic, reproducible, and feasible for incorporation in the commercial electrode manufacturing process.³⁹

A previous study identified microstructured platinum as a fibroblast repellent implant surface.¹⁵ Thus, we aimed at using platinum microstructured with grooves of approxi-

mately 5 μm width. This is of vast importance, since the electrode contacts need to be free of fibrotic tissue for effective stimulation. It would be favorable to guide and attach neurons or their extensions to electrode contact materials. Both types of linear structures investigated on platinum, that is, gradient and constant groove widths, determined a highly significant orientation of the PC-12 neurites parallel to the grooves. Interestingly, guidance of the neuronal-like PC-12 cells was more pronounced on gradient grooves when compared to constant grooves. Additional investigations with primary SGCs on constant grooves confirmed the neurite guidance effect of linear microstructures when compared to unstructured control surfaces. Other studies have investigated neuronal guidance potential of microgrooves in similar dimensions of a few μm-range, for example on PC-12 cells on poly(lactide-co-glycolide) (PLGA)²¹ and on copolymer 2-norbornene ethylene [cyclic olefin copolymer (COC)] foils³¹ or primary hippocampal neurons on poly(L-lactic acid) (PLLA).⁴⁰ Bigger groove dimensions seem to be ineffective for neurite alignment of astrocytes²⁷ but not of dorsal root ganglion cells.⁴¹

In addition to the alignment, the influence of surface topography on neurite length was investigated. On platinum, surface topography did not show any impact on neurite length in PC-12 cells. The neurite length in both cell types, PC-12 cells and SGCs, is also not significantly influenced by molded silicone microstructures. Direct ablation of silicone led to significantly shorter neurites of PC-12 cells, but not of SGCs. Interestingly, the neurite length of SGCs on microstructured platinum was also reduced, but not for PC-12.

FIGURE 5. Neurite length of PC-12 cells and primary SGCs on (a) silicone elastomer with molded microstructure, (b) silicone elastomer with direct laser ablated microstructure, (c) platinum. Both cell types showed no significant differences in neurite length between structured and non-structured surfaces on molded silicone.

TABLE II. Average of the Neurite Length on the Different Electrode Materials

Material	Procedure	Samples	<i>p</i>	<i>n</i>	Structured	<i>n</i>	Control
PC-12 neurite length (μm)							
Silicone elastomer	Ablation	11	<0.005	689	109 ± 13	417	123 ± 14
	Mold	8	n.s.	364	116 ± 14	159	130 ± 153
Platinum	Gradient	3	n.s.	137	139 ± 16	42	143 ± 14
	Constant 5 μm	5	n.s.	270	119 ± 11		
SGCs neurite length (μm)							
Silicone elastomer	Ablation	13	n.s.	373	135 ± 11	326	133 ± 18
	Mold	9	n.s.	254	217 ± 23	151	251 ± 18
Platinum	Constant 5 μm	12	<0.001	452	143 ± 17	76	212 ± 12

n: number of cells, *p*: level of significance, n.s.: not significant.

Based on these results, the influence of microstructured surfaces on neurite length cannot be judged appropriately. The influence of the surface structuring on the neurite length is indeed discussed controversially. Yao et al. also described a negative correlation between laser ablated microstructures and neurite length of PC-12 cells.²¹ By contrast, Li et al. described a reversed behavior of embryonic sympathetic neurons: aligned neurites were significantly elongated, whereas neurites of PC-12 cells showed only a tendency toward elongation.³⁸ Interestingly, Hansen described significantly elongated neurites of SGC explants on platinum when compared to silicone.⁴² However, in our study we could not corroborate these results. Hansen used a different silicone elastomer (Sylgard); however, in our study we analyzed silicone LSR-30 from Cochlear Ltd., used for the manufacturing of CI electrodes.⁴³ Thus, the impact of different silicone elastomers on neurite outgrowth should be addressed in additional future experiments.

Our results indicate that the overall behavior of PC-12 cells and SGCs is comparable. Therefore, conveniently the PC-12 cell line can be used for the cell culture tests instead of the more demanding SGCs. In future, *in vitro* experiments PC-12 cells should therefore be preferred for screening investigations of growth studies, representing neuronal behavior, followed by exemplary confirmation of the results with primary isolated neuronal cells. Furthermore, stable neuronal cell lines like PC-12 cells can be fluorescence labeled allowing live observation of cells as well as their growth on non-transparent materials.

In summary, surface microstructuring of auditory implant materials offers a guiding clue for neurites. The groove pattern most likely depends on the shape of the electrode contact. In case of the rod shaped AMI, where the electrical contacts are arranged cylindrically separated by silicone,¹⁷ the microstructure of the silicone carrier should be linear toward the platinum contacts. Once attached to the platinum surface the neurite should be kept on the electrode contact by a perpendicular orientated microstructure in order to enable stable long-term neurite-electrode interaction. In the case of other electrode contact designs like CI and ABI, we envision generating gradient grooves on silicone arranged as a stellate structure pointing to the platinum contacts at the centre, enabling the guidance of the neurites toward the electrode contacts.

CONCLUSION

As a model for primary isolated neuronal cells from the inner ear, PC-12 cells can be used for screening studies concerning surface neurite guidance. Microstructuring for the guidance of neurites and additionally for the reduction of fibroblast growth may enable an optimized nerve-electrode interaction. Parallel microstructuring on platinum and by molding techniques on silicone provides a guidance of neurites parallel to the grooves. However, this effect could not be observed on directly laser ablated silicone, assuming a distracting influence of the toxic metabolites and the overlay of debris on the microstructures. This is of major interest, since the molding technique presents an excellent applicability to be incorporated into a commercial electrode manufacturing process.

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