ADVANCED BIOMATERIALS

DOI: 10.1002/adem.201080023

Morphological Differentiation of Neurons on Microtopographic Substrates Fabricated by Rolled-Up Nanotechnology**

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Arrays of transparent rolled-up microtubes can easily be mass-produced using a combination of conventional photolithography, electron beam depositioning, and chemical etching techniques. Here, we culture primary mouse motor neurons and immortalised CAD cells, a cell line derived from the central nervous system, on various microtube substrates to investigate the influence of topographical surface features on the growth and differentiation behaviour of these cells. Our results indicate that the microtube chips not only support growth of both cell types but also provide a well-defined, geometrically confined 3D cell culture scaffold. Strikingly, our micropatterns act as a platform for axon guidance with protruding cell extensions aligning in the direction of the microtubes and forming complex square-shaped grid-like neurite networks. Our experiments open up a cost-efficient and bio-compatible way of analysing single cell behaviour in the context of advanced micro-/nanostructures with various biological applications ranging from neurite protection studies to cell sensor development.

The importance of micro- and nanostructures for the life sciences field has recently been realized.[1,2] It was found that cells can be effectively controlled and manipulated by micro-/nanodevices by responding to cues in their extracellular environment.[3–5] In addition, micro-/nanostructures can in certain ways mimic the in vivo environment of adherent cells and may thus play a significant role in tissue engineering and regeneration.[3,6] Therefore, studying the interactions between

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[**] *Acknowledgements: We acknowledge Dr. Frank Uhlmann for hosting our biological experiments, Dr. Ina Weisswange for imaging advice, Emica Coric and Anne Weston for SEM measurements and Guillermo Menendez and Anna Wade for kindly providing us with primary mouse neurons. This work was financially supported by a grant from the Volkswagen Foundation (I/84 072) and partial support from the Germany/Hong Kong Joint Research Scheme (Grant No. 426/hk-PPP-cab). MK is supported by funds from a Wellcome Trust University Award (077429/Z/05/Z).*

micro-/nanostructures and living cells is of great importance and highly demanded. The major limitation in this field has been the difficulty to effectively and economically fabricate micro-/nanostructures that are made of bio-compatible materials and at the same time provide interesting desired geometries. The recent invention of rolled-up micro-/nanotube technology has provided us with such a valuable tool as it has demonstrated a convenient way of fabricating regular arrays of microtubular structures made of virtually any material on practically any substrate [7,8] with many fully integrated functionalities (resistive,[9] optofluidic,[10] plasmonic,[11] magnetic,[12] catalytic[13]). For instance, the technology utilises conventional photolithography to obtain microtubes by simply releasing pre-stressed nanomembranes from polymer sacrificial layers.[8] Due to their ultra-thin and transparent nanolayers the microtubes as well as processes within them can easily be observed by optical microscopy. We have previously tested such rolled-up microstructures in biological applications and found that they are highly bio-compatible to living yeast cells. Yeasts are single cell organisms that are usually grown in suspension. Despite their free-living nature, baker's yeast cells could be successfully grown inside the confinement of the tubes where they kept dividing in a spatially controlled manner dependent on the mechanical interaction with the microtube.[14] Yeast is a well established model organism for studying evolutionarily conserved processes such as the cell cycle and chromosome biology. However, as a single cell organism, in many ways, it does not resemble the situation in higher eukaryotes, such as humans, where most cells are adherent and grow in the context of 3D defined tissues. For therapeutic applications it is therefore crucial to extend any biological studies to the behaviour of more complex cells.

Hence, we chose to investigate the interactions between tubular microstructures and mammalian neurons as an example of a highly specialised adherent cell type. Neurons are the basic units of the nervous system, and during development they form two types of long cell protrusions, axons and dendrites, collectively referred to as neurites that transmit/receive information to/from neighbouring cells, respectively. Due to their long, flexible and thin, rope-like nature, we were particularly curious to analyse the interaction of these structures with regards to the narrow tubular structures on our microarrays. It has been shown that neurites respond to extracellular cues in a highly dynamic fashion while their connections to neighbouring cells undergo constant changes. Importantly, malfunctions in these processes are implicated in psychiatric and neurodegenerative disorders such as Schizophrenia, Parkinson's and Alzheimer's disease. Previous research has shown that during embryogenesis the mobile tips of growing neurites (or growth cones) encounter a cornucopia of endogenous signals guiding the neurite outgrowth toward its appropriate destination.[15] This includes chemical gradients,[16] electrical gradients,[17] physical tension[18] and mechanical contact guidance by topographic cues of the culture surface.[19–25] Especially the latter has been a major research focus in recent years, and several studies have led to remarkable results, showing that cellular processes such as cell migration, cell adhesion and neurite extension[20–23] are strongly affected by topographical features of the culture surface.[26–29] For instance, Clark *et al.* [30–32] found that the direction of neurite outgrowth is highly correlated with the surface pattern the cells grow on.

We demonstrate here that our rolled-up, easily mass-producible microtube arrays are highly bio-compatible devices with well-defined topographic characteristics that provide an intriguing tool for neuron–substrate interaction studies. In the current work we were able to successfully culture primary mouse motor neurons and a variant of a CNS catecholaminergic cell line (CAD)[33] on topographic substrates containing rolled-up microtubes. Strikingly, we found that the surface features of our microchips affected the growth behaviour of both studied cell types. Our experiments demonstrate that axonal guidance can be affected by micropatterned surfaces and that it is highly interwoven with spatial sensation. Since it is a new approach, a quantitative analysis is presently beyond the scope of this work and requires further in-depth studies in the near future.

The diagrams in Figures 1(a–c) briefly describe the rolling-up process and the cell culture experiments (see Experimental section). As described in our previous work,[14] a SiO/SiO_2 bilayer was evaporated to a photoresist pattern by

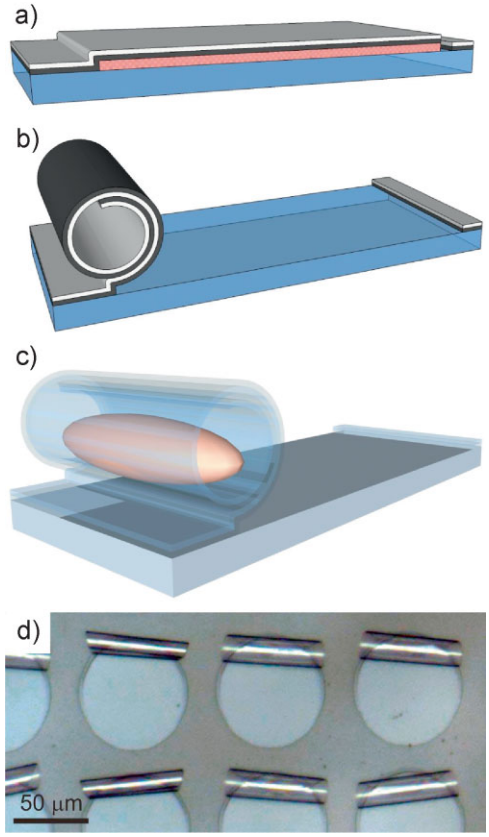

Fig. 1. (a, b) 3D diagram illustrating the fabrication process of rolled-up SiO/SiO_2 microtubes. (c) 3D scheme of a cell in a transparent microtube. (d) Optical microscope image of an array of microtubes fabricated on circular patterns.

electron beam evaporation with a glancing angle, which leaves a window open at the far end of the photoresist pattern due to the ballistic shadow effect [Fig. 1(a)].[8,14] We used acetone that penetrated through the window, removed the underneath photoresist layer and released the bi-layer, which bended up and formed a microtubular structure due to the existence of intrinsic stress [Fig. 1(b)]. Prior to the cell culture experiments [Fig. 1(c)], the microtubes were strengthened by 30 nm-thick Al_2O_3 layers on both inner and outer tube walls using atomic layer deposition (ALD). Figure 1(d) shows the optical microscope image of a rolled-up microtube array formed on circular patterns, demonstrating that this technology is suitable for mass-production. The microtubes exhibit a uniform distribution of diameters centred at 15 μm, which was adjusted to fit the cell size, and are all aligned into the same direction.[14] Due to the materials choice and the extremely thin walls the microtubes are transparent, and thus the bio-activities inside the microtubes can easily be observed by optical microscopy which will be discussed later.

We plated undifferentiated CAD cells, an immortalised cell line originally derived from the central nervous system, onto our microtube arrays and found that they not only attached on and around the microtubes but also squeezed into the *inside* of the tubes. Importantly, in this microenvironment the cells not only attached to the inner tube walls but also underwent cellular growth and proliferation. To prove this bio-compatibility of our microtubes, we compared the cell proliferation activities of CAD cells in- and outside the microtubes. Figures 2(a–e) display a sequence of optical microscope images showing the proliferation of CAD cells on the substrate. Although the cells inside the microtube were spatially confined due to the rigid wall of the microtube, all other conditions of the extracellular environment were initially comparable. As expected in a non-toxic environment, the proliferation process of the cells inside the tubes occurred in a temporally similar fashion to the cells beside the microtube. We also observed that after extended imaging times (around 60 h) our CAD cells eventually died due to phototoxicity (not shown). Interestingly, the cell inside the tube survived for a slightly longer period than the cells beside the tube suggesting that the interior of our microtubes might provide a survival advantage possibly due to its close 3D confined resemblance to tissues. Since our tubes are totally transparent in the visible range,[14] we can exclude light absorption as a cause for protecting the confined cells, which could hint new advantage of 3D culture scaffolds and may stimulate interest in cytology and physiology.

Immortalised CAD cells when grown in full medium can proliferate in cell culture as undifferentiated cells over weeks. In contrast, the motoneurons we used were dissected as primary cells from mouse embryos at a developmental stage before motoneuron differentiation. Once plated, they differentiate by protruding long neurites and stop dividing. Importantly, primary neurons are very sensitive cells and the most difficult cell type to culture in vitro. Figure 2(f) shows an SEM image of differentiated mouse motor neurons grown on a microtube array. Similar to CAD cells, the motor neurons also survived and grew after having attached to the microstructured substrate. The neurons not only attached around and on the outer surfaces of the microtubes but even extended their neurites into the interior of the tubes.

Our cell culture experiments demonstrate that both cell types, differentiated motor neurons and undifferentiated CAD cells, when cultured on microtopographic substrates

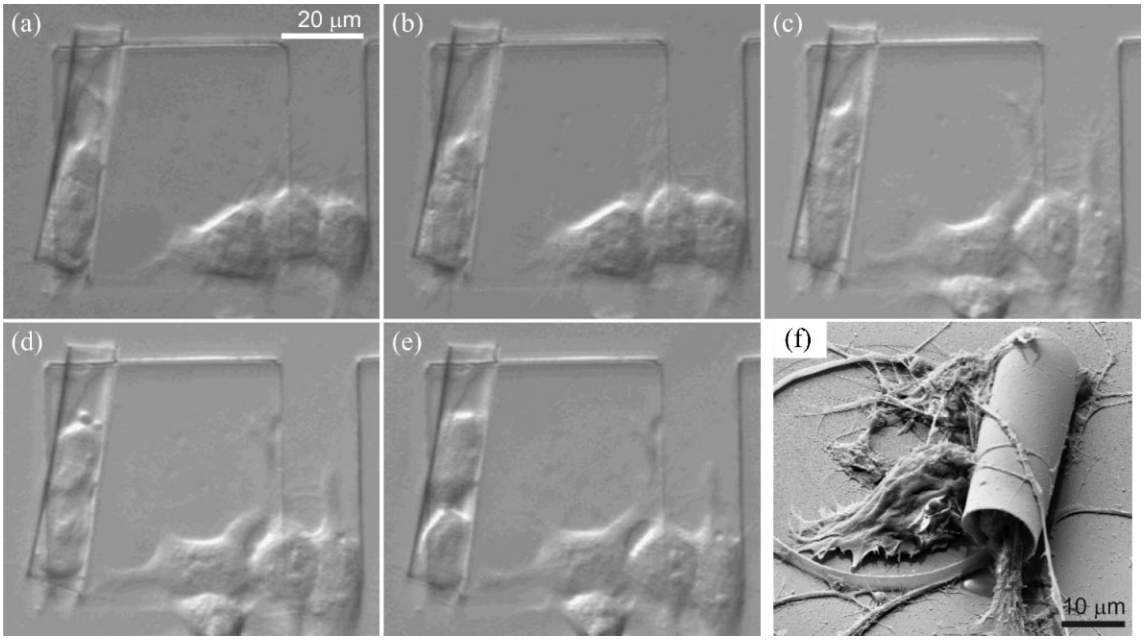

Fig. 2. (a–e) Sequence of optical microscope images showing the proliferation and increased photo-resistance of a CAD cell inside a microtube. CAD cells outside of the tubes proliferate within a similar time interval (a–e, lower right corner). (a) t = 0 min (b) t = 40 min (c) t = 360 min (d) t = 410 min (e) t = 470 min (f) SEM image of motor neurons extending their neurites onto/into a microtube.

show the expected levels of physiological activity in a non-toxic environment as all cells grow and differentiate, and, in the case of undifferentiated CAD cells, even proliferate. This proves that our Al_2O_3-coated SiO/SiO_2 microtubes did not have any inhibiting properties concerning cell growth and morphological differentiation. Taken together, not only the materials used for fabricating the tubes are bio-compatible, even the microtubes themselves appear to have no negative influence on cellular physiology but might even provide certain growth advantages. Before plating the cells, the microtube arrrays were coated with two extracellular matrix components, laminin and poly-ornithin, that facilitate and stabilise cell adhesion in tissues. Considering this bio-coating of the narrow inner tube walls in addition to the 3D confinement of the channels, our modified microtubes may provide an interesting novel microenvironment with features comparable to those of tissues. They may thus fulfil substantial pre-requisites for studying processes like cellular growth and proliferation under improved physiological conditions.

During nerve cell differentiation the outgrowing axons and dendrites seek their way to their appropriate targets where they build up a cellular network to transmit and exchange information with their neighbouring cells. Using immunostaining and fluorescence microscopy techniques we analysed the neurite outgrowth of mouse motoneurons with regards to the surface features of our microtube arrays. We used beta III tubulin and tau antibodies to stain whole neurons in red and axons in green, respectively. The images in the upper and lower row of Figure 3 show the results of mouse motoneurons grown on substrates either lacking or containing specific rolled-up surface features, respectively. When the neurons were grown on a smooth flat substrate surface, the arrangement of outgrowths appeared random (Fig. 3, upper row). In contrast, partial alignment of neurites due to the influence of surface structures can be observed in the lower row of Figure 3. Our antibody staining allowed us to distinguish between axons (red and green) and dendrites (only red). The alignment of axons appears more striking consistent with the fact that they are much longer than dendrites and extensively roam their environment, making them more likely to encounter guidance cues from the surrounding surface. To further investigate the microstructure-dependent axon guidance, we looked at the fluorescent microscope images under a higher magnification, as displayed in Figure 4. We noticed that two different types of structures, that provide geometric confinement on our microtopographic substrate, can influence the growth behaviour of axons. The first structures are vertical semicircular-shaped residual membranes that are left behind on the substrate due to the rolling-up process, as we have described previously.[14] In Figures 4(a–c) we found a neurite that gropes along the semicircular residual membrane and extends its tip into the predetermined direction. The neurite lights up both in the red and green channel identifying itself as an axonal outgrowth [see dashed arrows in Figs. 4(a–c)]. Our result proves the fact that the orientation of axons is highly influenced and significantly determined by surface morphology. The second type of structures that influence neurite growth behaviour are the microtubes themselves. Once the neuronal growth cones enter a microtube, this microenvironment directs the cellular extension along the inner tube wall, leading the neurites automatically towards the opposite opening of the tubes [see solid arrows in Figs. 4(d–f)]. The two kinds of microstructures both influence the direction of neurite outgrowth through topographical interaction but the effect of microtubes was more pronounced due to the increased spatial confinement of the microchannels. As shown in Table 1, we counted 64 cells on our rolled-up tubes on

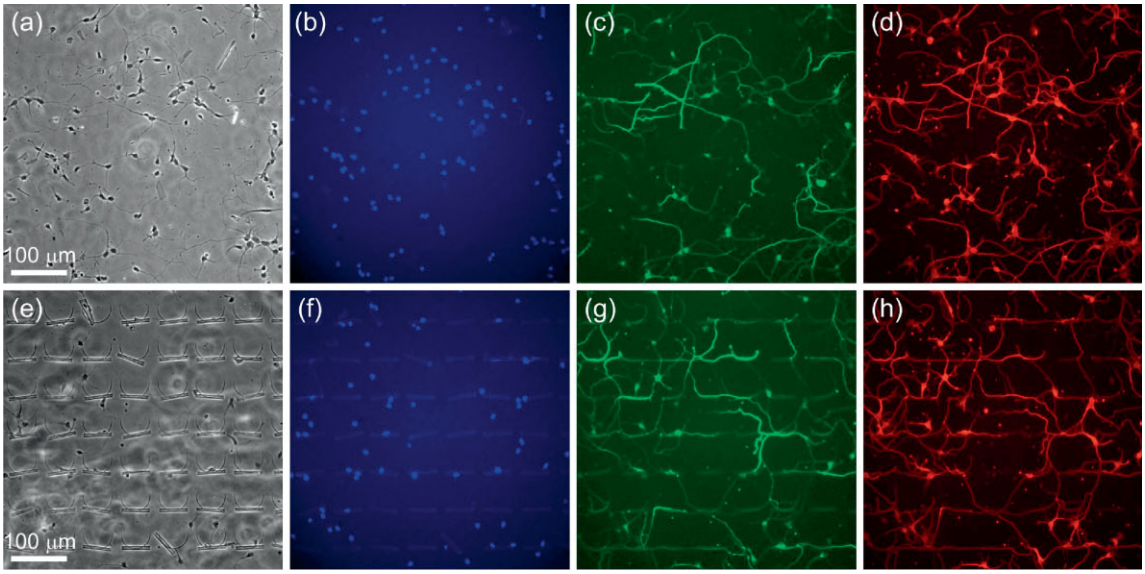

Fig. 3. Optical microscope images of fluorescently stained mouse motor neurons grown on substrate without (a–d) and with (e–h) microstructures. (a, e) Phase contrast image. (b, f) DAPI staining of nuclei. (c, g) AlexaFluor488-stained axons demonstrating the axon outgrowth guided by surface topography. (d, h) Neurons stained with AlexaFluor568 illustrating the neuronal outgrowth along the patterned substrate.

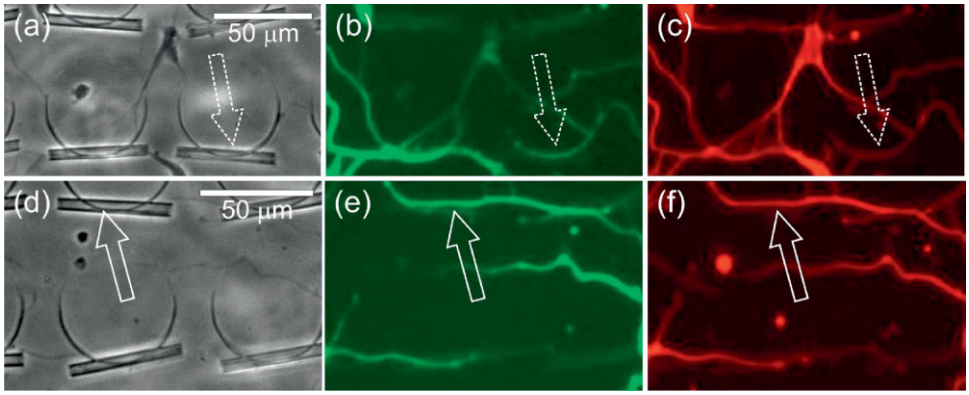

Fig. 4. The dependence of the oriented neurite outgrowth of mouse motor neurons on patterned substrates. (a–c) Neurite outgrowth influenced by circular pattern. (a) Phase contrast image. (b) Axons stained by AlexaFluor488. (c) AlexaFluor568-stained axons and dendrites. (d–f) Neurite outgrowth is guided by microtubes producing straightly aligned axons and dendrites. (d) Phase contrast image of neurites aligned to microtubes. (e) AlexaFluor488 fluorescently stained axons. (f) Aligned neurites stained by AlexaFluor568.

Table 1. Statistical analysis of cells on a microtube array showing the guidance by predefined patterns and rolled-up tubes.

	Guided by patterns	Guided by tubes	Guided by patterns and tubes	Total
Number of cells	1	8	55	64
Percentage	1.56%	12.50%	85.94%	100%

patterned substrates. All cells were guided by predefined patterns and/or rolled-up tubes, which confirms that such 3D microtubes can promote neurite guidance. Further more, the guidance from the rolled-up microtubes even led to the formation of complex square-shaped grid-like neurite networks, as one can see in Figures 3(g) and (h).

CAD cells, when induced to differentiate by starvation (depriving the medium of serum), also form neurites but the extensions are shorter compared to motoneuron axons and we could see them align less on our micropatterns (not shown). Amongst nerve cells, motor neurons have to bridge the longest distances as their axons have to connect their cell bodies in the central nervous system to the muscle cells in the peripheral parts of the body. The axonal growth cones of motoneurons may therefore need to extend and retract more frequently to find their destinations, a behaviour we could also observe in our experiment (not shown). One can imagine that this difference in length and behaviour may be responsible for the varying effects of our micropatterns on the two different cell types. To this end, our experiment shows that mouse motor neurons extend their axons and scan the surrounding micropattern for external stimuli similar to the way they do in their physiological environment.

In conclusion, we cultured two types of mammalian cells, primary mouse motor neurons and undifferentiated CAD cells, on microtopographic substrates, coated with rolled-up SiO/SiO_2 microtubes, to investigate how topographic features influence various physiological processes in these cells. The cell culture experiments demonstrate good bio-compatibility of the rolled-up microtubes. Compared to flat unstructured substrates, the microtubes successfully guide and direct the growth of axons into square-shaped grid-like neurite networks, and it can easily be imagined that not only neurite guidance but also other cellular functions are controlled by such environmental features. One example might be the increased photo-resistance we observed of CAD cells growing inside the tubes. It will be interesting to investigate whether the tubes can also serve as protective coats against other stress conditions arising from the environment. In addition, our microtube arrays offer a more general tool to investigate cell differentiation mechanisms in the context of tissue-mimicking microenvironments which can be envisioned to become important for medical applications such as topographically mediated nerve growth, tissue engineering and regeneration. Taken together, this present work provides proof of principle that high-density microtubes can be used for an array of intriguing biological applications. For instance they can be employed as integrative platforms for single cell/neurite manipulation and analysis since the microtubes can be combined with the functionalities of fluidic channels, optical resonators, remote controls and electronic circuits and can furthermore be utilised as hyperlenses.[34–36]

Experimental

Microtube Fabrication

The rolling-up process of microtubes is schematically shown in Figure 1(a). A glass cover slip (Menzel-Gläser) was uniformly coated with a photoresist (ARP-3510, Allresist GmbH) by spin-coating at a speed of 3500 rpm. Using conventional photolithography technology this layer was patterned into circular and square shapes of different sizes to

form a sacrificial layer for the rolling-up process. Then a SiO/SiO_2 bilayer was deposited onto the photoresist by electron beam evaporation with a glancing angle of 60°, which ensures a gap for the following etching step due to the ballistic shadow effect. A thickness ratio of 1:4 between SiO and SiO_2 was adapted in the evaporation process. Parameters as layer thickness and pattern sizes were adjusted for every single application to get dimensionally optimized microtubes. For the etching process the samples were placed in the chamber of a critical point dryer (CPD030, Bal-Tec AG) which was filled with acetone (BASF GmbH). The acetone passed through the window in the SiO/SiO_2 layer and removed the photoresist. Due to the intrinsic stress in the evaporated layers the SiO/SiO_2 nanomembrane rolled up and formed self-assembled microtubular structures [Fig. 1(b)]. Then the rolled-up microtubes were dried in the critical point dryer by using liquid CO_2 to avoid the microtubes to collapse. To enhance the constancy of the microstructures, Al_2O_3 was deposited onto both the inner and outer surface of the rolled-up nanomembranes by ALD (Savannah 100, Cambridge NanoTech Inc.).

Cell Culture

The glass cover slips with the microtubes on top were fixed in their positions in petri dishes using nail polish. For sterilization these prepared culture dishes were exposed to UV light for approximately 30 min. To ensure the attachment of cells and to promote neurite outgrowth both the microtubes and the bottom of the dishes were coated with poly-ornithin ($1.5\,mg \cdot ml^{-1}$, overnight at room temperature) and laminin ($1\,mg \cdot ml^{-1}$, 3–4 h at 37 °C). For our cell culture experiments, mouse motor neurons, dissected after a modified protocol from Banker,[37] and murine CAD cells were investigated. Motor neurons were cultured in DMEM/B27/2% horse serum medium supplemented with 0.5 mM · L-glutamine, whereas undifferentiated CAD cells were grown in DMEM/F12/10% FCS medium supplemented with 2 mM glutamine. Both cell types were grown in a humidified 5–7% CO_2 incubator. The immortalised CAD cells were passaged every 3–4 days.

Light Microscopy

Live cell modulated contrast/Hoffmann time-lapse sequences [see Figs. 2(a–e)] were acquired every 10 min over 3 days using a 20× MC objective, a Hamamatsu CCD-ER camera and a Nikon Diaphot 300 microscope coordinated by Kinetic Imaging software. Phase-contrast images [Figs. 3(a–e) and Fig. 4(a–d)] and fluorescent images of immunostained fixed samples [Figs. 3(b–d), 3(f–h) and Figs. 4(b–c), 4(e–f)] were acquired using a Nikon Plan Fluor ELWD Ph1 ADL, 20×/0.45NA objective, an Andor iXonEM+ DU-888 back-illuminated EMCCD scientific camera and Nikon ECLIPSE TE2000-E inverted wide field microscope with a motorised MS-2000 (ASI) stage, Lambda 10-3 (Sutter) filter wheels and shutters controller; and a Lambda LS xenon arc lamp (Sutter), all coordinated using MetaMorph Version 7.1.4.0 (Molecular Devices) software. Both microscopes are fitted with environmental enclosures regulated at 37 °C, and either 7% CO_2 in air was delivered to the immediate cell environment or medium was buffered with 40 mM Hepes/NaOH pH 7.0 to maintain a stable pH and avoid osmotic stress during acquisition.

Immunostaining

After fixing cells with 4% para-formaldehyde (PFA) for 10 min, PFA was washed off using PBS. To permeabilise the cell membrane for antibody staining, the cells were treated with 0.05% saponin plus 10% FCS in PBS for 10 min. The cells were then incubated with two primary antibodies, anti-beta III tubulin (Covance PRB-435P, rabbit polyclonal) and anti-tau (Millipore clone PC1C6, mouse monoclonal), for 1 h at room temperature. After washing three times with PBS, fluorescently labelled secondary antibodies were added: AlexaFluor568-labelled goat anti-rabbit IgG (Invitrogen) to stain whole neurons in red and AlexaFluor488-labelled goat anti-mouse IgG (Invitrogen) to specifically stain the axons in green. After washing off the unbound antibodies with PBS the cover slips were mounted with $1\,\mu g \cdot ml^{-1}$ DAPI in BioRad FluoroGuard antifade agent to stain the nuclei.

Scanning Electron Microscopy (SEM)

The cover slips containing the micropatterned surfaces with our cells were fixed for 0.5 h at room temperature using 2% glutaraldehyde in serum-free Eagle's DMEM. A second 1.5 h fixation step at room temperature followed in 2% glutaraldehyde/0.1 M sodium cacodylate pH 7.2 containing 0.1 M sucrose. After rinsing the cover slips in 0.1 M NaCacodylate pH 7.2 the samples were transferred to a holder and dehydrated in a step-wise ethanol series (30, 50, 70, 95% and 3× in 100%, 5 min each). For critical point drying (CPD) the cover slips were mounted on aluminium stubs and dried in liquid CO_2. Subsequently, the samples were coated with carbon using a sputtering technique followed by gold deposition through electron beam evaporation. The morphology of the samples was observed with a Zeiss NVision 40 workstation.

Received: February 3, 2010
Final Version: May 13, 2010

[1] S. J. Huang, A. B. Artyukhin, J. A. Martinez, D. J. Sirbuly, Y. Wang, J.-W. Ju, P. Stroeve, A. Noy, *Nano Lett.* **2007**, *7*, 3355.
[2] W. Sun, J. E. Puzas, T.-J. Sheu, X. Liu, P. M. Fauchet, *Adv. Mater.* **2007**, *19*, 921.
[3] M. A. Nandkumar, M. Yamato, A. Kushida, C. Konno, M. Hirose, A. Kikuchi, T. Okano, *Biomaterials* **2002**, *23*, 1121.
[4] G. Kumar, C. C. Ho, C. C. Co, *Adv. Mater.* **2007**, *19*, 1084.

[5] J. Nakanishi, Y. Kikuchi, T. Takarada, H. Nakayama, K. Yamaguchi, M. Maeda, *J. Am. Chem. Soc.* **2004**, *126*, 16314.
[6] M. Kato, M. Mrksich, *Biochemistry* **2004**, *43*, 2699.
[7] O. G. Schmidt, K. Eberl, *Nature* **2001**, *410*, 168.
[8] Y. F. Mei, G. S. Huang, A. A. Solovev, E. Bermúdez Ureña, I. Mönch, F. Ding, T. Reindl, R. K. Y. Fu, P. K. Chu, O. G. Schmidt, *Adv. Mater.* **2008**, *20*, 4085.
[9] F. Cavallo, R. Songmuang, O. G. Schmidt, *Appl. Phys. Lett.* **2008**, *93*, 143113.
[10] A. Bernardi, S. Kiravittaya, A. Rastelli, R. Songmuang, D. J. Thurmer, M. Benyoucef, O. G. Schmidt, *Appl. Phys. Lett.* **2008**, *93*, 094106.
[11] a) E. J. Smith, Z. Liu, Y. F. Mei, O. G. Schmidt, *Nano Lett.* **2010**, *10*, 1; b) E. J. Smith, Z. Liu, Y. F. Mei, O. G. Schmidt, *Appl. Phys. Lett.* **2009**, *95*, 083104.
[12] E. Bermúdez Ureña, Y. F. Mei, E. Coric, D. Makarov, M. Albrecht, O. G. Schmidt, *J. Phys. D Appl. Phys.* **2009**, *42*, 055001.
[13] A. A. Solovev, Y. F. Mei, E. Bermúdez Ureña, G. S. Huang, O. G. Schmidt, *Small* **2009**, *5*, 1688.
[14] G. S. Huang, Y. F. Mei, D. J. Thurmer, E. Coric, O. G. Schmidt, *Lab Chip* **2009**, *9*, 263.
[15] J. B. Recknor, D. S. Sakaguchi, S. K. Mallapragada, *Biomaterials* **2006**, *27*, 4098.
[16] T. E. Kennedy, T. Serafini, J. R. de la Torre, M. Tessier-Lavigne, *Cell* **1994**, *78*, 425.
[17] S. Ingvar, *P. Soc. Exp. Biol. Med.* **1920**, *17*, 198.
[18] S. Chada, P. Lamoureux, R. E. Buxbaum, S. R. Heidemann, *J. Cell Sci.* **1997**, *110*, 1179.
[19] S. Krayanek, S. Goldberg, *Dev. Biol.* **1981**, *84*, 41.
[20] P. Aebischer, V. Guénard, R. F. Valentini, *Brain Res.* **1990**, *531*, 211.
[21] B. Wójciak-Stothard, Z. Madeja, W. Korohoda, A. Curtis, C. Wilkinson, *Cell Biol. Int.* **1995**, *19*, 485.
[22] J. B. Recknor, J. C. Recknor, D. S. Sakaguchi, S. K. Mallapragada, *Biomaterials* **2004**, *25*, 2753.
[23] X. Zang, S. Prasad, S. Niyogi, A. Morgan, M. Ozkan, C. S. Ozkan, *Sens. Actuators, B* **2005**, *106*, 843.
[24] J. S. Goldner, J. M. Bruder, G. Li, D. Gazzola, D. Hoffman-Kim, *Biomaterials* **2006**, *27*, 460.
[25] M. Scholl, C. Sprössler, M. Denyer, M. Krause, K. Nakajima, A. Maelike, W. Knoll, A. Offenhäusser, *J. Neurosci. Methods* **2000**, *104*, 65.
[26] D. F. Newgreen, *Dev. Biol.* **1989**, *131*, 136.
[27] I. Nagata, N. Nakatsuji, *Development* **1991**, *112*, 581.
[28] S. Stokolsa, M. H. Tuszynski, *Biomaterials* **2004**, *25*, 5389.
[29] J. Li, T. A. Rickett, R. Shi, *Langmuir* **2009**, *25*, 1813.
[30] P. Clark, P. Connelly, A. S. G. Curtis, J. A. T. Dow, C. D. W. Wilkinson, *J. Cell Sci.* **1991**, *99*, 73.
[31] P. Clark, P. Connelly, G. R. Moores, *J. Cell Sci.* **1992**, *103*, 287.
[32] P. Clark, S. Britland, P. Connelly, *J. Cell Sci.* **1993**, *105*, 203.
[33] Y. Qi, J. K. T. Wang, M. McMillian, D. M. Chikaraishi, *J. Neurosci.* **1997**, *17*, 1217.
[34] E. J. Smith, Z. Liu, Y. F. Mei, O. G. Schmidt, *Nano Lett.* **2010**, *10*, 1.
[35] G. S. Huang, S. Kiravittaya, V. A. Bolaños Quiñones, F. Ding, M. Benyoucef, A. Rastelli, Y. F. Mei, O. G. Schmidt, *Appl. Phys. Lett.* **2009**, *94*, 141901.
[36] K. Dietrich, C. Strelow, C. Schliehe, C. Heyn, A. Stemmann, S. Schwaiger, S. Mendach, A. Mews, H. Weller, D. Heitmann, T. Kipp, *Nano Lett.* **2010**, *10*, 627.
[37] G. A. Banker, *Anat. Rec.* **1976**, *184*, 351.

