Guided Neuronal Growth on Arrays of Biofunctionalized GaAs/InGaAs Semiconductor Microtubes

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We demonstrate embedded growth of cortical mouse neurons in dense arrays of semiconductor microtubes. The microtubes, fabricated from a strained GaAs/InGaAs heterostructure, guide axon growth through them and enable electrical and optical probing of propagating action potentials. The coaxial nature of the microtubes – similar to myelin – is expected to enhance the signal transduction along the axon. We present a technique of suppressing arsenic toxicity and prove the success of this technique by overgrowing neuronal mouse cells.

Optical read-out of the interactions of protein arrays and cellular networks form the backbone of high-bandwidth parallel information processing techniques [1, 2]. Hence, it is essential for bio-electronic circuitry - such as microtubes discussed in this work - to provide optically active materials, such as III/V-semiconductors. This enables optical tracing of propagating action potentials in cellular networks. Apart from the detection and stimulation of action potentials, sucessful realization of neuronal guidance is the first step towards the designing of neuronal networks in vitro. Guidance has previously been achieved by using chemical guidance cues [3, 4] as well as by exploiting the geometrical properties of the growth substrate [5, 6]. It has been shown [7, 8] that arrays of micrometer-sized silicon-based tubes can sucessfully direct the outgrowth of neurons, where the neurites show a remarkable attraction towards the tube orifices.

So far, these microtubes have been fabricated from a strained bilayer of Si/SiGe and Si/SiO_2, respectively, whereas we use a combination of GaAs and InGaAs as a base material for the fabrication of arrays of tubes. The usage of GaAs, an optical III-V semiconductor, for microtubes offers a variety of advantages over Si: It exhibits a tunable, direct bandgap, which makes it suitable for experiments with optogenetic neurons. The growth of axons through optical bottle resonators [9] or the exploitation of surface plasmons [10] could spawn a new method of action potential detection.

Additionally, the electron velocity and mobility in GaAs are generally higher than in Si, resulting in lower noise levels of electronic devices. Together with other features such as its piezoelectric properties, GaAs offers a whole new range of sensing mechanisms of action potentials in neurons.

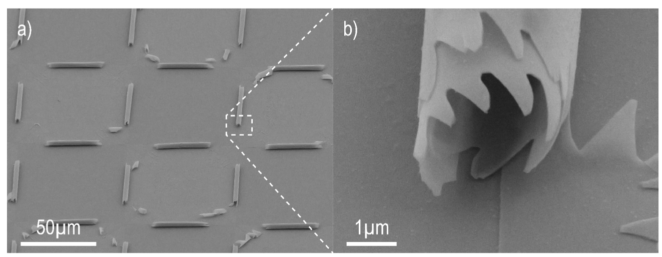

Figure 1: (a,b) SEM images of arrays of GaAs/InGaAs heterostructure microtubes. The tubes are arranged in a square array with tube lengths of 50 µm and distances between opposing tube orifices of 40 µm. (b) The membrane at the end of the tubes exhibits nonregular protrusions which are generated along the crystal structure during the rolling-up process.

The basis for the fabrication of microtubes is a strained heterostructure grown by molecular beam epitaxy on top of the $\langle 100 \rangle$ surface of a GaAs substrate. It consists of a 40 nm AlAs sacrifical layer covered with layers of $In_{0.19}Ga_{0.81}As$ and GaAs. The layers are grown pseudomorphically, thus the different lattice constants of $In_{0.19}Ga_{0.81}As$ (5.73 Å) and GaAs (5.65 Å) causes a strain in the top bilayer, which is released and leads to the rolling up of the bilayer when the sacrificial layer is selectively etched away using HF. The diameter of the microtubes is precisely tunable via the choice of these different layer thicknesses. The diameter of our tubes, determined by means of SEM imaging, is in the range of about 2 to 5 μm. In Fig. 1(a,b), an array of such

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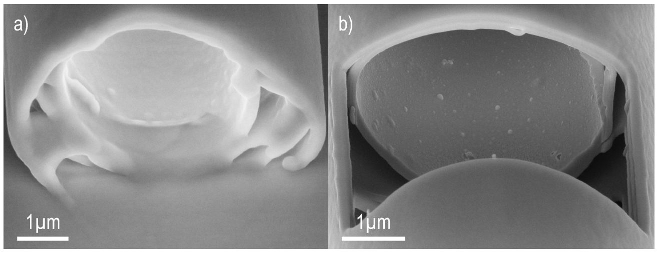

Figure 2: SEM images of the parylene-C coating of microtubes. (a) Tube orifice coated with 160 nm. (b) SEM image of the same parylene coated 100 μm long microtube cut open in the middle using a FIB. The bubbles on the inner tube surface are regions of parylene agglomeration thus proving the diffusion of parylene gas into the tubes.

microtubes is shown. The rate of successfully fabricated microtubes on a sample was close to 95% with about 5000 intact tubes, thus it is possible to form highly complex neural networks on the sample.

The arsenic component of GaAs, however, renders the material highly toxic to cells [11, 12]. The 3-5 nm thick, native oxide layer consists of a nonuniform mix of As_2O_3, Ga_2O_3 and elemental As [13]. In aqueous solution, these oxide layers dissolve and, due to their reformation with the aid of oxygen in the water, a continuous etching process occurs. Pure, cleaned GaAs corrodes in 140 mM NaCl solution under incubating conditions (37°C, 5% CO_2) with etching rates of ∼200 nm/day [14]. Typical neuron cultures utilize Neural Basal Medium with a concentration of 3 g/l NaCl, which corresponds to 50 mM NaCl. An efficient method of suppressing arsenic toxicity is therefore imperative.

Due to the release of arsenic compounds into the medium, the entirety of the sample has to be protected from corrosion. This was achieved by placing the samples onto semi-cured PDMS. After curing, the PDMS with the wafer pieces was cut out to squares. The top part was covered with parylene-C using the *Specialty Coating Systems PDS2010* parylene coater at a thickness of 160 nm, as determined by means of atomic force microscopy. The conformal CVD process ensures a diffusion of the parylene monomer gas to the inner surface of the tubes, where polymerization on the tube walls occurs. Fig. 2(a) shows the increase in the tube membrane thickness due to the parylene coating (c.f. Fig. 1(b)). Fig 2(b) proves the diffusion of parylene into the tubes and its polymerization at the inner tube walls.

The samples were sterilized under UV light for 30 min and coated in 0.1 mg/ml Poly-D-Lysine (PDL) and, after one hour at room temperature, they were rinsed three times with sterilized DI water. Dissected embryonic primary cortical E15.5 mouse neurons (prepared following a previously published protocol [15]) were plated without glia at a density of 5000 cells/cm^2 onto the samples together with control substrates where the PDMS coating was omitted. After incubation for 1 hour at 37°C and 5% CO_2, the plating medium was exchanged with serum-free

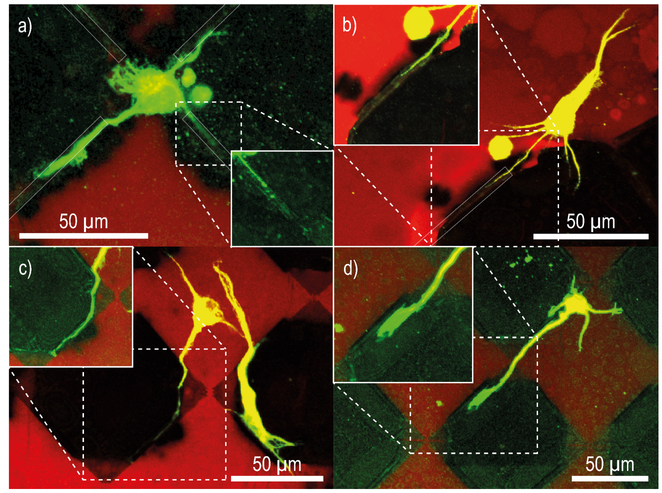

Figure 3: Fluorescent microscopy images of cortical mouse neurons grown for 1 day on a rectangular array of microtubes (marked by the gray transparent lines) coated with 160 nm of parylene. (a) A soma attached to the substrate right in front of the orifices of the four microtubes. The three extending neurites grew through the tubes or along the tubes (see inset). (b) A neurite grows through a microtube at the edge of the tube array. (c) After extending along a tube, the neurite eventually finds a tube orifice to grow through. (d) In some cases, the neurites do not outgrow through rather than alongside the tubes.

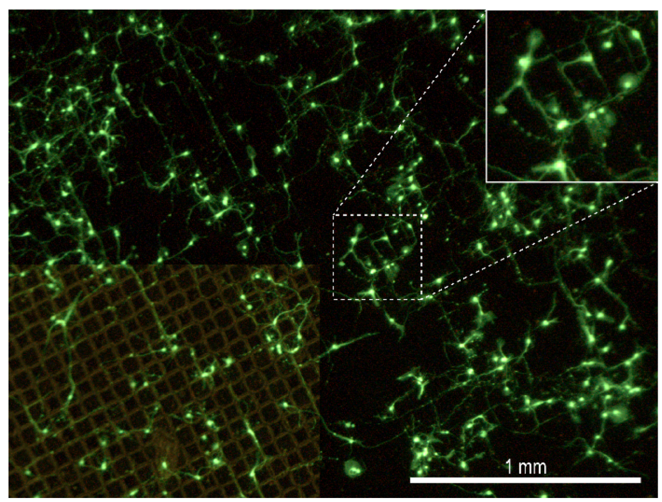

Figure 4: Fluorescent microscopy image of a neuronal network after growth for 9 days on an array of microtubes coated with 160 nm of parylene. The underlying square-shaped tube structure is resembled in the outgrowth of the neuronal network (see inset on the lower left, where the fluorescent image and the image of white light microscopy are overlaid).

medium. The cells were incubated for up to 9 days where one third of the medium was exchanged every three days.

Despite the toxicity of GaAs, the neurons showed good viability after culture for 1 day and 9 days, respectively. The viability was assessed by fluorescent optical microscopy, where the live cells were stained with the green-fluorescent calcein-AM and the dead cells were stained with the red-fluorescent ethidium homodimer-1. The tubes attract the outgrowth of neurites as shown in Fig. 3(a,b,c), where neurons have grown neurites through microtubes. In some cases, the neurites grew alongside the tubes rather than through them, as Fig. 3(d) demonstrates.

Growth on control substrates without the PDMS coating lead to the death of all cells. This indicates the necessity of preventing the corrosion of the sides and bottom of the growth substrate.

In contrast to recent results by others [7, 8], we fabricated tubes with a smaller diameter, which is similar to the size of a neuronal axon (2 to 3 μm) to ensure that only single axons grew through the tube. The reduction of the tube diameter did not have a negative effect on the attraction of neurites.

Figure 4 shows a neuronal network on an array of microtubes after culturing for 9 days. The long-term culture shows a high density of living cells, and the overall appearance conforms to the network of tubes (see inset in Figure 4) proving both the suppression of GaAs toxicity and cell guidance through microtubes.

In this letter, we demonstrated that tube structures made of a GaAs/InGaAs heterostructure can be used to guide the outgrowth of neuronal networks where, as a first step, the highly toxic effects of arsenic compounds were successfully suppressed using a combination of parylene and PDMS coating. For future work, it is necessary to compare the viability of neurons on GaAs-based microtubes to those based on Si/SiGe via statistical analysis. Also, for a better outgrowth of the neural network, the cell attachment has to be confined to the area in front of the orifices of the microtubes, which could be achieved by PDL patterning.

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