Microelectronic Engineering 135 (2015) 52–56

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

Transfer of vertical nanowire arrays on polycaprolactone substrates for biological applications

Inga von Ahnen [a,b,c], Gaëlle Piret [a,b,d], Christelle N. Prinz [a,b,e,*]

[a] Division of Solid State Physics, Lund University, Box 118, 22100 Lund, Sweden
[b] Nanometer Structure Consortium (nmC@LU), Lund University, Box 118, 22100 Lund, Sweden
[c] University of Hamburg, Department of Physics, D-20355 Hamburg, Germany[1]
[d] INSERM, Clinatec, Minatec Campus, 17 rue des Martyrs, 38054 Grenoble Cedex 09, France[1]
[e] Neuronano Research Center, Lund University, Sölvegatan, 221 84 Lund, Sweden

ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form 11 February 2015
Accepted 5 March 2015
Available online 12 March 2015

Keywords:
Nanowires
Polycaprolactone
Biocompatibility
Polymer
Gallium phosphide
Cells

ABSTRACT

We used two methods, namely *stamping* and *printing*, to transfer arrays of epitaxial gallium phosphide (GaP) nanowires from their growth substrate to a soft, biodegradable layer of polycaprolactone (PCL). Using the *stamping* method resulted in a very inhomogeneous surface topography with a wide distribution of transferred nanowire lengths, whereas using the *printing* method resulted in an homogeneous substrate topography over several mm^2. PC12 cells were cultured on the hybrid nanowire-PCL substrates realized using the *printing* method and exhibited an increased attachment on these substrates, compared to the original nanowire-semiconductor substrate. Transferring nanowires on PCL substrates is promising for implanting nanowires *in-vivo* with a possible reduced inflammation compared to when hard semi-conductor substrates are implanted together with the nanowires. The nanowire-PCL hybrid substrates could also be used as biocompatible cell culture substrates. Finally, using nanowires on PCL substrates would enable to recycle the expensive GaP substrate and repeatedly grow nanowires on the same substrate.

1. Introduction

Semiconductor nanowire arrays are used in a growing number of bio-applications ranging from biosensing [1–13], tuning cellular growth and adhesion [14–21], to transfecting cells [22,23]. A particularly promising application would consist of using nanowires to improve current neural implants, which are limited by the formation of a scar around the implant electrodes [24]. This scar is composed of glial cells, which isolate the electrodes from the intended recorded (or stimulated) neurons, resulting in implant loss of function. Many studies, both *in-vitro* and *in-vivo,* have demonstrated that nanowires are a promising tool for neural implant applications. *In-vitro*, nanowire arrays have been shown to promote neuronal adhesion and neurite outgrowth to an exceptional extend, while glial cell proliferation has been shown to be limited on these substrates [14,16,19,25]. Nanowire arrays have also been shown to enable simultaneous, multiple cellular signal measurements with great sensitivity in cultures [8,26–28]. *In vivo*, nanowire-based electrodes were able to measure neuronal activity in the rat cortex in acute experiments, and gallium phosphide nanowires injected in the rat brain were shown to be non-toxic [3,29]. The main obstacle to transposing the nanowire array platform from *in-vitro* studies to *in-vivo* applications is their stiff and thick substrate (Young's modulus of 150 GPa [1]), which would elicit a strong inflammatory reaction, resulting in a glial scar if used on a chronic basis [3]. When using nanowire photodetectors, for retinal prosthesis applications for instance, the substrate is not needed, except for providing mechanical stability during its insertion. Therefore, there is a need for resorbable substrates, which can provide mechanical support during nanowire implantation in neural tissues. Polycaprolactone, is a biodegradable polymer with a Young's modulus of 300 MPa [30], which is softer than GaP but still significantly harder than neural tissue (1 kPa [31]) and is therefore of interests since it is expected to maintain the implant integrity during insertion before being degraded *in vivo*. Here, we tested two different methods for transferring nanowires from their original substrate to a thin PCL substrate. The morphology of the substrate after transfer and the lengths of nanowire segments protruding from the polymer were investigated using scanning

* Corresponding author at: Division of Solid State Physics, Lund University, Box 118, 22100 Lund, Sweden. Tel.: +46 46 222 4796.
E-mail address: christelle.prinz@ftf.lth.se (C.N. Prinz).
[1] Present address.

http://dx.doi.org/10.1016/j.mee.2015.03.007
0167-9317/

electron microscopy (SEM). PC12 cells were cultured on nanowire-PCL substrates and immunocytochemistry was used to assess cell adhesion and differentiation, which were compared to the ones of PC12 cells cultured on control PCL substrates and on the original nanowire semiconductor substrates.

2. Materials and methods

2.1. Nanowires

Gallium phosphide (GaP) nanowires were grown using Metal Organic Vapor Phase Epitaxy (MOVPE) as described earlier [2]. Briefly, 80 nm gold nanoparticles were deposited randomly, at an average density of $1/\mu m^2$ on GaP (111)B substrates (Girmet, Russia) using an aerosol setup. The substrates were placed in a MOVPE reactor (Aixtron 200/4, Germany) where the nanowire growth was conducted at 10 kPa, 470 °C using trimethylgallium and phosphine as gas precursors. The precursor gas molar fraction were 4.3×10^{-6} and 8.5×10^{-2} for $Ga(CH_3)_3$ and PH_3, respectively, in a hydrogen carrier gas flow of 6 L/min. The resulting nanowire diameter was 80 ± 5 nm and the nanowire length was adjusted using the growth duration to 3.6 μm and 5.5 μm (referred to as *short* and *long* nanowires, respectively), depending on the samples (see Fig. 1).

2.2. Nanowire transfer in PCL substrates

Pellets of PCL (Sigma) were dissolved in dichloromethane to a final concentration of 4% (weight/volume). Two mL of the PCL solution were poured in a container (lid of a 1" fluoroware box) and heated up to 70 °C for 10 min to let the solvent evaporate. The resulting film of PCL (approximatively 200 μm thick) was cooled down to room temperature and cut in approximately 5 by 5 mm pieces.

2.2.1. Stamping method

The PCL substrate was heated up to 110 °C in an oven for 2 min. The GaP nanowire substrate was then placed upside down on top of the PCL. Both substrates were placed in the oven at 110 °C for 2–5 min and then allowed to cool down at room temperature. When the PCL substrate became opaque, the GaP substrate was peeled-off, leaving the nanowires in the PCL substrate (see Fig. 2)

2.2.2. Printing method

This method was adapted from a study published recently [32]. Briefly, the GaP nanowires were heated up to 110 °C in an oven for 30 min. The PCL substrate was put in an oven at 110 °C until the PCL became transparent. Both the GaP nanowire and the PCL substrates were then taken out of the oven to cool down. While cooling down, the transparent PCL substrate was placed on top of the nanowire substrate. After cooling down completely the PCL was peeled off, ripping all nanowires off the GaP substrate (Fig. 3).

2.3. PC12 cell cultures and differentiation

Frozen PC12 cells (ATCC) were thawed and transferred into 9 mL growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen Life Technologies) with 10% heat-inactivated horse serum (Invitrogen Life Technologies), 5% fetal bovine serum (Invitrogen Life Technologies) and 1% L-glutamine–penicillin–streptomycin solution (Sigma–Aldrich). The cell suspension was centrifuged for 12 min at 200 *g* at room temperature. After one wash step in growth medium, the cells were resuspended in 10 mL growth medium and seeded onto a 75 cm^2 culture flask (VWR). The growth medium was exchanged every second day. After four to six days, the PC12 cells were subcultured. After changing the complete growth medium the cells were detached from the flask surface by pipetting up and down ten times. The resulting cell suspension was split into four parts of 2.5 mL each to which 7.5 mL of medium were added before seeding the cells on new culture flasks. The cells were differentiated two days after subculturing them by replacing the medium by DMEM containing 100 ng/mL neural growth factor (NGF) and 1% L-glutamine–penicillin–streptomycin solution. The cells were then detached from the culture flask by pipetting up and down ten times and then 2 mL of the cell suspension were seeded onto 35 mm polystyrene petri dishes (SARSTEDT) in which the test substrates were placed and coated with poly-D-lysine (PDL) and laminin beforehand (see section below for detailed experimental procedure). The differentiation medium was changed every second day. After seven days of differentiation, the cells were fixed for 30 min in 4% paraformaldehyde in phosphate buffered saline (PBS) and then rinsed three times for 5 min in PBS.

2.4. Sample coating with PDL and laminin

The test substrates were placed at the bottom a petri dish and were coated with PDL and laminin before differentiating PC12 cells on the substrate. PDL (Sigma–Aldrich) was dissolved in sterile water at a concentration of 50 μg/mL. The dishes were incubated with 2 mL of this solution for 2 h at room temperature and then rinsed in sterile water to remove the excess PDL. The petri dishes were subsequently incubated with 2 mL of a 10 μg/mL laminin (Sigma Aldrich) in PBS solution for 3 h at room temperature.

2.5. Immunocytochemistry and confocal microscopy

After fixation, the cell F-actin was labeled by incubating the cells with Alexa Fluor 488-Phalloidin (Invitrogen, Life Technologies) 1/200 in PBS containing 0.25% Triton X-100 and 0.25% bovine serum albumin (BSA) for 1 h at room temperature.

The cells were then rinsed three times in PBS and incubated with 1 μg/mL bisBenzimide Hoechst 33342 trihydrochloride (Sigma–Aldrich) in PBS for 1 min at room temperature to label the cell nuclei. The cells were then washed three times in PBS before visualization using a confocal microscope (Zeiss LSM 510).

2.6. Scanning electron microscopy (SEM)

After fixation, the cells were dehydrated in ethanol series and air-dried. A thin layer of Au/Pd (6 nm) was sputtered on the samples (Polaron E5100 DC, AXIMA, Sweden) in order to obtain a conducting layer on the substrates. Scanning electron microscopy was performed using a SU8010 SEM (Hitachi).

3. Results and discussion

Short and long GaP nanowires were transferred to PCL using the *stamping* and *printing* method (see method section). SEM was used to verify that the nanowires break at their base from the GaP substrate (Fig. S1). When using the *stamping* method, most nanowires are not oriented vertically on the PCL substrates (Fig. S2) and very broad length distributions were measured for the nanowire segment protruding from the PCL for both short and long nanowires (see Fig. S3).

For the *printing* method, most transferred nanowires are oriented vertically on the PCL substrate (Fig. 4) and the topography is homogeneous over several mm^2. In general, the topography of both nanowire substrates (GaP and PCL) is very similar except for the nanowire length. The length distribution of the transferred