SILICON NANOWIRES AS BIOCOMPATIBLE ELECTRONICS-BIOLOGY INTERFACE

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ABSTRACT

Silicon nanowires (SiNWs) represent new opportunities for developing electrical biosensors due to their inherent properties, including large surface-to-volume ratio, rapid signal response and nanoscale footprint comparable to biomolecular and subcellular structures. Still, fabrication of nanosized electrodes is time-consuming, pricey and might be only scarcely compatible with the Complementary-Metal-Oxide-Semiconductor integrated circuits (CMOS-IC) technology. To take a step forward, we introduced an innovative approach to fabricate small, high-density SiNWs with a low-temperature (200 °C) and CMOS-compatible method. In this work, the fabrication process and the preliminary results showing biocompatibility and neutrality of SiNWs used as seeding substrate for cultured cells are presented.

KEYWORDS

Silicon nanowires, CVD, biointerface, biosensor, CMOS compatible, Vapor-Liquid-Solid growth.

INTRODUCTION

Recently, there has been an increasing progress in developing nanotechnologies for biological applications. These technologies can be defined as functional devices or structures with at least one dimension on the nanoscale (~100 nm). Critical components involved in their production can include bottom-up synthesis of nanostructures, manipulation and/or assembly of these structures, and nano-fabrication methods, which can define and connect structures on a nanoscale and larger (i.e. chips). New developments are reaching a level of sophistication that may impact standard electrophysiological recording methods, such as patch clamp. In fact, in most configurations patch-clamp relies on accessing (thus perturbing) the interior of single cells, which limits the recording output both in duration and overall number of examined samples. In order to overcome such limitations, different extracellular recording methods (e.g. Multi Electrode Arrays (MEA) [1] and multi-transistor arrays [2]) have been explored. However, these methods sacrifice the direct correspondence between cells and electrodes and might offer recordings with low signal-to-noise ratio. A valid alternative is represented by optical methods based on ion indicators and voltage-sensitive dyes [reviewed in 3]. With these technologies it's possible to perform parallel recordings, yet they might still suffer for perfectible resolution and applicability. Overall, both pharmacological screening of ion-channel drugs and high-resolution investigation of cell excitability are still usually

executed by means of low-throughput, intracellular recording methods [reviewed in 4].

In this scenario, assembling an all-electrical device for electrophysiological imaging has long been a major pursuit in bioengineering applied to neuro- and cardio-experimental physiology [5]. Notably, one of the most important challenge for this approach is represented by the interface between the cell membrane and the nano-electrodes which is affected by the electrical screening of biological signals along even very short distance (e.g. ~10 nm) [6]. Recently, adoption of nanowire transistors [7], nanotube-coupled transistors [8] and laser-induced optoporation MEA [9] did pave the way to a new technology with significantly improved signal resolution during recordings from various living cells. Nano-electrodes with different geometries based on CMOS-ICs could be used to record action potentials from cardiomyocytes in culture over a long period of time with an upgraded signal resolution [10]. Still, CMOS-based technology and nano-scaled electrodes have not been combined yet into an all electrical, low-cost device due to the scarce compatibility between CMOS technology and the nanotechnology required to grow on site nanowires. Trying to fill this gap, we designed a new approach to fabricate nano-scaled, high-density and CMOS-compatible silicon nanowires. The possibility to growth such nano-detectors directly onto the ICs allows an extremely tight coupling between the cell membrane and the recording device, theoretically minimizing the electrical screening of biological signals.

By introducing this new technology, we hope to overcome the limitations suffered insofar in the field and aim to produce a device for massively parallel electrophysiological imaging of biological samples at high-resolution.

FABRICATION

Silicon Nanowires Growth

So far, the two major techniques developed for nanowires preparation have been the "top-down" and "bottom-up." For top-down technique, nanowires are shaped from the bulk of Si wafers and patterned through microfabrication processes (e.g. lithographic techniques combined with dry and wet etching) [11]. The production of bottom-up nanowires starts with the growth of SiNWs, which are normally grown on the substrate with the help of metal catalysts. Our SiNWs were synthesized with a bottom-up technique using the Vapor Liquid Solid (VLS) mechanism (first demonstration of this mechanism in 1964 [12]). The main advantage of using this technique is the possibility to customize the process parameters in order to obtain the desired nanowires features. In this case, nanowires diameter can be tailored by the sizes of

the metal-catalyst clusters. Indeed, for our technique the metal droplets formed do function as nano-susceptors for nanowires which in turn, once the metal-semiconductor eutectic temperature has been overcome, build-up in presence of inflowing silane. So, the metal catalyst choice is crucial. The most commonly used metal catalyst for silicon wires growth has been gold and it is still frequently employed [13,14]. However, many efforts have been made to find a valid alternative, as using Au induces deep level defects in the Si structure [15], thus making this catalyst scarcely compatible with the CMOS technology standards. For our deposition process, Sn has been chosen as catalysts material. According to [16], both the impurity levels in Si bandgap and minimum temperature for VLS growth meet the compatibility requirements.

Figure 1: Schematic of the MWCVD deposition chamber. The substrate with the Sn layer is located on the crucible support and due to the tangential electric field focused on its surface, Sn droplets are heated up by MWs and work as nano-susceptors.

Figure 1 shows the deposition chamber. The main structure is a cylindrical tube used as waveguide for TE₁₀ mode propagation of the electromagnetic field generated by a magnetron at 2.5 GHz. Since the growth is performed in high vacuum conditions, a quartz disc is interposed between the MW generator and the chamber, allowing MW passage while guaranteeing the preservation of deposition chamber settings. The sample holder is positioned at the bottom of the whole structure and can be controlled along z-axis in order to modify the waveguide conditions. In order to locally damp the intensity of the generated field and to prevent energy dissipation towards the conducting crucible, a nitride spacer is positioned between the crucible surface and the sample substrate.

First step for SiNWs fabrication was the substrate baking in vacuum conditions (1×10^{-6} mbar) and at 400 °C with a 3200 °C/h regime. Those conditions, thanks to the coalescence mechanism, ensure the establishment of small Sn droplets (~30 nm) on the substrate surface as reported in Figure 2 (a). For all the heating steps, the temperature was monitored using a thermocouple in contact with the backside of the sample holder. After the baking, the

substrate was cooled to 200° C and exposed to a SiH₄ environment for several minutes in order to obtain the desired nanowires shape and length. The resulting nanowires are depicted in Figure 2 (b).

Figure 2: SEM images of two deposition steps. In (a), Sn droplets on Si substrate before the deposition process (~30 nm diameter) and in (b) SiNWs after the full fabrication procedure (20-30 nm diameter). In (b) the metal droplet is still evident at nanowires tips. (Scale bars: (a,b) 100 nm).

Cell Cultures, Patch-clamp, Ca²⁺ imaging and SEM

Cell lines were grown in standard conditions (10% FBS, 1% P/S DMEM), plated on uncoated substrates (glass coverslips or SiNWs) and used after 24-48 hrs. Primary cultures from neonatal mice were kept in standard conditions for 11 days (neurons) or 7 days (mixed glial culture). Microglia was seeded on SiNWs after purification from the mixed glial culture. In patch-clamp experiments we used standard external (NaCl-based) and pipette (K-gluconate-based) solutions to investigate passive and active neuronal properties and the expression of voltage-activated Inwardly or Outwardly Rectifying (IR or OR) K⁺ channels in microglial cells. To quantify the peak-current density, the cell capacitance was estimated from the amplifier capacitance compensation dial. For Ca²⁺ imaging, we loaded BV-2 cells with the Ca²⁺-sensitive fluorescent dye Fura-2 (2μM) and recorded the epifluorescence (380 nm exc., 510 nm em. wavelengths) time course in response to the fast application of 1mM ATP (3-sec long application). Cells grown on nanowires were prepared for SEM using a standard protocol consisting of fixation in glutaraldehyde (2,5% in PBS for 40 min at room temperature) and sample dehydration in rising concentrations of ethanol followed by overnight treatment in Hexamethyldisilazane. Fixed substrates were coated with few nanometers of chromium (*sputtering*) to increase SEM contrast. All recordings were at room temperature. Results are shown as mean ± s.e.m.

RESULTS

The cellular membrane/nanostructures interface regulates all the biological processes and it's crucial for design of bio-devices. Despite the fact that recent advancements in the fabrication of artificial bio-interfaces have yielded an enhanced understanding of this interface, there remain open questions on how the cellular membrane behaves in the presence of sharp objects at the nanoscale. Therefore, to first characterize this behavior in a fixed condition, a SEM characterization of cell membrane-nanowires interaction has been made.

In Figure 3 SEM images of BV-2 cell cultures on SiNWs are showed. As a first observation, we notice that the SEM image resolution and contrast are sufficient to discern clearly between the cell/cell membrane and the SiNWs. More importantly, the overall cell morphology appears unaltered and the cellular membrane can be seen adhering to the substrates very tightly (Figure 3, b). The images show that independent of the nanostructure size and orientation the cellular membrane tends to adhere to the substrate along the full profile, including the sharp edge at nanowires base.

Figure 3: SEM images of BV-2 cell on SiNWs. (a) shows a portion of cell culture (scale bar 20 μm). The magnification (b) demonstrates the intimate contact between the cell and the nano-structures (scale bar: 200 nm).

As BV-2 cells were successfully grown on engineered substrates we stepped forward and tested their physiological properties using patch-clamp and Ca^{2+} imaging. Thus, to investigate whether SiNWs could alter the pattern of K^+ ion channels expressed on these cells (compared to glass coverslips as standard substrates; [17]) we recorded whole-cell membrane currents elicited by voltage steps and quantified their peak-current density. No apparent difference was found between control (Ctrl) and silicon substrates (3 and 4 cells, respectively; Figure 4, a). To further demonstrate that cells grown on SiNWs do express a pattern of membrane receptors similar to those present in physiological conditions we performed Ca^{2+} imaging experiments on BV-2 cells on SiNWs and found that both basal intracellular $[\text{Ca}^{2+}]$ and 1 mM ATP-elicited $[\text{Ca}^{2+}]$ rise were typical of these cells in normal

culture conditions (Figure 4, b; [18]). In line with these findings, primary microglia from neonatal mice could be grown on SiNWs with no apparent alteration of their morphology (Figure 4, c).

(a) Patch-clamp

(b) Calcium imaging

(c) Immunofluorescence

Figure 4: Voltage-clamp recordings, Ca^{2+} imaging and immunofluorescence on microglial cells. (a) typical transmitted (IR-DIC) image of BV-2 cells on SiNWs (left) and current response to applied voltage steps (V_{cmd} -130 to +30 mV, 1-sec long, 20 mV/step; holding potential -70 mV). (c) typical optic field depicting fura-2 AM loaded cells (left). The arrow indicates a cell responsive to the fast application of 1 mM ATP. Right, time course of the fluorescence response (indicating $[\text{Ca}^{2+}]$ rise) to two consecutive applications of ATP (arrows). Typically, we found three-to-four responsive cells per optical field (6 fields analyzed across different substrates, no difference found). (c) Magnifications of immunofluorescence depicting microglial cells in culture on SiNWs (green, anti-phalloidin labelling microglia; blue, Hoechst labeling every cell nuclei). (All scale bars: 25 μm)

Similar to microglial cells, also neuronal cells could grow on SiNWs. We cultured NG108CC15 cells, a hybrid cell line showing neuronal properties [19] and in current-clamp experiments we found that in response to steps of hyperpolarizing or depolarizing injected currents NG cells had both passive properties (resting membrane potential; membrane capacitance; voltage sag and input resistance) and active response (firing profile) unaltered by the presence of SiNWs (3-to-4 cells; Figure 5, a). SiNWs were also tested for biocompatibility using primary hippocampal neurons from neonatal mice with no

apparent alteration of their morphology (Figure 5, b).

Figure 5: Current-clamp recordings and immunofluorescence of neuronal cells. (a) Typical IR-DIC images of NG18CC15 cells (left) and their voltage response to steps of injected current (right) showing unaltered morphology and electrophysiological properties in presence of different seeding substrates. Injected current steps: I_{inj} -200 to +600 pA, 50 pA increments, 1s-long; inter-sweep-interval 1.5 sec; HP -80 mV. (b) Mouse hippocampal neurons after 11 days in culture on uncoated SiNWs (red, anti-TuJ1 labeling neurons; blue, Hoechst labeling every cell nuclei). (All scale bars: 25 μ m)

CONCLUSIONS

We described a novel technology for the fabrication of small, high-density Silicon NanoWires, which can theoretically be grown directly on CMOS IC-equipped substrates. Our method is fast, inexpensive and works at relatively low temperature. Of note, using a multidisciplinary approach (SEM; patch-clamp; Ca^{2+} imaging) we demonstrated that our SiNWs are neutral to living cells and thus potentially amenable to pass electric signals both from and onto cells in tight contact.

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