Biocompatibile Silicon Nanowires for CMOS-IC Based Sensors

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State of the Art/Methods

Ionic currents across membranes are crucial in both excitable and non-excitable cells; their accurate measurement requires efficient coupling between cell membrane and measuring electrodes. An elective approach to investigate membrane currents and potentials in details, from network to single-channel activity, is the patch-clamp technique [1]. However, in most configurations patch-clamp relies on accessing, thus perturbing, the intracellular milieu and is usually performed on single cells. Hence, patch-clamp can provide a high-resolution recording output, though intrinsically limited in duration and n value. Extracellular recording methods, such as multi-electrode arrays (MEA) [2] and multi-transistor arrays (MTA) [3], are noninvasive and allow long-term and multiplexed measurements. However, extracellular recording not only sacrifices the one-to-one correspondence between cells and electrodes, but also suffers significantly in signal strength and quality. So, high-resolution investigations of the molecular mechanisms underlying cell excitability and pharmacological screening of ion-channel drugs is still usually performed by low-throughput, intracellular recording methods [4].

In this work we demonstrate the possibility to grow biocompatible silicon nanowires (20-30 nm-diameter, Figure 1) using a Vapor-Liquid-Solid (VLS) based, low temperature deposition process. This temperature is compatible with CMOS integrated circuit, which thus can be used as electronic acquisition board interface of the cell membrane electric signals. Recently, has been shown that vertical CMOS-based high density MEA nanopillars can record extracellular and intracellular action potential of hippocampal neuronal culture [5]. Here, the bio-compatibility of these SiNWs using both primary cultures (hippocampal neurons or microglial cells obtained from neonatal mice) and cell lines (NG108CC15 or BV2 cells, respectively a hybrid cell line with neuronal properties and a murine microglial cell line) is tested.

After performing electrophysiological (patch-clamp) and Ca^{2+} imaging analysis of neuronal and glial immortalized cells it has been found that cells i) can be kept in culture for several days on uncoated SiNWs, ii) show typical morphology and membrane ionic currents and iii) are amenable for standard patch-clamp recordings.

Experimental Results

Our preliminary results show that different cell types have unaltered morphology and functional properties when seeded on SiNWs compared to control condition. A first evaluation, in a fixed condition, has been made with a SEM characterization of cell membrane-nanowires interaction. Figure 2 shows SEM image of BV-2 microglial cell cultured on SiNWs. The overall cell morphology appears unaltered and the cellular membrane can be seen adhering to the substrates very tightly. 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. Voltage-clamp experiments revealed same membrane current profile and density across different substrates (Control and nanowires). Typical recordings are depicted in Figure 3. We also tested BV-2 cells grown on SiNWs (both silicon and silicon oxide substrates) using Ca epifluorescence imaging and found that both basal intracellular [Ca^{2+}] and ATP-elicited [Ca^{2+}]$_i$ rise were typical of BV-2 cells in physiological conditions (Figure 4). Primary hippocampal neurons and microglial cells from mice tested by immunofluorescence also exhibited bio-compatibility with SiNWs substrate (data not shown).

Altogether, Silicon NanoWires do not appear to alter normal survival and functional properties of both microglial and neuronal cells in vitro thus resulting amenable for non-interfered biological measures and conditioning.

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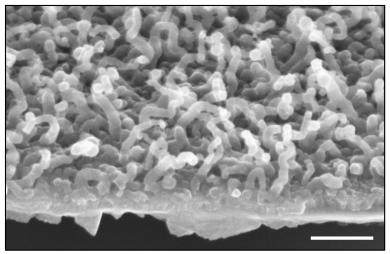

Figure 1. The silicon nanowires grown at low temperature (200°C) used as cells seeding substrate (Scale bar: 100 nm).

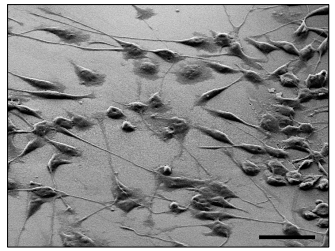

Figure 2. SEM images of BV-2 cell cultured on SiNWs substrate (Scale bar: 20 μm).

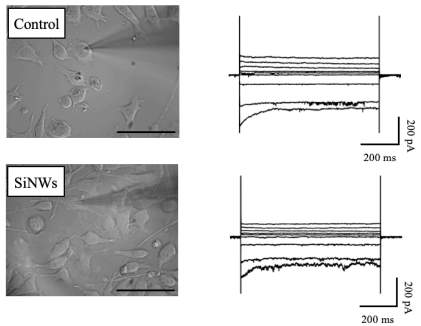

Figure 3. Transmitted (IR-DIC) image of BV-2 cells on SiNWs (left) and current response to applied voltage steps (V_{cmd} –130 to +30 mV, 1-sec long, 20 mV/step; holding potential –70 mV). Unaltered electrophysiological properties have been recorded.

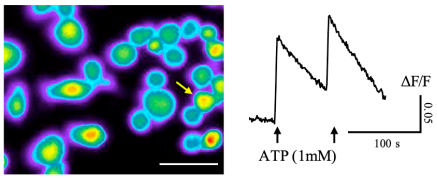

Figure 4. Typical optic field depicting fura-2 AM loaded cells (left). The arrow indicates a cell responsive to the fast application of 1 mM ATP. Right, time course of the fluorescence response (indicating [Ca2+i] rise) to two consecutive applications of ATP (arrows). Typically, we found three-to-four responsive cells per optical field (6 fields analyzed across different substrates, no difference found).