Effects of III-V Semiconductor Nanowires on Human Lung Cells

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ABSTRACT

Due to unique electronic and optical properties, semiconductor nanowires (NW) are widely used in various opto – electronic devices. Concerns have been raised about using NW since they share morphological similarities with nanofibers, such as carbon nanotubes and asbestos. However, the effects of NW exposure on humans remain unknown and there is a pressing need to assess any possible NW toxicity. During the NW development and large scale production, the most probable exposure route is inhalation. In this study, we subjected cultured human small airway epithelium (SAE) cells to epitaxial grown gallium phosphide (GaP) NW and assessed the NW uptake by the cells. We observed that SAE cells engulf NW, cells stay viable 7 days after exposure and the NW remain inside the cells after cell division.

Keywords: semiconductor nanowires, human small airway epethelium cells, toxicity, nanowire uptake

1 INTRODUCTION

III-V semiconductor nanowires (NW) are one-dimensional structures with a diameter on the nanometer range (<100 nm) and a length on the micrometer range. Their geometrical structure and chemical composition are critical for electronic and photo-electrochemical performance, as well as for bioapplications [1]–[5]. The occupational safety and hazard organizations draw attention to NW for their high aspect ratio geometry, which resembles the morphology of asbestos fibers and carbon nanotubes [6]. However, the effects of NW to humans and ecosystem are not fully understood [7]–[10]. We have identified that, during the NW technology development and large-scale production, the highest risk of human exposure is through inhalation. Therefore, we need to study how NW effect human lung cells.

Here we have studied how the presence of free-floating gallium phosphide (GaP) NW in cell culturing media influences normal human small airway epithelium cells.

2 METHODS

Nanowire fabrication. Galium phosphide (GaP) nanowires were synthesized using metal organic vapor phase epitaxy (MOVPE) from 80 nm diameter catalytic gold nanoparticles as described previously [11]. Size-selected gold particles were generated using spark dischange - aerosol technology and randomly deposited on GaP (111B) substrate at 1 particle/μm^2 density [12]. GaP NW were grown in a MOVPE reactor (AIXTRON 200/4). During the first step, in the hydrogen and phosphine atmosphere at 650 °C, surface native oxide was removed and Au particles were anneled to the substrate. Then the temperature in the reactor was reduced to 450 °C and hygrogen was exchanged to trimethylgalium for 8 min under low pressure (10 kPa) to produce GaP NW. Unintended radial growth was prevented by using HCl during the crystal growth step [13]. The obtained nanowires were homogeneous in length and diameter and oriented perpendicularly to the surface (Figure 1 A). To obtain homogeneous nanowire surface chemistry, all nanowires were coated with a 10 nm layer of aluminum oxide (Al_2O_3) using atomic layer deposition (Savannah-100 system, Cambridge Nanotech, USA). The resulting NW were 5.7±0.4 μm long and 108.8±6.1 nm in diameter.

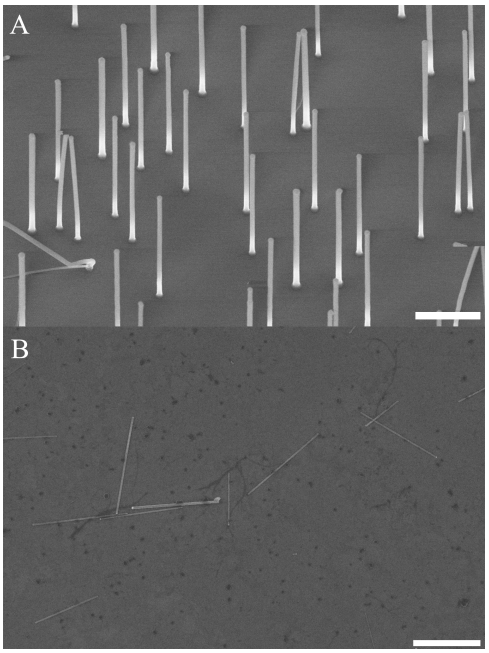

Figure 1: GaP nanowires used for SAE cell exposure. **A**. Scanning electron microscopy (SEM) image of GaP nanowires, used for exposure. Stage tilt 30°, scale bar 2 μm. **B.** SEM image of nanowires broken from the substrate surface using sonication. Stage till 0°, scale bar 4 μm.

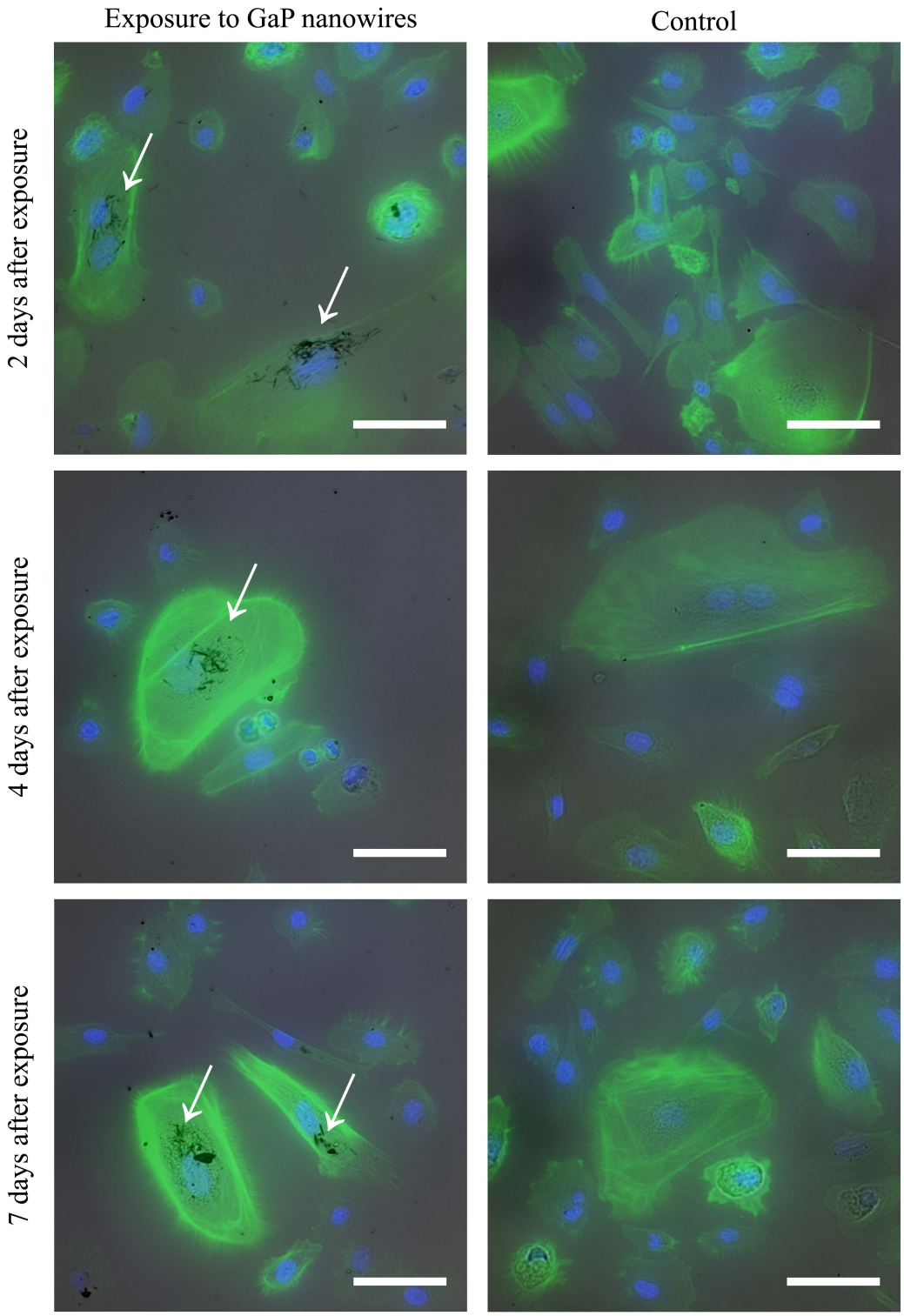

Figure 2: SAEC after 2, 4 and 7 days of exposure (left column) and controls (right column). White arrows show NW accumulation in the cells. Actin is labeled with AlexaFluor488® phalloidin (green) and DNA is labelled with Hoechst 33342 (blue), black needles are GaP nanowires visualized using bright field microscopy. Scale bar 50 µm.

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Cell culture and exposure. Normal human small airway epithelial cells were purchased from Merck Millipore (USA) and cultured in EpiGRO® medium (Merck Millipore, USA) according to the protocol provided by the distributor.

GaP nanowires were broken off the substrate and suspended in sterile ultra-pure deionized water (18.2 MΩ/cm, MilliQ) using ultrasonication for 3 min (Figure 1B). Afterwards, the nanowire suspension was mixed with cell medium and SAE cells were exposed to NW at a concentration of 1 NW/nL and incubated with NW for 2 days. The cells were subsequently washed: one part was fixed for 20 min in 4% paraformaldehyde solution, labeled for cell F-actin with AlexaFluor488® phalloidin (0.033 μM, ThermoFisher Scientific) and cell DNA was labelled with Hoechst 33342 (1 μg/mL, Sigma Aldrich), while the other part was cultured further. The cultured cells were split into 2 groups: the first group was cultured for 2 more days (4 days in total, including the exposure) while the second group was cultured for 5 more days (7 days in total). Afterwards, cells were fixed and stained as described above.

The cells were subsequently imaged using fluorescent microscopy. All images were aqcuired using Nikon Eclipse T*i* microscope, equiped with x100 oil immersion objective. GaP NW were visualized using bright field microscopy.

The image analysis was performed using open source ImageJ software [14].

3 RESULTS AND DISCUSSION

In this work, we have performed single SAE cell exposure with GaP NW at 1 NW/nL concentration for 2 days and let the cells grow for up to 7 days. We observed that SAE cell uptake the GaP NW after 2 days and the NW remain inside the cells for the entire exposure and culturing time (4 days and 7 days, Figure 2).

We observed a co-localization of most of the NW with the SAE cell cytoplasm and perinuclear area (Figure 2). When comparing exposed to NW group with control group fluorescent images, we did not observe any change in SAE cell morphology and cell nuclei morphology. However, we observed NW inside in the cells 7 days after exposure, which suggests that NW are shared between daugther cells. There is therefore a probability for NW to interact with the cell DNA during mitosis. In two studies, L. M. Sargent *et. al.* have shown that carbon nanotubes (CNT) are capable to interact with nuclei components due to CNT fibrous structure, which was capable to reduce the mitotic aparatus elasticity [15], [16]. Although we did no find any accute effect of GaP NW exposure on the cells after 7 days, we cannot rule out that DNA – NW interactions in longer cultures would introduce toxicity.

After analysis of the NW exposed cell images, we observed that cells engulfing the NW, appear to have a higher surface area compared to the cells with few or no internalized NW. This suggests that the cell population is heterogeneous, containing not only epithelial cells but including other lung cells, such as macrophages, clara cells, and ciliated cells [17]. In the future, we are planning to identify possible different cell types in the culture using immunomarkers, such as CD68 for macrophages and CC10 for clara cells.

4 CONCLUSIONS

GaP NW are engulfed by cultured primary human SAE cells after 2 days and stay inside the cells for at least 7 days. With this work, we have established a platform suitable for future studies of the effects of NW concentration, size and distribution to SAE cells and their organelles. In the future, we plan to assess the effects of NW on cell motility and migration by employing phase holographic microscopy, as well as the cell viability and the presence of reactive oxygen species cells.

5 ACKNOWLEDGMENT

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