

# Morphological Investigation of Cell Culture and their Contact patterns on the Surface of the Gold Nanowire Templates

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## ABSTRACT

Gold nanowire arrays are fabricated inside nanoporous alumina templates. The wires are vertically arranged in a parallel alignment and are synthesized by means of electrochemical deposition inside porous aluminum oxide templates. Successful cultures of various cell lines are demonstrated on the top surface of such arrays. Cells have been imaged by means of fluorescence microscopy measurements and luminescence imaging by two-photon Keck microscopy studies revealing healthy cell growth on the surface of the wires. Fixed cells have been imaged by using scanning electron microscopy (SEM), which reveal good contact between the nanowires and the cell surface. In the case of neuronal cell line cultures on the nanowire arrays the cells are tightly interfaced with the nanowire surface.

**Keywords:** nanowires, cell culture, myeloma, fibroblast and hippocampus neuron

## 1 INTRODUCTION

Biological and clinical applications of nanomaterials are receiving increased research attention, for example, applications of metal nanoparticles in drug and gene delivery,<sup>1-7</sup> DNA analysis,<sup>8</sup> nanomaterials for neural interfacing,<sup>9</sup> metal nanoshells for cancer treatment.<sup>10-13</sup> Gold nanoparticles in particular have been increasingly used for biocompatibility and easy synthesis procedure. In our work, we studied the use of gold nanowire (GNW) arrays for bio-applications. We investigated the viability of different cell lines on the surface of the Au-nano templates. The gold-nano particles are almost nontoxic to human cells and could be used *in vivo* without any side effect.<sup>14</sup> In contrast, the *in vitro* cell lines cultures on the gold nano surface may exhibit some degrees of toxicity which depends on the surface charges of the gold nano particles.<sup>15-18</sup> We used GNWs for the experiments because of their optical and electrical functionalities, chemical and biological stabilities,<sup>19-21</sup> imaging<sup>22</sup> and biosensing<sup>23-24</sup> capabilities and their capabilities to interact as a base for the viability of different cell lines *in vitro* culture. In addition, the use of gold array based structure allows for the potential development of nanobiodevice structures integrating nano and biomaterials with important clinical applications, such as in implant

devices, drug deliveries, electrical impulses etc. In this regard, it is first important to understand the interaction of biomaterials with the nanomaterials.

Here, we report on the use of GNW array-based surfaces for various *in vitro* cell lines cultures. Morphology of three different cell lines cultures are compared to understand the influence of the nanowire based cell cultures on their viabilities. Our results will help us further to investigate the use of nano-particles in *in vivo* investigations and as catalysts for cell contacts and connectivity.

## 2 EXPERIMENTAL DETAILS

GNW arrays are fabricated by means of electrodeposition inside nanoporous aluminum oxide templates. The process involves anodization of a commercial aluminum foil under a 10V DC voltage with Al foil as the anode and a platinum (Pt) mesh as cathode in 3% oxalic acid or 5% phosphoric acid or 15% sulfuric acid electrolytes. This results in the formation of alumina films at the surface of Al, containing cylindrical pores aligned vertically in a parallel arrangement. The pores are uniformly arranged across the template with a pore diameter of the order of 15-75 nm. Au nanowires are electrodeposited inside the pores by means of AC electrodeposition. For this, an AC voltage of 15V at 250Hz is applied across the Pt mesh and the porous alumina template. To fabricate an array of Au nanowires, an aqueous 0.1M solution of gold hydrogen tetrachlorate (HAuCl<sub>4</sub>) is used as electrolyte.<sup>25</sup> During every negative half cycle Au ions are reduced to Au atoms ( $Au^{3+} + 3e^- = Au$ ) and deposit inside the pores. The longer the time of deposition, the wire length is increased. The diameters of the nano-wires correspond with the diameter of the pore.

In order to prepare Au-nanowire array based templates for cell culture, first the templates are etched slightly in 5% phosphoric acid at the top of the pores for 10-15 mins in order to expose the deposited gold nanowires. The templates are then rinsed thoroughly in ethyl alcohol followed by air drying or critical point drying and plasma cleaning. The top surfaces of the templates are then used for the cell culture experiments.

Three types of cells have been cultured, immortal myeloma cells, fibroblasts and neuronal cells, which allowed for a comparison of growth behavior and morphology on the wire surface. Myeloma cells and fibroblasts were cultured using standard culture techniques. For suspended cell lines (myeloma cell lines) we used 10% Fetal Bovine Serum (FBS)

and 1% Pencillin-streptomycin antibiotic in RPMI 1640 media and for adherent cells like fibroblast cell lines we used 10% FBS and 1% antibiotic containing DMEM media (GM and FLO-1). The rat neuroblastoma cell lines were cultured using primary cell culture lines, namely embryonic 18-day gestation Sprague Dawley rat hippocampal tissues, purchased from BrainBits. Tissues were mechanically dissociated and spun at 1100 rpm for 1-2 mins. The neuron cells were then cultured in the serum free B27/Neurobasal medium. The pellets of the neural cell lines were cultured on the surface of the Au-nanowire templates. For comparison, these cell lines were also cultured on the top of the glass cover slips, titanium oxide (TiO<sub>2</sub>) and blank porous aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) templates.

The viability of the cells was studied using a 20X optical microscope connected to a CCD camera. The images of the cells on the nanowire surfaces were obtained by Fluorescent microscopy (Nikon Optiphot 200D) and the luminescence imaging was obtained by two-photon Keck microscopy. We also used SEM imaging for the detection of the cell-nanowire contacts and interactions. For SEM imaging, the cells must be 'fixed'. The cells were fixed on the nanotemplate in 4% formalin (Sigma) followed by soaking in 0.1M sodium cacodylate buffer, for 1 hr at room temperature. The samples were incubated with 1% osmium tetroxide in the same buffer, for 1 hr at room temperature. Dehydration was performed in an ethanol series (70%, 80%, 95%, and 100%) and samples were then critical point dried. The samples with the Au-nano templates were then covered by a thin gold/palladium layer and observed under the SEM.

### 3 RESULTS AND DISCUSSIONS

Myeloma cells, also known as myeloma plasma cells are immortal bone marrow cancer cells produced in the white blood cells. These malignant cancer cells affect the immunity system of the body by causing disruption of antibody production in humans and vertebrates. These cancer cells are derived from bone marrow reproduce rapidly, and are transported through the lymphatic system to destroy the immunity of the body. Myeloma cells were cultured on the surface of Au-nanowires in order to study the interaction of the nano-particles with the cancer cells. The growth and viability of these cells were studied under the fluorescence and Keck microscope after 2 days of culture upto 'day 7'. The density of cells on the glass cover slip, were found to be relatively higher than that on the nano templates. Cell growth and viability on the surface of the GNW were significant. The shape of the cells were distinct and spherical and the size ranges from 2-5 $\mu$ . It was found that more cells persisted on the surface of the nano templates on day 2 but the numbers gradually decreased during successive days (Fig. 1). At day 7, the number of cells abruptly decreased. The reasons for the mortality of the cells are the external influences in the nanotemplates e.g. temperature fluctuations, environmental degradation, toxicity and media deterioration. In contrast, the cells grew very well on the

surface of the glass cover slips up to 11 days. For determination of the connectivity of the cancer cells with the GNW arrays, we carried out SEM measurements. It was found that the myeloma cells conjugated well with the gold-nano wires even in the absence of bio-conjugation materials or adhesion proteins. Fig. 1 indicates that there is a strong interaction and bonding between myeloma cells and GNWs. This result could be helpful to use this conjugation method to diagnose and treat the cancer cells by *in vivo* transplants. We also cultured myeloma cells on the surface of coverslips, TiO<sub>2</sub> and the blank Al<sub>2</sub>O<sub>3</sub> nano templates. SEM image reveals that myeloma cells also grew significantly with good viability and growth on the surface of the Al<sub>2</sub>O<sub>3</sub> nano templates and TiO<sub>2</sub> templates (Fig. 1). We visualized the interactions of the cells and gold-nanowires by Nikon Optiphot 200D and two-photon Keck microscope with sub-micrometer resolutions.<sup>26-31</sup> The size of the myeloma cells varied as a function of time. Gradually the size of the cells increased on the 'day 4' and the cells were seen to settle down on the surfaces of the nanowires. After day 7 the cells began to die, perhaps the external environment is no more suitable to grow these cancer cells on the surface of the nanowires. There may have been cytotoxicity introduced on the surface of the nanowires causing the apoptosis. This may give us clues to halt the stability and rapid production of cancer cells *in vivo* system by using Au-nano wires or particles.

Fibroblast cells make the structural fibers and ground substance of connective tissues. They are also called fibrocytes and are smaller and spindle shaped cells. They can give rise to other cells, e.g. smooth muscle cells, bone cells, fat cells etc. The fibroblast cells (immortal cell lines) were seen to grow rapidly, initially on the surfaces of the GNWs, but gradually in course of time, the number of cells was found to decrease. The images of fibroblast cells on the nanowire surface and cover slip are shown in Fig. 2. It has been reported that the viability of the 3T3 muscle fiber cells transfected after 24 h on the Au-nano wire were 65-90%. 30% of the gold nanowires could be internalized by the 3T3 cells within 8 h.<sup>14,32</sup> The fibroblast cancer cells were cultured on the surface of the nanowires without using any bio-conjugation materials. The viability of the cells was significant on the surface of the gold nano-wires.

Rat hippocampal neuron cells were cultured on the surface of the Au-nano wires and glass cover slips. We tested several different cell adhesion molecules (CAMs) that enhanced the stability, electronic coupling and mode of interaction between the device and the cells. The role of synthetic biomaterials is the instructive extracellular microenvironments that actively determine the cell fate in neuronal tissue engineering applications.<sup>33</sup> Proteins (CAMs) are located on the cell surface and are involved with the binding with other cells or with the extracellular matrix (ECM) in cell adhesion. Poly D-lysine (PDL), Enactin-Collagen IV-Laminin (ECL) cell attachment matrix from Engelbreth-Holm-Swarm (EHS) mouse tumor (Millipore), and laminin (LMN) from EHS murine sarcoma basement membrane (Sigma) were used to study the attachment

properties of primary E18 hippocampal neuron cells on the GNW templates. Each of these surfaces were coated with PDL, ECL or LMN. The neurons were cultured for two weeks and visualized by fluorescence, Keck and scanning electron microscopy. Our studies showed that while PDL, laminin and ECL are excellent matrices for the cell attachments. PDL proved to be a good attachment protein molecule for the neuronal cells (Fig. 3). Laminin is also known to promote a better cell outgrowth<sup>34</sup> and survival on the glass cover slips and nanowire arrays. It has also been reported that the patterning of adhesive molecules results in polarization of neurons and axon orientation.<sup>35,36</sup>

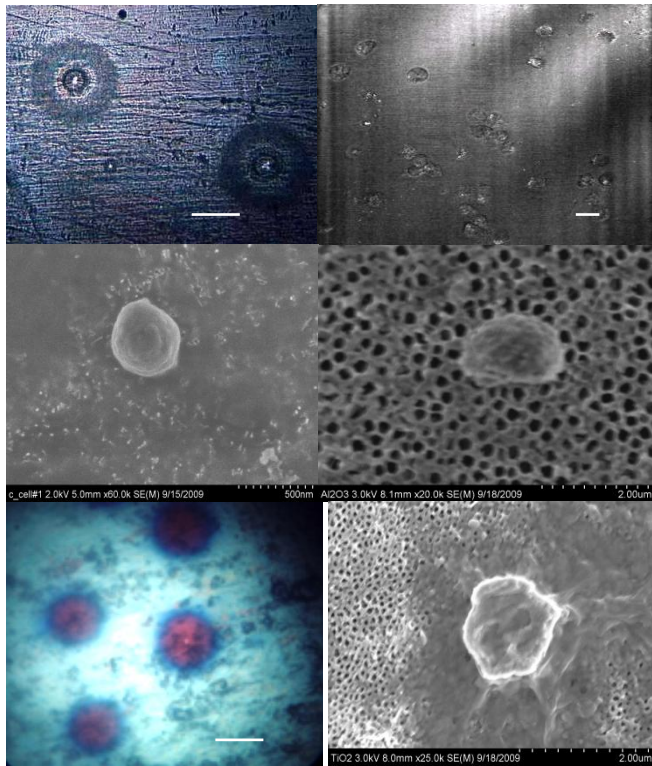


Figure 1: Myeloma cells on the surface of different nano-wires templates and cover slip. Top left panel shows cells growing on the Au-nano surface imaged by Nikon Optiphot 200D microscope, scale bar 10 μm; top right panel has 4d old cells on a coverslip, imaged by Keck microscope, scale bar 10 μm; middle left panel is a 6d old cell on the gold nano wire surface imaged by SEM, scale bar 500 nm; Middle right panel shows a cell on the Al<sub>2</sub>O<sub>3</sub> template surface under SEM, scale bar 2 μm; bottom left panel shows cells on the TiO<sub>2</sub> surface, imaged by Nikon Optiphot 200D microscope, scale bar 5 μm; bottom right panel shows a cell on the surface of the TiO<sub>2</sub> under SEM, scale bar 2 μm.

One of the biggest advantages of using Au nanowire arrays for investigation of neural activity is that they can be easily functionalized with specific bio-recognition molecules for the neuronal attachment. Au has a strong affinity for thiol groups to form strong bonds and this has been exploited to

attach various molecules. The main objective of our experiments is to measure the interactions and contact patterns of hippocampal neurons on the surface of the Au-nano templates. Some nanowires are seen to penetrate the neuronal cell membrane indicating that from such nano-wires intracellular recordings could be possible.

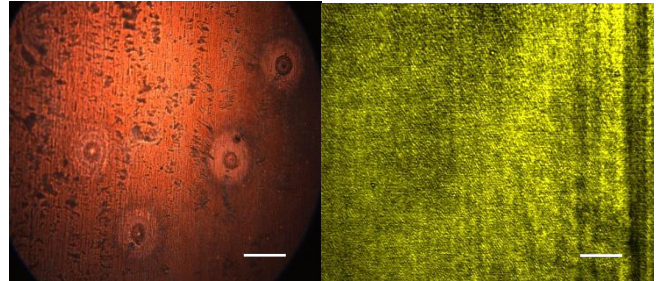


Figure 2: Fibroblast cells cultured on the surface of the gold nano wires and coverslip. Here in left panel 4d old fibroblast cells are seen to grow on the surface of the gold nano wire, imaged by Nikon 200D microscope, scale bar 10 μm; right panel shows 3d old cells on the cover slip surface, imaged by Keck microscope, scale bar 10 μm.

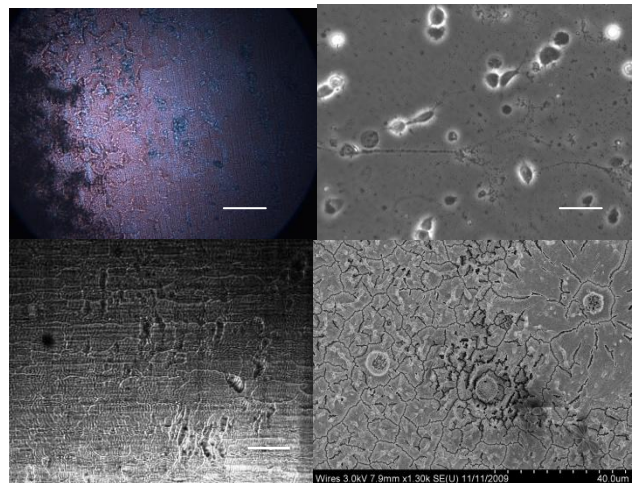


Figure 3: Rat hippocampal neuronal cells on the surface of the gold-nanowire and coverslip. Top left panel shows 5d old neurons on the surface of the Au-nano wire, scale bar 10 μm; top right panel shows 6d old neuronal network on the cover slip imaged by optical microscope, scale bar 10 μm; bottom left panel is a 7d old neuronal network on the surface of the Au-nanowire, imaged by two-photon Keck microscope, scale bar 10 μm; 7d old neuronal cells on the gold nano surface, imaged by SEM, scale bar 40 μm.

## 4 CONCLUSIONS

By studying different cells cultures on the surfaces of nanowires, it is revealed that there is a good contact between the nanowires and the cells. This result will allow us to implement the hypothesis that the signal patterns from the

living cells could be measured using GNWs. From such nanowires it may thus be possible to obtain minimally invasive quasi-intracellular recordings which may provide new kinds of biophysical information that will be helpful in resolving finer subcellular electrical details from cells and neural networks.

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