

Biocompatible Electrospun Titania-composite Nanofiber Networks for Whole Cell Sensing

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Abstract

Biosensor technology includes the detection of generated biological functionality. One whole-cell biosensor concept leaves specific receptor molecules in their natural environment to be optimized and adapted for efficient molecular recognition during evolution, and to use intact cells as biologically-active elements. In this project, a basic nanomaterials platform for whole cell sensors is produced by electrospinning composite nano- and microfiber networks. The resulting polymer-metal oxide (titania) "nano-mats" are fabricated on a conductive indium tin oxide (ITO) coated glass substrate for epithelial or other cell incubation and future incorporation into bioelectronics. Impedance spectroscopy is used to characterize cell interactions with the nano-mat to detect changes in cells with proliferation or when exposed to chemical stimuli, such as lactic acid. Cell response to media conditions can include growth, attachment and spreading, motility and migration, and cell death.

Keywords: *electrospinning, whole-cell sensing, biosensor, titania nanofibers, indium tin oxide, impedance spectroscopy*

Introduction

Biosensors have been widely used in nanotechnology for different areas of medical applications. This is due to its very high selectivity of biological reactions and binding. A biosensor consists of both a bioreceptor that recognizes the target analyte and a transducer which converts the recognition event into a measurable signal. The simplicity and the speed of measurements that require no specialized laboratory skills are the main advantages of a biosensor [1-3]. One type of biosensor, a whole-cell sensor, can be used to monitor various aspects of living cells in culture. Dependent on how the experiment is designed and in which mode the electrical measurements are performed, conditions can be adjusted to be tailor-made for the problem to be solved [4, 5]. Cell-based sensors are used for their high sensing efficiency, rapid response, and ability to detect various forms of substances. They can also provide information on the physiological effects of an analyte. These type of sensors are low cost since there is no need to separate and purify proteins embedded in the cell membrane [6-8].

Various methods such as electrodeposition and template synthesis have been used to fabricate nanowires, nanofibers, and other nanostructures [7, 8, 27, 28]. Electrospinning is a century old method that has been re-designed by many researchers for nanofabrication of different materials such as polymer-based nanofibers [11-17]. It has a simple and versatile set-up that produces a significant amount of ultrafine fibers in a short period of time. Another advantage is the room temperature processing and wide range of appropriate precursor materials. It makes use of electrostatic forces to stretch the solution as it solidifies [18-20]. The set-up collects randomly oriented nanofibers as nonwoven mats. This method uses a polymer-based solution with any metal oxide to provide a mat of nanofibers on a conductive substrate. Electrospun wires have been more resistant to water when baked at high temperatures. This has an advantage for sensing when introducing the wires to various aqueous environment for testing [15-17, 20].

Many nanomaterials have been used to fabricate a variety of nanostructures such as carbon nanotubes [27],

silica nanowires [34], and gallium nitride nanowires [31]. These nanostructures have been manipulated and redesigned to provide the best-suited conditions for specific sensing capabilities [28-33]. Among widely used nanomaterials, titanium oxide has been most popular in medical applications such as implants. Titanium's biocompatibility is mainly due to its oxide layer that keeps it inert in the human body [23, 35]. Researchers have focused mainly on the use of metal oxide nanowires because of their versatility [21, 22]. High aspect ratio oxide nanowires are obtained and used as sensing elements. Titania composite nanowires has played an important role in environmental applications and sensing technology [23-26].

Several techniques have been developed that are capable of measuring the various aspects of cell-substrate interactions optically, electrically or even mechanically [34, 36, 39-41]. Impedance spectroscopy has been used to characterize specific behaviors and responses of cells [2, 8-10]. Electrochemical techniques have been used to analyze cell attachment and spreading on conductive substrates [37]. Electric cell-substrate impedance sensing (ECIS) monitors the dynamics of cell attachment and spreading. Attachment and spreading of cells on surfaces is an enormously complex and versatile process. Cells tend to behave like insulating particles forcing the current to flow around the cell body on cellular pathways [38-40]. The interactions of mammalian cells with in vitro surfaces have attracted more and more interest throughout the years from a broad range of biomedical applications that are based on cells immobilized on solid substrates. Processes like tumor metastasis, wound healing, or cell migration in general focus on the stationary and dynamic interactions of cultured cells with a particular substrate [40].

In this paper, titania nanofiber mats electrospun on indium tin oxide (ITO) plates are subjected to a monolayer of epithelial (HeLa) cells. Lactic acid is introduced to the cells at different stages of incubation. Impedance measurements are taken from the surface of the ITO plates to observe the growth of cells on the surface and the effect of lactic acid.

Materials and Methods

Chemicals. The following chemicals were used for the titania-composite nanofiber solution: acetic acid, glacial (Fisher Scientific); ethyl alcohol, 200 proof (AAPER Alcohol & Chemical Co.); polyvinylpyrrolidone (PVP), MW 1,300,000 (Sigma-Aldrich); and titanium (IV) isopropoxide, titanium precursor (Fluka, Sigma-Aldrich).

Electrospinning. Titania-composite nanofiber networks were fabricated using the electrospinning

method [25]. This method uses an electrical charge to form a mat of fine fibers on a conductive substrate. The set-up consists of a syringe pump (Advance Series 1200), high voltage power supply (HVPS, EMCO High Voltage Corp.), two 1ml syringes (BD) with 27G 1 ¼ metallic needles (BD), and the substrate collector, indium tin oxide (ITO) plates (Delta Technologies Ltd.). The ITO plates are made with unpolished float glass with a measurement of 18 x 25 x 1.1mm and are SiO₂ passivated. The HVPS is connected to the metallic needles and the substrate [Figure 1]. The distance between the tip of the needles and the substrate is 5cm [15-17].



Figure 1: This is the electrospinning set-up used to make titania nanofiber mats; (clockwise from bottom) syringe pump, high voltage power supply (HVPS), conductive collecting substrate.

Combining acetic acid, ethanol, PVP, and titanium precursor, makes a polymer/sol-gel solution to be loaded in the syringes [13, 25]. First, Solution A is made by combining the following chemicals in a small vial: 2.5ml ethanol and 0.15g PVP. Place the vial in a sonicator (Sharpertek) for 30 minutes until thoroughly mixed. Then, Solution B is made in another small vial by mixing 0.52 ml titanium precursor, 1ml acetic acid, and 1ml ethanol and stir for 10 minutes. Carefully pour Solution B in Solution A and stir for 1 hour. This is the titania solution that will be loaded into the syringes. Any unused amount of titania solution should remain in the vial and continuously stirred. This keeps the mixture consistent and prevents solidification.

Electrospinning is done at room temperature [25-27]. At a specific flowrate of 10ml/min, the syringe pump was programmed to drive the titania solution to the needle tip and form a droplet. When the HVPS is turned on, a voltage of 8kV is applied to the needles; the droplet appears to stretch into a structure called the

Taylor cone. Eventually, an electrified liquid jet is formed. The jet is then elongated and whipped continuously by electrostatic repulsion and deposited on the grounded collector. This takes about 3 seconds. Whipping due to a bending instability in the electrified jet and evaporation of solvent allows this jet to be stretched to nanometer-scale diameters.

The plates with the electrospun titania nanofibers are then placed in a standard lab oven (VWR International) and baked at 200°C for 24 hours. These should be annealed for another 24 hours to allow the nanofibers to properly set on the plates. Baking keeps the titania nanofibers in place when introduced to any aqueous environment [25, 35]. A 10nM solution of fibronectin is placed on the surface of the wires and allowed to dry. A light rinsing of DI water removes any salts that have collected on the surface. A control set of samples were not coated with fibronectin.

Cell preparation and incubation. HeLa epithelial (skin) cells are then added on the surface of the plates with titania nanofibers. HeLa cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, penicillin-streptomycin antibiotic at 37°C in 5% CO₂ [9, 20, 39, 40]. For this experiment, cells were plated at 8×10^4 cells per 35 mm well in 2 ml of growth medium. The cells were dosed with 1μl of lactic acid, 85% (Mallinckrodt Chemicals) and allowed to grow at 37°C in 5% CO₂. A control batch of samples that were not dosed with lactic acid were also prepared. These were incubated for 2 days at 37.5°C and 5% CO₂.

Impedance measurement. Electrical impedance was measured at 24 hours, 48 hours, and 72 hours after plating for first set of titania nanofibers to observe growth effects [40]. For the lactic acid experiments, only 24 hour and 48 hour measurements were taken. Electrical measurements were conducted with a Keithley 4200 Semiconductor Characterization System (SCS) connected to a VersaSTAT3 System (Princeton Applied Research). The samples were placed on a probing station [Figure 2]. Two tungsten probes are lowered to touch the surface of the ITO while a reference electrode was submerged in the media. The V3 Studio computer program was set to take galvanostatic electrochemical impedance spectroscopy (EIS) measurements [39-41]. The properties of the AC measurements are as follows: 1) Start frequency of 100,000 Hz, 2) End frequency of 1 Hz, and 3) Amplitude of 100 μA.

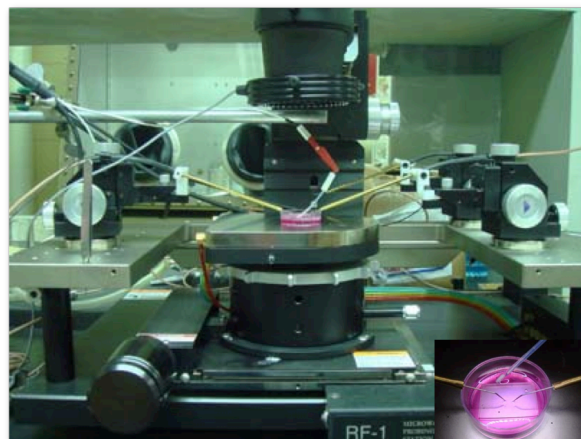


Figure 2: This is the probing station set-up with 2 tungsten probes touching the surface of the ITO plates in a culture dish of HeLa cells.

Optical imaging. Imaging of the titania nanofibers and cells were done using an optical microscope (Olympus). The growth and attachment of the HeLa cells to the titania nanofibers were observed. Live HeLa cells have a flattened and elongated appearance as opposed to the round shape of dead HeLa cells [Figure 3].

Results and Discussion

A total of 36 samples of ITO plates with titania nanofibers submerged in media with HeLa cells were tested per day.

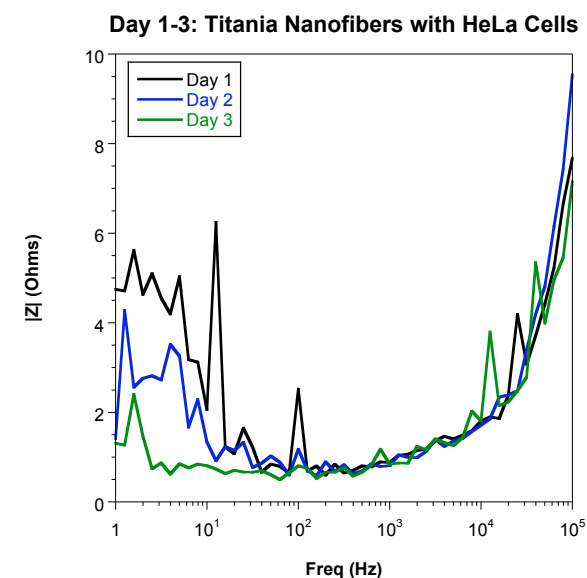


Figure 3: This shows the growth of HeLa cells on titania nanofiber mats from Day 1 to Day 3. Cells begin to overcrowd and die on Day 3.

The first set of impedance measurements show the growth of HeLa cells on ITO plates with titania nanofibers, without a fibronectin layer [Figure 3]. An average of all samples were recorded.

As shown in Figure 3, the resistance decreases each day the measurements were taken. After 72 hours, a large amount of cells have grown and tend to overcrowd leading to death.

The second set of impedance measurements show the effect of lactic acid on HeLa cells [Figure 4]. The average of all samples were also recorded. On Day 1 of lactic acid dosage, the cells begin to attach and grow on the nanofiber mat. A minimal amount of live HeLa cells was observed and dead cells start to appear due to the lactic acid. As expected on Day 2, the incubated HeLa cells started to overcrowd and dead cells were apparent.

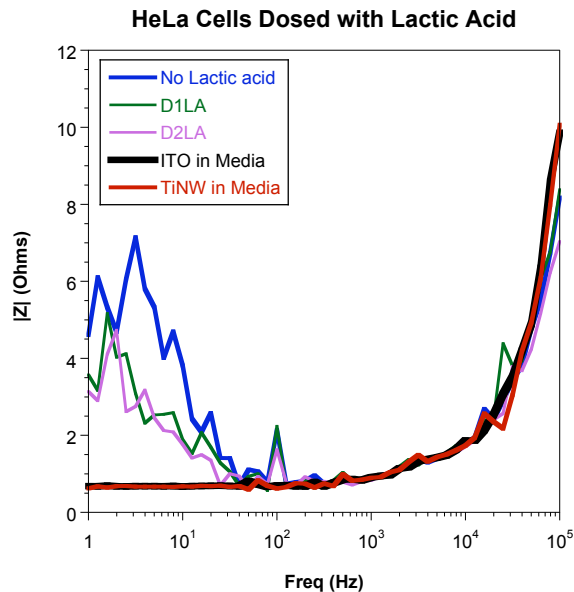


Figure 4: The graph shows the effect of lactic acid on HeLa cells when applied on Day 1 (D1LA) and Day 2 (D2LA). The control batch indicated by the blue line has no lactic acid. These are compared against the baselines of an ITO plate in DMEM, and ITO with the titania nanomats in DMEM.

As shown in Figure 4, the impedance data for the plates without lactic acid, Day 1 lactic acid dose, and Day 2 acid dose were compared with an ITO plate and ITO plate with titania nanofibers in DMEM, the media used for the cell cultures. This indicates that the addition of HeLa cells and lactic acid to the surface of the titania nanofibers produces a significant noisy signal. Comparing the plates with no lactic acid and the plates dosed with lactic acid, a slight decrease in resistance is observed. This again could be due to the cell death on the surface.

HeLa cells start out as flattened and elongated cells [Figure 5]. As they grow and overcrowd the surface, they die and appear round. The attachment and spreading of cells on surfaces is a complex and versatile process and results in growing contact area [41]. With time the adhesion state to the substrate alters due to the strength of adhesion, changes in number of cells and alteration of the cytoskeleton.

When lactic acid was introduced to the monolayers of HeLa cells, the pH of the media immediately changed. The 1 μ l lactic acid dose appeared to already kill the cells present before incubation, but as observed, live cells were still present after 24 hours. Future experiments should include a specific dosage that will immediately lead to cell death.

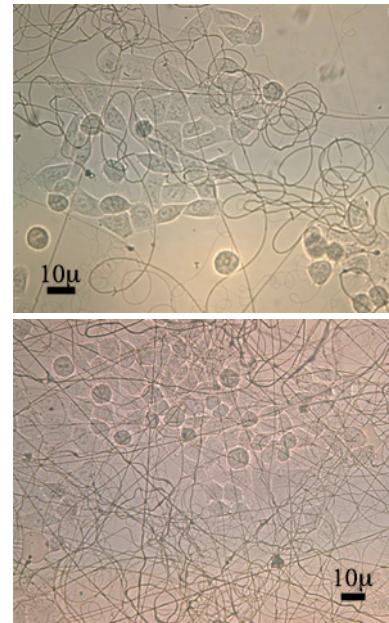


Figure 5: The images of titania nanofibers with HeLa cells dosed with lactic acid is shown. Flattened, elongated cells are the live cells while the rounded cells are dead. The image of Day 2 (bottom) of lactic acid dose shows cell overgrowth compared to Day 1 (top) of lactic acid.

Preliminary results show evident cell growth on the surface of titania nanofiber mats. Fibronectin is not necessarily needed for the attachment of the cells on the surface of the titania nanofiber mat. It is merely a protein that helps cells internalize the wires. Electrospun layers of titania nanofibers and the layers of HeLa cells have an effect on the ITO substrate's electrical properties. The cells tend to have an effect on the impedance measurements. The noisy signal could be a response whether it may be cell attachment to the titania nanomats or cell death.

Conclusions

The addition of fibronectin on the surface of the titania nanofibers does not contribute a lot of difference in impedance data. Fibronectin simply aids in cell internalization of the wires.

Changes in the impedance spectrum in the growth experiments may have indicated cell death. Lactic acid increases cell death, but after observing the cells after incubation, not all cells have immediately died. The lactic acid dosage of 1 μ l was not enough to kill the cells in 2ml of DMEM. Thus the dosage could be increased to a more significant amount to readily observe the signal the cells transmit once killed.

Preliminary tests show cell response with an increase in resistance when added to the surface of the titania-composite nanofiber mats. Cell growth and death response has also been shown with a decrease in resistance after a 72 hour incubation period. Cell death response is further shown with the addition of lactic acid. These are encouraging for future cell-based sensors for detecting growth, attachment and spreading, and cell death.

Work is ongoing to replicate the lactic acid dosage and cell incubation periods to establish protocols for quantitative analysis of cell growth and standardize device and testing.

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