A Cell-Sensory Bioscaffold of Biocompatible Titanate Nanofiber

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ABSTRACT

To date, little has been reported in literature about turning a bioscaffold into an electrochemical sensor, because often the bioscaffolds are electrochemically inactive. An ideal cell-sensor criteria is to be simple, sensitive, reliable, directly adaptable to pathological clinic, and easily fabricated in a large scale at low-cost. For over a decade, the titanate nanowire has been proven as a bioscaffold of the new type for applications in the implants, cosmetic, and pharmaceutical fields. In the present work, the titanate nanofibers were first of all grown on top of an implantable titanium metal, and characterized by means of SEM, XRD, etc. In an aqueous phospate buffer saline (PBS), the as-made bioscaffold showed a characteristic electrochemical impedance spectrum as a baseline. After being incubated with the human breast cells, a new impedance spectrum was recoded, suggesting that upon binding onto the bioscaffold the cells have altered the surface charge-density across the nanofiber-bioscaffolds. This exciting and long-overdue change in the electrochemical signal has been realized for the first time on such a bioscaffold. This new method can be potentially used in various important applications in future cancer screening and monitoring in vitro and in vivo at ultra-low-cost and in real-time, which seems promising and exciting.

Keywords: Cell sensing, bioscaffold, Titanate, nanofibers.

1 INTRODUCTION

Electrchemical cell-sensing is an active forefront in research that has been progressing rather slowly. For decades, however, the electrochemical cell-sensing is often realized on a surface of an electrochemically active material in a physical or chemical environment far from that of a typical bioscaffold. For example, the electrical cell-substrate impedance sensing (ECIS) was demonstrated on the gold-electrode as a noninvasive method by Giaever and Keese to study the cell-substrate interaction [1]. Thus, developing a bioscaffold-based electrochemical cell-sensor has been a long-unmet challenge in both the biomaterials and the biosensing fields.

Recently, nanowires of different types have attracted wide attentions thanks to their protentials for developing new biosensors, some showing a high sensitivity for detecting even a single molecule [2]. Among these smart nanowires, the biocompatible titanate nanowire of wide-bandgap semiconductor has been grown on titanium metal and fabricated into the new bioscaffold for new appllications in bioimplants, cosmetics, pharmaceutics, etc. For instance, the electrochemical detection of bacteria such as slmonela and lesteria have been realized directly on the titanate nanowires [3]. This is because thus-made titanate nanowires can have their bandgap varying widely between 1.8 eV and 4.1 eV, making them ideal for impedance sensing. Further, the titanate nanowires have been grown directly on top of the titanium metal as the bioscaffolds that can support the stem-cell prolifereation [4], and this nanosynthesis is relatively easy, simple hence scalable. In addition, the ideal cell-sensor should be simple, sensitive, reliable, directly adaptable to pathological clinic, and easy for mass-production in a large scale at ultralow-cost. This unique set of the properties for the long-overdue cell-sensors can be practically realized on the titanate nanowire-based bioscaffold surface in theory.

In this study, the biocompatible titanate nanowires were first of all grown into the bioscaffold for developing such a long-overdue type of label-free electrochemical biosensor for direct detection of different kind of human cells. In an aqueous phospate buffer saline (PBS), the as-made bioscaffold showed a characteristic electrochemical impedance spectrum as a baseline. Then, the human breast cells (MCF10A) exhibited a quantitatively and reproducible measure in the electrical conductance and capacitance using electrical impedance scanning (EIS) method. This result suggests that the breast cells have altered the surface charge-density on the nanowire bioscaffolds upon bounding on the surface. These new data have proven the electrochemical property tunnable on the bioscaffold by the cell, which is potentially useful in important applications in monitoring

and quantifying cells *in vitro* and *in vivo* on the bioscaffolds at ultralow-cost and in real-time.

2 MATERIALS & METHODS

2.1 Preparation of Self-Assembled Titanate Nanowires

In the nanosynthesis [4], the titanium foil (from Alfa Aesar) was cut into 0.5cm × 1cm in size, sonicated for 10 min in 10 mL of acetone at room temperature, and then rinsed with the deionized (DI) water. Then, the Ti foil was placed in a Teflon-lined lave container pre-loaded with 10 mL of 1.0 M NaOH solution, then sealed and hydrothermally heated at 240 ℃ for 4 –12 hours, which is summarized in the flowchart below (**Fig. 1**.).

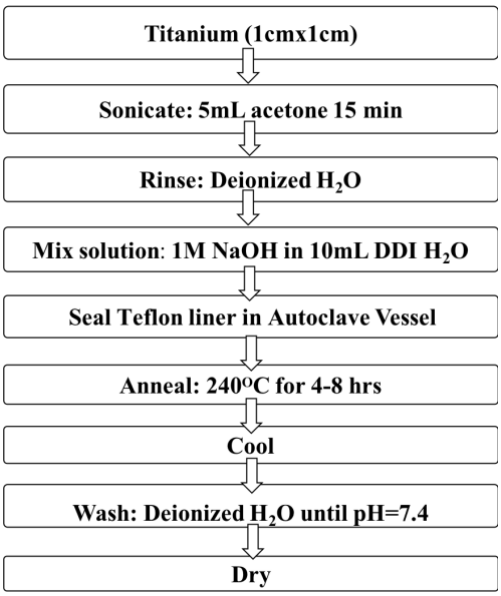

Fig.1. Flowchart for the nanosynthesis of the titanate nanowire-based bioscaffold.

Thus-treated Ti folis, covered by the titanate nanowires scaffolds, were finally collected, rinsed with the DI water, and dried in air [4]. As illsutrated in the **Fig. 2**, each thus-treated foil had its top part together with the back side being masked with the electrically insulating epoxy, leaving the bottom part of the bioscaffold in the front to interact with the cells for performing the cell-sensing.

2.2 XRD and SEM Characterizations

The crystal structure and phase purity of the nanowires were characterized by the powder X-ray diffraction (PXRD) on a Rigaku Miniflex X-ray diffractometer using the Cu Kα (λ=1.5405Å) as the radiation source scanning from angle 10 to 60 (2θ) at a speed of 2/min (**Fig. 3**). The morphology of the nanowire scaffolds' surface and cross section were examined under a scanning electron microscope (SEM, Tescan SEM VEGAII SBH) performed at 20kV (**Fig. 4**).

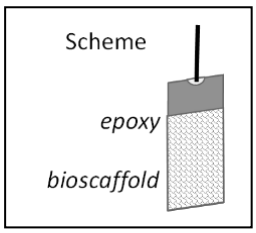

Fig.2. The schematics of the electrode configuration.

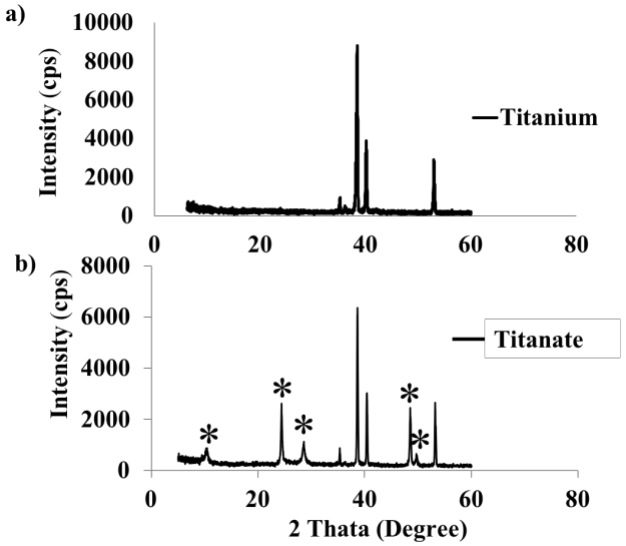

Fig. 3. PXRD patterns. For the Ti metal (a), and the titanate nanowires (b) by the asterisk-denotion.

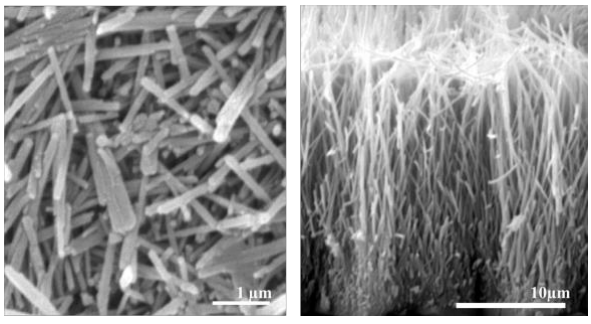

Fig. 4. The titanate nanowire-scaffold's surface and corss-section veiw.

2.3 Cell Impedance Sensing

The breast cell being tested here is from the normal human epithelial cell line (MCF 10A) from the ATCC, USA. The cells were suspended in 1mL of 1x phosphate buffer saline (PBS) with $1x10^6$ cells/ml. The cell count was determined by using hemocytometer chamber and stained with 0.4% Trypan-blue (from ThermoFisher Scientific, USA). The impedance-based cell-sensing was realized on three such biocaffolds with the similar baseline in their impedance spectra. Each of the bioscaffolds was incubated first in the 10ml 1xPBS solution containing 1×10^5 live cells/ml for 35 minutes and then taken out.

The cell-sensing was done immediately using the two-electrode method in which the bioscaffold is the working electrode (WE), and a platinum plate is the counter electrode (CE) on the machine i.e. Gamry Refrence 600 (by Gamry Instrument, USA). The parameters was setup by varying the frequency starting from 300 kHz to 1MHz (**Fig. 5**).

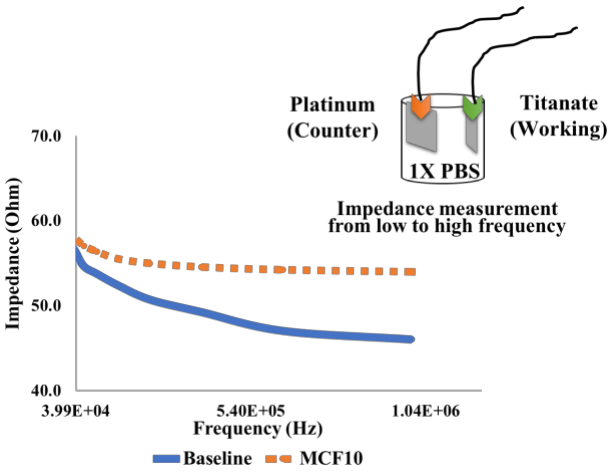

Fig. 5. Impedance measurements of titanate (base line) and MCF-10A, over a frequency range (30 KHz - 1.0 MHz). The results are an average of five trials with standard deviations been determined.

3 RESULTS & DISCUSSION

The **Fig. 5** shows two curves. The blue one is the baseline, the organge one shows a characteristic impedance signature of the human brest epithelial cells, and each measurement is averaged out of five trials. The orange cruve has indeed quantitatively characterized the capacitive nature of the cell lines, with respect to activity of the cell-membrane proteins. In general, the magnitude is decreased with the increase in the frequency. As can be seen in the **Fig. 5**, there is a significant difference between the normal MCF-10A and the bioscaffold in the same 1× PBS, and the difference is indeed the basis for the electrochemical cell-sensing. This result seems in line with a similar cell-sensing but again on a glod electrode [5], since the surface of the gold electrode is different than that of a bioscaffold. Even on the gold surface, the progression stages of cancer cells were quantified in the label-free manner using the impedance method [6,7], which is highly inspiring. Moreover, Yang *et al*. realized a real-time impedance quantification of the cellular activities to distinguish oral cancer cells from the normal epithelial esophageal Het-1A cells [8]. In addition, Cui *et al*. recently reported the electiccal cell substrate impedance to monitor the skin wound healing on nano-grooved electrode *in vitro* [9]. These studies have sugegsted that the direct electrochemical cell-sensing on a bioscaffold is important in science but difficult in practice, hence seldom reported in lietrature to date.

4 CONCLUSION

The nanowire-entangled bioscaffold has for the first time been successfully used to directly quantify the impedance signature of the normal human breast tissue cells. Like the cell-sensing on the gold-electrode, this sensory bioscaffold could potentially lead to the development of a powerful tool for detecting different cells e.g. bacteria, and quantifying the cells behavior when in contact with different therapeutic drugs, which are highly encouraging.

5 ACKNOWLEDGMENTS

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