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Primary cortical neurons on PMCS TiO_2 films towards bio-hybrid memristive device: A morpho-functional study

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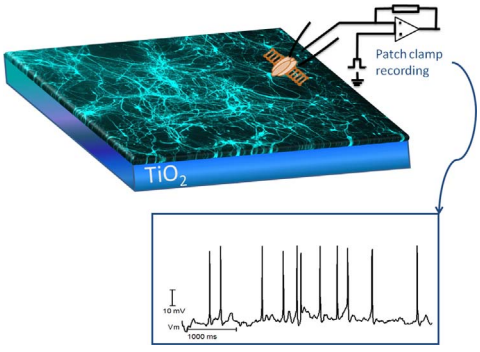
HIGHLIGHTS

- Stoichiometric or oxygen over-stoichiometric films were produced.
- Murine cortical neurons were successfully grown on TiO_2 films.
- The bioelectrical activity of adhesive neurons was enhanced by TiO_2 films.

GRAPHICAL ABSTRACT

Murine cortical neurons were grown on TiO_2 film prepared by PMCS technique and a detailed morpho-functional analysis was performed



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ABSTRACT

We report a comprehensive study of the biocompatibility and neurocompatibility of titanium dioxide films (TiO_2) prepared by Pulsed Microplasma Cluster Source (PMCS). This technique uses supersonic pulsed beams seeded by clusters of the metal oxide synthesized in a plasma discharge. The final stoichiometry of the TiO_2 thin films is tuned changing the gas mixture, achieving stoichiometric or oxygen overstoichiometric films. All the films showed consistent biocompatibility and a spontaneous absorption of poly-D-lysine (PDL) that favors the adhesion and growth of murine cortical neurons. Moreover, the bioelectrical activity of the neuronal culture grown on the TiO_2 film can be modulated by changing the chemistry of the surface. This work paves the way to develop a bio-hybrid neuromorphic device, where viable nerve cells are grown directly over a titanium dioxide film showing a network of memristors.

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1. Introduction

Titanium alloys and titanium dioxide are widely used in medicine. Due to their corrosion resistance and biocompatibility combined with high mechanical performances, they have been used in long term implantable devices, in particular for teeth and bone surgery [1].

Besides medical uses, one of the more promising applications of TiO_2-based materials is related to the realization of memristive devices [2,3]. Memory resistors or memristors were conceptually designed in 1971 by Leon Chua [4]. They are characterized by a pinched hysteresis loop in the current-voltage domain when excited by a bipolar periodic stimulus. Memristors have adaptive learning ability and could be used as computing elements able to perform both data storage and processing, as actually occurs in natural neuronal networks.

In the last decade, not only the scientific community but also the industrial world invested time and resources in the development of nanoscale synaptic devices integrated into neuromorphic circuits [5–7], emulating the behavior of natural synapses.

Neuronal cells communicate through both electrical and chemical signals and can form very complex networks by interacting with each other, making them one of the most complex biological systems in nature. This high degree of interaction allows neural cells to constitute “computing units” with a large parallel processing power, greatly overcoming the capacity of Von Neumann based computing devices [8]. Neurons modify their conductive and resistive capacity depending on their previous “stimulatory experience”, allowing the information to proceed only when the received stimuli exceed the voltage threshold of the axon hillock.

A memristor has a functional resemblance to a synapse [9]. In fact, the electrical resistance of a memristor depends on the history of current that had previously flown through the device. The resistance state can be maintained, paving the way for realization of resistive RAM devices that can be controlled by overcoming a dynamic threshold by the signal transmission.

While organic semiconductor like polyaniline (PANI) showing memristive properties have demonstrated to be viable for neuron-like cells growth [10,11], up to now the direct growing of nerve cells on inorganic memristors is poorly reported and understood. The coupling of human neuroblastoma cells to a memristor through a MEA (Multi-Electrode Array) device in 2013 was a first attempt [12]. Stimulating results in this direction have been recently obtained by Gupta et al. [13], placing a high number of electrodes below the neuronal culture with a solid state TiO_x memristive device.

The in-situ growth of neuronal network is a relevant and challenging goal, possibly overcoming the strong limitations that present approaches based on biocompatible electrodes coupled to conventional electronics are facing to study brain properties.

The first step in this direction is the evaluation of the neuronal biocompatibility with the metal oxide layer (titanium dioxide in our case). The inorganic film is here obtained in vacuum using a supersonic beam seeded by clusters of metal or metal oxides, the Pulsed Microplasma Cluster Source approach (PMCS). This technique was previously utilized to develop supercapacitors [14], optical devices [15,16], sensor arrays [17–19], solar cells [20] and gas sensors [21–23]. Most of all, PMCS has shown the ability to control film stoichiometry, i.e. oxygen content, achieving a high degree of nanocristallinity at room temperature growth, without any thermal treatment [24,25]. For these reasons this technique is highly promising for development of memristive devices [26].

The biocompatibility of the titanium dioxide films deposited by the PMCS technique has been previously demonstrated using human fibroblasts and melanocytes [27]; other applications such as platform for viral vectors immobilization [28] or for protein arrays [29,30] have been reported.

Even if examples of neuronal cell growth on different kind of TiO_2 films can be find [31], to our knowledge, no previous experiments have been reported on primary neuronal cultures growth on PMCS-derived TiO_2 films.

The chemistry and the morphology of the surface can strongly affect cell adhesion, proliferation and differentiation [1]. For this reason, we evaluated these parameters using molecular and high-throughput microscopy approaches.

In this work we performed the morphological and functional evaluation of a culture of cortical neurons grown on TiO_2 films. The neuronal morphology and electrical activity were studied through functional parameters (such as neurite’ length, number of extremities, cell density) and patch-clamp electrophysiology at two different developmental stages (7 and 14 days in vitro, 7 DIV and 14 DIV respectively). In parallel we analyzed the chemical and morphological aspects of the titanium dioxide surface. X-ray photoelectron Spectroscopy (XPS), atomic force microscopy (AFM), static contact angle (CA), profilometer and fluorescence microscopy were utilized to check the quality of the PCMS-derived films.

In this work we lay the basis for the development of a TiO_2-based bio-hybrid memristive device, where neuronal networks grow directly on the inorganic surface. This work paves the way for the development of a bio-hybrid TiO_2-based memristive device that supports the interaction with a neural network. This layout would bring enormous advantages in research fields like neurobiology and cognitive sciences, where the study of brain properties is still tied to biocompatible electrodes coupled to standard electronics. The application of this technology could span from the modeling of artificial neural networks to the construction of novel surgical devices.

2. Materials and methods

2.1. Materials

Fluorescein-labeled poly-L-lysine (PLL-FITC, P3069), Cytosine β-D-arabinofuranoside (C1768), Dnase (D5025), Paraformaldeide (P6148) and monoclonal Anti-β-Tubulin III (neuronal) antibody produced in mouse (T8578) were purchased from Sigma Aldrich (Milan, Italy). Neurobasal (21103–049) plus B27 supplement (17504–044) and polyclonal anti-mouse IgG Alexa Fluor® 488 produced in goat (A11017) were purchased from Life Technologies (USA). Papain solution (LS003119) for cells dissociation was purchased from Worthington Biochemical Corporation (USA). Sterile PBS pH 7.4 (AM9624) was purchased from Ambion (USA).

2.2. TiO_x films deposition

TiO_x thin films were grown on a 15 mm diameter microscope glass slide (Marieenfield 01115 50) by a pulsed microplasma cluster source (PMCS). This source is particularly well suited when the material to be deposited can hardly be sublimed, as titanium. In particular, this deposition process is based on the ablation of a titanium rod by a pulsed plasma, created by the ionization of an inert gas trigged by an electric discharge. After the ablation of the cathode, metallic ions thermalize with inert gas and achieve clusterization. The mixture of clusters and inert gas is extracted in vacuum through a nozzle to form a seeded supersonic molecular beam. Three different mixtures of inert gas with an increasing percentage of oxygen were used: 0% (pure helium), 0.1% and 1.0% of O_2 as reported in Table 1. Using a mixture including oxygen, the oxidation process is promoted already in the source, forming TiO_x clusters during the deposition. In 0% TiO_2 case, the titanium thin film must be exposed to air at room temperature in order to have transition from metallic phase to metal oxide structure.

2.3. Poly-L-lysine adsorption

The adsorption of Poly-L-lysine–FITC Labeled (PLL-FITC) was performed at 0.01 mg/ml in sterile PBS for 45 min at 37 °C. After incubation, the substrates were washed manually three times and the signal was acquired using a fluorescence microscope Leica DMLA, equipped with a mercury lamp and fluorescence filter L5 (Leica Microsystems, Germany). All samples were observed with a 20 × magnification objective and measured with a cooled CCD camera (DFC 420C, Leica Microsystems, Germany). The measured signal was quantified using the ImageJ software [32].