DEVELOPMENT OF PIEZOELECTRIC NANOSTRUCTURES FOR CELL STIMULATION

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ABSTRACT

This paper describes the first insights on the use of piezoelectric two-dimensional nanostructures or nanoflakes (NFs) to electrically stimulate living cells. In the past years, piezoelectric nanostructures have been widely used to fabricate power generators or energy harvesting devices. However, the number of biological applications of this kind of materials is more reduced. We have demonstrated that mechanical interaction produced between the cell and the nanostructures generates a local piezopotential that can induce changes in the intracellular calcium concentration. This observation makes possible the use of nanodevices based on piezoelectric materials for in-situ stimulation of electrically responsive cells like neurons or muscle cells.

INTRODUCTION

Since 2013, GSK's Bioelectronics R&D unit is pursuing a relatively new scientific field that could one day result in a new class of medicines that would not be pills or drugs but miniaturized, implantable devices [1], [2]. It has been proved that the electrical stimulation can be successfully used as clinical therapies [3]. Implantable electrodes are the most common application, however this solution is invasive and lacks of spatial resolution. Recent advanced in nano- and micro-technology allows the exploration of less invasive treatments or improving the spatial resolution [4]. Novel smart materials are also attracting a lot of attention, due to the new functionalities that can offer. Piezoelectric materials for instance create a coupling link between mechanical and electric domains. Some biological application of piezoelectric materials have been already explored, but mainly focused on generator or sensors [5], [6]. However, the application of piezoelectric materials and nanostructures to stimulate living cells has not been fully explored yet. In this work, we have first tested the use of piezoelectric NFs as generators of local electric fields in the surrounding of living cells to electrically stimulate them.

METHODS

In this work, we propose the use of ZnO NFs as piezoelectric generator at cell-level. The working principle that we envisaged is focused on the generation of an electric field in the immediacies of the cell when they are in close contact. This electric field is the result of the mechanical stimuli generated by the inherent cell forces transduced by the piezoelectric element.

We have chosen the ZnO NFs due to the high aspect ratio and reduced thickness of this nanostructure. These geometrical features provide a great sensitivity, allowing the deflection and the consequent charge separation due to very small forces. The ZnO NFs are synthetized by means of a simple and low-temperature hydrothermal method. Our ad-hoc procedure can be processed at wafer-level and allows selective-area patterning [7]. It is mainly based on a hydrothermal process at temperatures lower than 100 ºC by using an aqueous solution of zinc nitrate ($Zn(NO_3)_2$) and hexamethylenetetramine (HMTA) [8]. In order to obtain the NF morphology, we have use AlN catalyst layer over an (100) silicon substrate. The hydrolysis of the AlN creates a local pH gradient that favors growth of the crystalline and piezoelectric NFs [7]. The AlN thin layer is deposited by reactive RF magnetron sputtering on top of a thin layer of Ti/Pt that improves the AlN crystallinity [9].

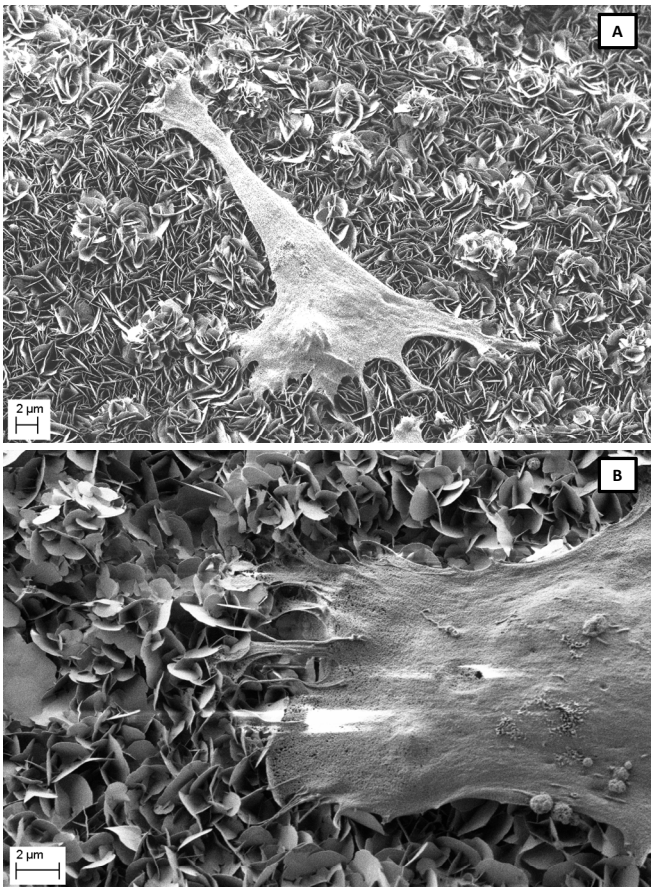

Figure 1: SEM images of Saos-2 cell cultured over a substrate full of nanoflakes (a) and detail of cell prolongations connecting to the surrounding nanoflakes (b).

Then, the substrate was diced into 5 mm by 5mm samples. On top of them, we cultured human osteosarcoma Saos-2 cells (ATCC) in Dulbecco's modified Eagle medium (DMEM) (Invitrogen) with 10% FBS under standard conditions. The samples (NFs and control substrates) were sterilized with absolute ethanol and individually introduced into a 4-well plate. Around 50,000 cells were seeded into each well and cultured for 24 and 72 h under standard conditions for viability studies.

RESULTS

We have found that cell viability, proliferation and differentiation were similar between the cells cultured on NFs and control substrates. In addition, we have observed that cells are able to proliferate and differentiate for up to 14 days after seeding on the piezoelectric NFs. Saos-2 osteoblast-like cells present voltage-gated calcium channels (VGCCs) in their membranes [10]. We have demonstrated that the presence of piezoelectric nanostructures in close contact with the cell opens its calcium channels, resulting in rapid and reiterative increases of intracellular calcium concentration ($[Ca^{2+}]$). Also, we have observed that cultured cells emitted short and long projections that firmly attached to individual NFs (Figure 1). The inherent mechanical stress generated by the cells when moving or adhering to the surface can be the input stimulus that feed the piezoelectric transduction and results in the electrical stimulation of the cells (Figure 2).

This cell-scale electrical stimulation can be used not only in osteoblast-like cells but in other excitable cells, such as neurons or muscle cells. The use of these nanostructured smart materials opens the door to the development of future bioelectronic medicines based on controlled and localized electrical impulses instead of chemical drugs.

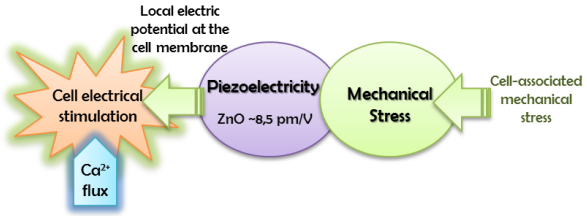

Figure 2: Proposal of working principle of the electrical stimulation of human cells by using piezoelectric nanostructures.

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