Different size microdevices with nanostructured ZnO integrated for electrical cell stimulation

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Introduction

Electrical stimuli have been proved to induce different responses in cells. Among them, proliferation, differentiation and migration are the most studied and they can vary depending on the type of cell, duration and intensity of the stimulus. Other physiological processes that are due to electrical stimuli are nervous impulses and muscle contraction.

To study how cells respond to these electrical stimuli and activate cellular pathways, electronic devices are used to deliver these electrical signals to cells. A safe way to deliver the electrical signal is to use devices with piezoelectric materials integrated [1, 2]. This way, cables -generally used when working with MEAs and other electrode matrices- and highly energetic electromagnetic pulses -that alter the cells and their environment- can be avoided. Ultrasounds (US) can be used as a wireless way of communication with the piezoelectric devices; even if implemented its use as an implant inside of our body [2], where US in the biomedical range (MHz) could deliver the signal safely. The mechanical stress produced by the pressure of the sound wave generate an electric dipole along the surface in piezoelectric materials because of the reversible reorganization of the charges on the atoms of the material [1].

To prove everything stated above, microparticles smaller than a cell were fabricated using microfabrication techniques [3]. Later, a piezoelectric layer of nanostructured zinc oxide (ZnO) was grown on top of them through hydrothermal synthesis. Saos-2 osteosarcoma cells were cultured with the microdevices to analyse their cytocompatibility. Currently, we are working on the effect of the electric fields generated by the microdevices on cells by stimulating them using ultrasonic pulses [1, 2].

Materials and methods

First, a microparticle array to be used as a template for the microdevices was designed following a microfabrication process in the clean room. Three different templates were designed: with microparticles of a size of 3 x 3 um^2, 6 x 10 um^2 and 12 x 18 um^2. In the microfabrication processs, the main steps were the metallization with a layer of aluminium nitride (AlN), the photolithography to define the particle and the etching to create the microparticles and their stand. The layer of AlN serves later as a seed layer to grow the piezoelectric ZnO in the form of nanosheets (NSs) through hydrothermal synthesis [4]. After this chemical synthesis, the microdevices were peeled-off, washed several times and suspended finally in ethanol, ready for its forthcoming use.

Secondly, a first approach to the biological characterization was performed by culturing the microdevices with Saos-2 human osteosarcoma cells. Microdevices of a 3 x 3 um^2 size were used. The experiments performed encompass a cytocompatibility test at days 1, 3 and 7, using calcein and ethium iodide; also, an internalization assay, where the cells were stained with phalloidine (actine fibers) and Hoechst dye (nuclei). Cells were observed at the confocal laser scanning microscope (CLSM) and, the microdevices in contact, quantified. In parallel, these cells were studied under the scanning electron microscope (SEM) to assess the positions taken. The intermediate size and bigger size microdevices were also tested for their cytocompatibility at day 3 and their internalization was also analysed after 24h without quantification.

Experiments stimulating these microdevices using ultrasonic pulses (ULTRASONIDO Sonic-Stimu Basic, Nu-Tek) are being performed on these Saos-2 cells and their cytocompatibility and calcium response using Fluo-4AM (calcium dye) under fluorescence microscope studied.

Results and Discussion

Once the microfabrication process in the clean room was finished, we obtained microparticles fabricated in Figure 1A. As it can be seen in the image, discrete microparticles with different sizes anchored to the substrate though a fragile stand were fabricated. The sizes of the microparticles were measured, finally being of 3.58 ± 0.04 µm x 3.56 ± 0.05 µm (small), 6.68 ± 0.06 µm x 10.96 ± 0.05 µm (medium) and 13.45 ± 0.64 µm x 19.27 ± 0.27 µm (large). After the hydrothermal growth step, the microparticles were covered by the ZnO NSs layer, increasing the size of the microdevice around 2 µm (Figure 1B). The ZnO NSs thickness obtained was different depending on the microparticle size, changing from a 17 nm thickness in the small ones to around 23 nm in the medium and 32 nm in the

large ones. The whole process was highly reproducible and scalable.

After the peel-off step, the percentage of recovery of the microdevices was of 82% for the 3 x 3 μm^2 area and below a 30% for the medium size (29%) and large size microdevices (26%). However, millions of microdevices are recovered from a single processed wafer despite the lower obtention of intermediate and bigger size microdevices [3].

After adding the microdevices into the cell culture in a 2:1 (small), 1:1 (medium) and 0.5:1 (large) proportion, cell viability remained above 85% for all of them, with respect to the control that was 96.5%. The internalization of the microdevices, as expected, decreases as the size increases. For the small microdevices, that were studied the most extensively, three positions are taken: internalized, NSs top down and NSs bottom up.

According to the results, these microdevices can be considered cytocompatible. They can also achieve a position outside the cell membrane with the ZnO NSs facing the membrane, an interesting position to trigger the VGCCs in the membrane [1].

Conclusions

Microfabrication techniques together with hydrothermal synthesis allowed to fabricate reproducible microdevices with different sizes and a piezoelectric layer of ZnO nanostructures. Their cytocompatibility was proved by culturing them with Saos-2 cells. The positions shown by the microdevices with respect to the cells were interesting to induce the electrical response in the cell. This is necessary for our upcoming work, as ultrasound actuation is currently being validated with promising expectations.

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Figures

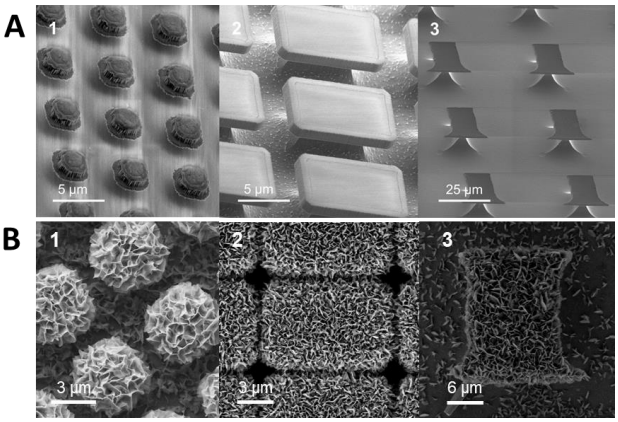

Figure 1. Microdevice fabrication. A) SEM image of the microparticle array: microparticles with 3 x 3 µm2 (1), 6 x 10 µm2 (2) and 12 x 18 µm2 (3) area. B) SEM images of the microdevices with the ZnO NSs piezoelectric layer.