Fabrication of gold nanopillars on gold interdigitated impedance electrodes for biological applications

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Abstract

Gold nanopillars have been successfully fabricated on top of interdigitated gold electrodes deposited on a Pyrex substrate and these were used for cytotoxicity monitoring on immortalized cells through the electrochemical impedance spectroscopy technique. These nanopillars were fabricated via contact metal deposition. E-beam lithography was used to define the pattern of nanopillars with dimensions of 150nm diameter and 500nm of distance between their edges in a honeycomb-like structure. These dimensions together with a, relatively, low aspect ratio (~50nm tall) have been chosen in the hope that cell adhesion will be promoted. Cell adhesion to these novel nanopillars is important as their ultimate use will be for cytotoxicity testing of cell cultures. This novel tool could potentially increase the sensitivity of this kind of analysis compared to its plain counterpart.

Introduction

Currently nanostructures, and more specifically nanopillars, are the centre of interest to many research groups working on the fabrication of electrodes for many different applications. The main applications of these devices are in the fabrication of solar cell and in the biological-medical field.

Structures like nanopillars are being used in the solar cell applications as they can reduce optical reflection and enhance both the absorption of incident photons and the carrier collection efficiency [1]. Rider et al. [2] report photovoltaic cells with nanopillar arrays have enhanced performances over their nanostructure deficient counterpart. In the biological and medical field, nanopillars have often been used to modify the substrate for growth of neuronal cells [3, 4, 5, 6], DNA separation and analysis [7, 8], measuring and monitoring biological reactions [9, 10, 11] and as a tissue culture substrate [4, 12, 13, 14, 15, 16]. The main reasons for focusing research on these kinds of nanostructures combined with different kinds of electrodes are that they can potentially achieve higher sensitivity given by the increased working surface area and allow for enhanced mass transport [10, 17, 18].

The gold nanopillars are fabricated on the surface of interdigitated impedance gold electrodes which are deposited on a Pyrex substrate using a combination of contact

metal deposition, e-beam lithography and metal evaporation techniques. These electrodes are subsequently packaged on a PCB in order to create a well of 12 mm^3 in which cells will be cultured, using the flip-chip method.

Fabrication

The contact metal deposition is done by the standard lift-off process. Pyrex wafers are first dipped in 5:1 BOE solution for 5 seconds to clean and promote resist adhesion. HMDS adhesion promoter is then spun on at 3000 rpm for 50 seconds. The same procedure is then adopted for the lift-off resist MicroChem LOR3A before baking the wafers on the hot plate at 150°C for 3 minutes. MicroChem S1805 imaging resist is spun on at 3000 rpm for 50 seconds and wafers are hot-plate baked again at a temperature of 115°C for 2 minutes. They are then aligned with their mask and exposed to UV in a Karl Suss MA1006 mask-aligner with an exposing dose of 40mW/cm^2. The wafers are subsequently developed in Microposit MF319 developer for 60 seconds and rinsed in DI and then oven baked at 90°C for 30 minutes. They are then loaded in Temescal FC2000 e-beam evaporator and the chamber is evacuated to <5x10-7 Torr and Ti:Au (10:200nm) is evaporated onto the wafers. Surplus Ti:Au and photoresist are lifted off in Microposit R1165 resist stripper, leaving the desired metal contact pattern on the Pyrex wafers. A 50 nm thick layer of Silicon Nitride is deposited on the wafers in the STS PECVD (Plasma Enhanced Chemical Vapour Deposition) system. E-beam lithography is then employed to define the nanopillars pattern on top of the Silicon Nitride overlying the metal fingers. The wafers are prepared for this step with a coating of a layer of 1:1 ratio mix of ZEP 520A Resist with ZEP A Thinner which is spun first at 500 rpm for 10 seconds and increased to 3000 rpm for 60 seconds. To allow better alignment and thus reducing error with the e-beam process, it was necessary to evaporate a layer of 200nm of ITO on the opposite side of the wafers to reduce the high transparency typical of the Pyrex material. Next, the beam draws the desired patterns (which have been previously designed on dedicated software and recalled at the moment of the procedure) on the interdigitated finger electrode. After this step, the wafers are dipped in ZED N50 developing solution for 30 seconds, then immersed in IPA for further 30 seconds and finally dried using a nitrogen gun. The Silicon Nitride is etched through the metal fingers in the STS RIE (Reactive Ion Etching) system where the nanopillars patterns are defined by using the e-beam photoresist as a mask. Wafers are then again loaded in to the Temescal evaporator and evaporated with Ti:Au (5:65nm) to fill the holes created with the RIE. The excess metal and the photoresist are removed using again the Microposit R1165 resist stripper leaving nanopillars of, approximately, 50-60nm in height (Fig. 3 and 4).

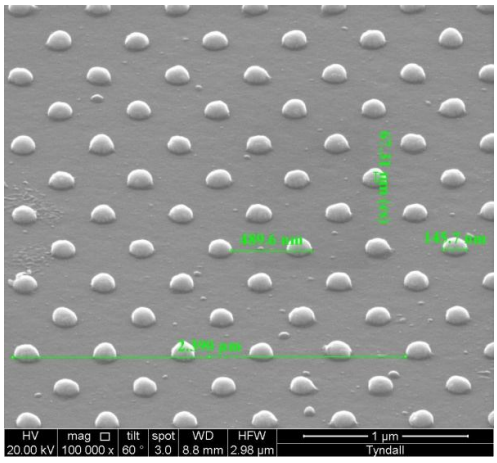


Fig.1: SEM image of the nanopillars on the finger electrode. The dimensions drawn in the picture are approximate; their nominal dimensions are 150nm in diameter, 500nm pitch and 50-60nm in height

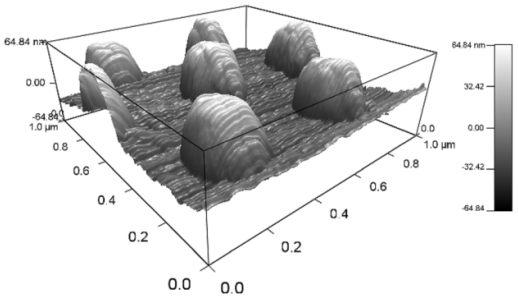


Fig. 2: AFM image of the nanostructured surface

Characterization

A contact angle machine was used to test the hydrophobicity and hydrophilicity of the electrode surface (Contact Angle System OCA, DataPhysics Instruments, Fig. 3 and 4). Using the sessile drop method (part of the SCA202 software installed on the system) the following results were obtained and they are represented in table 1, 2 and 3 for the nanostructured electrode, the flat electrode and the bare Pyrex substrate:

Nano	
CA left	CA right
44.2	42.4
49.7	47.8
45.7	48.3
46.4	50.1
49.1	51.6
47.02	48.04

Table 1: Contact angle results on the nanostructured electrode

Flat	
CA left	CA right
41.7	40.9
42.2	43.3
43.4	40.8
45.0	44.0
43.3	43.3
43.12	42.46

Table 2: Contact angle results on the flat electrode

Bare substrate	
CA left	CA right
39.2	38.8
40.7	36.9
42.8	40.3
41.6	39.7
36.6	36.6
40.18	38.46

Table 3: Contact angle results on the bare Pyrex substrate

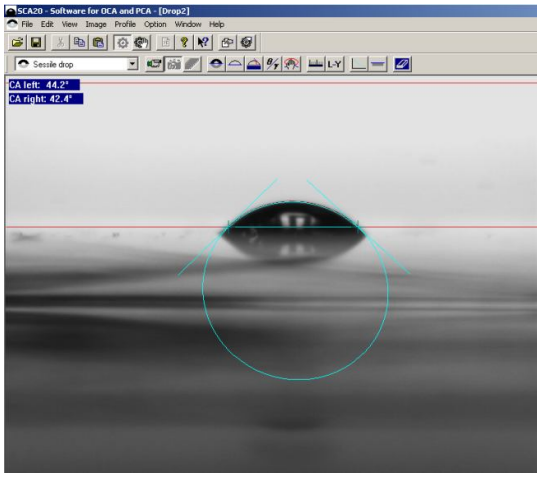


Fig. 3: Contact angle measurement on the nanostructured electrode

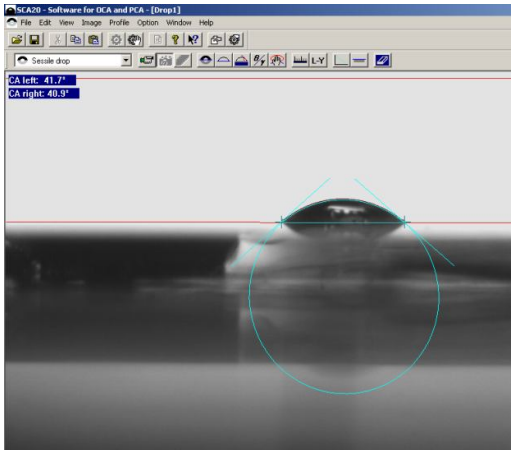


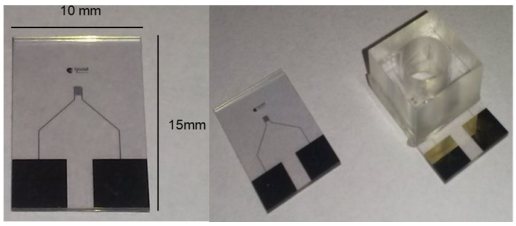
Fig. 4: Contact angle measurement on the flat electrode

These contact angle figures suggest that the presence of the nanostructures on the electrode surface introduce a small increase in hydrophobicity of the device. Both the average angles for the surfaces are well below the threshold of 90°, over which would define

them as hydrophobic surfaces. The difference between the angles measured for the two samples is not huge but it shows that the flat surface is slightly more hydrophilic as expected.

Packaging

After the fabrication, the wafer is covered with a thin dicing-protective layer of silicon nitride and sent to the dicing laboratory where the chips were cut in rectangles of 10mm by 15mm as shown in figure 5a. The protective layer was then dissolved by soaking the devices for 5 minutes in acetone heated at 60°C and rinsed in DI water. Then a Perspex well of 10mm in length, 10 mm in width and 6mm in height was attached to the Pyrex chip using a double-sided medical grade adhesive to create a volume of approximately 28mm^3 where the cells and the media for their growth will be placed. The diameter of the central hole is 6mm.



Cell Culture

A5495 cells were cultured in sterile growth medium. The basal media DMEM (Sigma, Ireland) was supplemented with 10% fetal bovine serum (Sigma, Ireland) and 1% L-Glutamine.
HBSS without calcium and magnesium (Sigma, Ireland) was used in the culturing process as a rinsing agent. Trypsin /EDTA (Sigma, Ireland) was used to detach the cells from the surface of the tissue culture flask. The process was as follows: spent media was removed and discarded. The flask was rinsed with 10mls warmed HBSS twice. 3mls of trypsin-EDTA was added and the flask was incubated for 5 mins at 37^{o}C.

An excess of routine media (7mls) was added and transferred to a 15ml falcon tube and centrifuged at 1000rpm for 5 minutes. Supernatant was removed and discarded.

The pellet was resuspended in routine media and transferred to new flask with a sub-cultivation ratio of 1/3 to 1/8. 30ml of media was required for the final volume. Media renewal was every 2-3 days. The cells were grown at 37 °C in a humidified 95% air/5% CO2 environment.

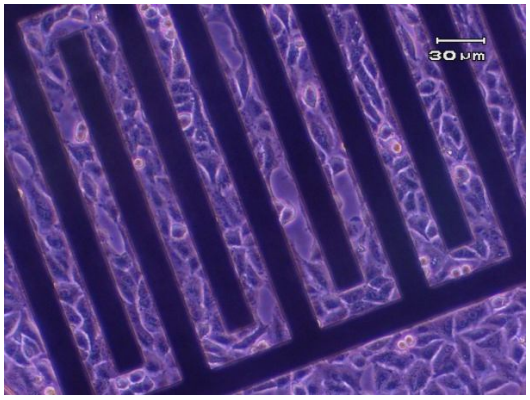


Figure 6: A549 cells growing on a nanostructured device (phase contrast microscope)

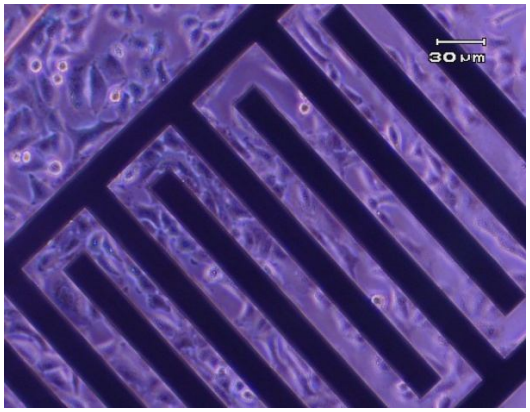


Figure 7: A549 cells growing on a flat device (phase contrast microscope)

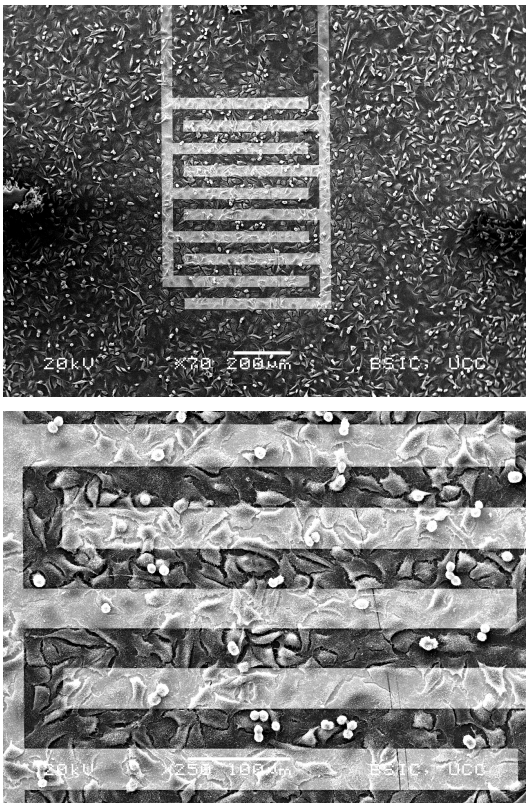


Figure 8 and 9: A549 cells growing on a nanostructured device (Scanning electron microscope)

Cytotoxicity preliminary experiments

A549 cells were used to perform cytotoxicity tests using an extract of Antrodia Camphorata which is a particular mushroom growing only in Taiwan. It has been used as a traditional medicine for protection of diverse health-related conditions and in our case we are interested in its anti-cancer proliferation properties. [19, 20].

Cells were cultured on five devices of each type. One was used as a positive control, one as a negative control and the remaining three were used to test the mushroom extract. The cells on the positive control were let grow for the entire length of the experiment, i.e. 48 hours. After 24 hours, a drop Triton™ X100 (Sigma-Aldrich, Ireland), a detergent for bio hazardous surfaces, was pipetted into the well containing the cell culture with the intention of being sure of killing the cells seeded on the device and this sample was assumed as a negative control. Again, after 24 hours, the remaining three cell cultures were exposed to 500µM of Antrodia Camphorata extract.

Impedance values (in our case we considered the resistive part) for the two sets of 5 devices, were recorded after 24 hours to give them time seed, spread and duplicate. At 48 hours impedance figures were recorded again in order to evaluate the effects of the mushroom extract. In the graph below it is possible to note that the positive controls show minimal or no changes; the cells used in the negative control died as expected; the cells treated with the extract of Antrodia Camphorata have figures almost equal to those of the negative controls at 48 hours.

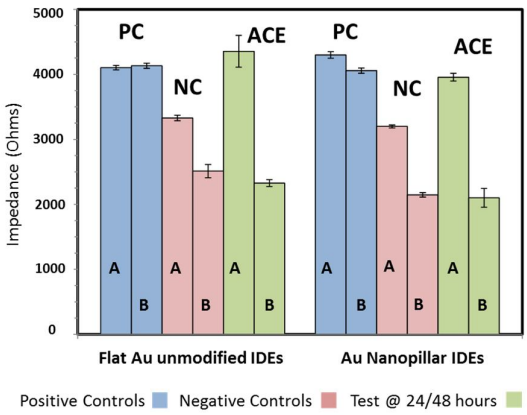


Figure 10: Graph showing the positive control (PC), negative control (NC) and Antrodia Camphorata Extract (ACE). A refers to values recorded at 24hours, B to those recorded at 48hours.

It is not possible, though, to define which one of the structure would give a better sensitivity in the experiment as the values are very close for both sets of devices.

Conclusions and future work

Gold nanopillars have been successfully fabricated on interdigitated gold electrodes deposited on a Pyrex substrate using techniques like contact metal deposition, RIE, PECVD and patterning a photoresist mask via e-beam lithography. The nanopillars have dimensions of 150 nm in diameter, pitch of 500 nm and are 50-60nm in height. Several configurations have been investigated, however the nanopillars with the described figures and the honeycomb-like structure has been preferred over others as the resist stripping process for these was more satisfactory giving better results.

Analysis with the contact angle system showed an expected increase of the hydrophobicity of the nanostructured surface. The average contact angle measured on the nanopillars is larger, approximately 5-6°, than that measured on the flat surface electrode.

The electrodes were packaged using a Perspex well which made possible the culture of cells for 48 hours (and longer in other experiments), creating a sufficient volume for containing the cell media.

A cytotoxicity test was also carried out using an extract of Antrodia Camphorata on A549 cells. Results showed the expected trend in impedance figures but did not evidence a big difference between the recorded with nanostructured and flat electrodes. Further studies will be conducted to find out whether it will be possible or not to achieve better sensitivity with nanostructured electrodes in this kind of assay.

At the moment, electrodes with taller nanopillars (100nm) are in fabrication and nanostructured electrodes with a reference electrode embedded on the Pyrex substrate have been fabricated and under testing for electrochemical detection of species in solution. The last ones worked very well and gave a higher sensitivity in the amperometric detection but further tests are needed to complete the study.

Other materials (i.e. ITO, Pt, etc) for the fabrication of the nanostructured electrodes will be also investigated.

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