STUDY OF SURFACE MORPHOLOGY CONTROL FOR A CELL-IMPLANT ONTO AN ALL-IN-ONE CHIP

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ABSTRACT

A novel fabrication method is presented for making a surface energy tunable bio-chip array with an investigation of cell behavior subjected to morphology of the substrate. Fine tuning of the shape and size of Au nanoparticles on an ITO surface controls the proliferation and spreading of HeLa cells on the chip without any chemical pretreatment. The adaptation of the cells to the substrate is enhanced by finely tuning the size of deposited structures within a range of a few nanometers to a few micrometers. Further increase of this morphology value aggravates cell-implant affinity due to reduced binding sites for cells on the rougher regions.

KEYWORDS: Electrochemical deposition, gold, ITO, cell adhesion, bio-chip.

INTRODUCTION

Controllable cell-sorting [1], the characterization of cell-adhesion to substrate [2], and some other investigations on biological cells that require designed and controlled implant surface properties have attracted significant attention. Apart from biocompatibility, the surface morphology of the material is crucial for growth and viability of the cultivated cells [3]. Therefore, it is important to attain designed surfaces of well-ordered and very fine tunable morphologies without disturbing the cell-substrate interactions. Electrochemical deposition of HAuCl₄·3H₂O and polyvinyl-pyrrolidone (PVP) solution onto ITO patterns is one of the simplest and most cost-effective methods to achieve such reliable and diverse surfaces.

In this work, a new fabrication method and surface characterization of a well-organized, surface energy tunable micro-array chip that consists of trans-scale nano to micro structures is presented. The cultivation of HeLa cells on the prepared platform shows a specific property in terms of cell adhesion and spreading.

FABRICATION AND CHARACTERIZATION

Combining top-down and bottom-up methods to pattern trans-scale features provides the modification of only designed local regions. As a first step, a pre-cleaned ITO layer with a 145 nm thickness on a glass substrate was patterned with a conventional photo-lithography and subsequent wet etching processes. It was then employed as the working electrode of a two-electrode system. The monolayer and multilayer of
Au particles were electrochemically synthesized on the conductive patterns of the ITO in an aqueous solution containing HAuCl₄ and PVP.

Figure 1 shows a 3×4 array of 6.5×6.5 mm² patterns. Starting from a bare ITO layer on glass, within a few seconds of deposition, mono-layered Au particles of a few nanometers and merged clusters up to 100nm were created. For longer deposition time, bigger clusters of micro-lumps were formed as depicted in Fig. 1(d). The surface energies of the modified patterns were characterized by contact angle measurements that are pictured in the insets of Fig. 1. The contact angle on the bare ITO was measured to be 60.2°. This angle tended to decrease in the order of 39.2°, 31.5°, and less than 5° for the deposition times of 1 minute, 5 minutes, 1 hour, and 2 hours, respectively. The porous nature of electro-synthesized Au tends to be super-hydrophilic without an extra chemical surface treatment.

Figure 1: HAuCl₄ modified 3×4 ITO pattern array on a glass substrate (center). SEM images and corresponding contact angle measurements of the patterns after an electrochemical deposition time of (a) 1 minute, (b) 5 minutes, (c) 1 hour, and (d) 2 hours.

CELL CULTURING AND EXPERIMENTAL RESULTS

HeLa cells were cultured in a DMEM medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin G (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air.

Analyzing the number of cells, cell size, and expansion of fibrous networks among the cells, it is clear that morphology-controlled trans-scale nano-structures promote cell adhesion and spreading as shown in Fig. 2(a) and 2(b). However, “the rougher the better” concept fails beyond a critical point; hence too much rough surface adversely decreases focal binding sites, which are key ingredients to sustain good adhesion between the substrate and cells (Fig. 2(c) and 2(d) with illustrations).
Figure 2: F-actin staining confocal images and corresponding illustrations of HeLa cells cultivated on electrochemically modified Au trans-scale structures with an electrochemical deposition time of (a) 1 minute, (b) 5 minutes, (c) 1 hour, and (d) 2 hours.

CONCLUSIONS

Different surface morphologies were achieved with multi-scale nanoparticles using top-down and bottom-up approaches together. Location, shape, and size of these nanostructures are precisely controlled for the fabrication of a cell culturing bio-array. Accordingly, the viability and adaptability of cultivated cells on the implant were improved on the synthesized structures. This fabrication technique can be attractive for a wide range of applications where the precise and local tuning of surface properties is essential.

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