Super Hydrophobic ZnO Nanostructured Surfaces for the Enhanced Formation of Cancer Spheroids

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Introduction

High throughput 2D chemotherapy drug testing is highly effective for the screening of drug efficacy and toxicity in many different types of cancerous and healthy cells simultaneously. However, this type of in vitro experimentation does not accurately reflect the results subsequently observed in clinical trials, since it is based on 2D monolayer cultures of cells [1].

Surface topography, wettability, and chemistry all govern cell adhesion to surfaces and other cells. So, How do we fabricate a hydrophobic surface that minimizes cell-substrate adhesion?? [2]

1. High surface roughness
2. Low wettability
3. Negative surface charge

By plating cells on hydrophobic surfaces, you can greatly reduce their interactions with the substrate, and instead promote cell-cell binding.

Spheroids grown on more hydrophobic surfaces exhibit higher compactness [3].

The goal of this project was to utilize nanolithography to optimize and fabricate hydrophobic nanostructured surfaces within microwell devices to reliably grow colorectal cancer spheroids.

Spheroid Gold Standard: Aggrewell 800

Advantages:
• Relatively cheap
• Easy to use
• Capable of producing a large number of spheroids

Disadvantages:
• Difficulty in forming tight spheroids in some cell lines
• Depend on an anti-adherence coating, which isn’t always uniform

Figure 1. A) SEM image of Aggrewell 800 plate and B) profilometry measurements.

Our Device

Fabricate a ZnO nanostructured surface which is superhydrophobic, nontoxic, and promotes tumor spheroid growth with:
• Silicon wafer base
• Subdivided into microwells
• Inside each well is a layer of Zinc Oxide nanorods treated with a hydrophobic surface coating

Figure 2. Overview of device design

Figure 3. Overview of the microfabrication process

Step 0: Wafer cleaning
Oxygen-plasma treatment

Step 1: S1813 lithography
Grid deposition to allow for blank spaces for microwell walls

Step 2: ZnO thin film deposition
ZnO NP solution is sonicated with a dispersant, and spin coated onto the wafer at 1500rpm for 30 seconds

Step 3: Dissolve grid & anneal
Lift off S1813 to reveal clean grid and then anneal wafer at 500°C for 1 hour in Argon gas

Step 4: Nanorod growth
Grow ZnO nanorods from the nanoparticle seeds in an HMTA and Zn(NO3)2 [4]

Step 5: SU8 lithography
Build the microwell walls using ultra-thick SU8 lithography to achieve 300μm resist thickness

Step 6: C8F8 deposition
Deposition of a Teflon-like non-sticky polymer onto the device surface

Results

Figure 4. A) Light microscope images of S1813 grid deposition. B) Light microscope and C & D) SEM images of ZnO thin film deposition after S1813 lift off.

Figure 5. SEM images of A & B) ZnO nanorods, C) full device before C8F8 deposition, and D) and nanorods after C8F8 deposition.

Figure 6. Water contact angle measurements of A) polystyrene coated with the anti-adherence solution, B) Si wafer after C8F8 deposition, and C) ZnO nanorod surface after C8F8 deposition.

Figure 7. Fluorescent images of HCT116 spheroids grown with A, B, & C) the Aggrewell 800 plate, and D & E) our device.

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References