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Evolvement of cell–substrate interaction over time for cells cultivated on a 3-aminopropyltriethoxysilane (γ-APTES) modified silicon dioxide (SiO_2) surface

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ABSTRACT

Since cell–substrate interaction is directly related to the traction force of the cell, the cell property can be judged from the imprint it leaves on the soft substrate surface onto which the cell is cultured. In this letter, the evolvement of the cell–substrate interaction over time was observed by cultivating cells on a 3-aminopropyltriethoxysilane (γ-APTES) modified silicon dioxide (SiO_2) surface for different periods of time. The cell–substrate interaction property as a function of time can then be found from the post-cell-removal surface morphology profiles determined by atomic force microscopy (AFM). Different surface morphology profiles were found between normal cells and cancer cells. It was found that the cancer cells tend to form deeper trenches along the circumference of the imprints, while the normal cells do not. In addition, our results indicated that normal cells involve cell–substrate interaction mechanisms that are different from those for cancer cells.

1. Introduction

The ability of cells to adhere to the substrate onto which they were cultured depends not only on the characteristics of the cell but also on their cultivation conditions. The adhesion ability of cells to the substrate also play a critical role in many of the fundamental cell-involvement processes such as embryonic morphogenesis, angiogenesis, inflammation and wound healing [1–3]. Consequently, cell–substrate interaction has raised the interests of many researchers [4–6] whose works have taught us additional facts about cell properties, such as traction force, migration, and even the metastasis ability [7–9]. Cell–substrate interaction has been recognized as an indication that cells will generate a local force via so-called cell–extracellular matrix (ECM) interactions. It also has been confirmed that it is the traction force caused by actin polymerization at the cell's leading membrane edge that transmits the contractility force to the ECM via the primary mediators' focal adhesion protein integrins [10–12]. In addition, reports have shown that not only the cell's leading membrane edge but also its central region plays an important role in cell–substrate interaction [5,13]. The literature shows that many researchers used fibronectin (FN) for the study of cell–substrate interaction [10,12,14] since FN can bond with integrins to form the traction force at the cell's leading membrane edge after actin polymerization. Since actin exists in both the cancer cells and in normal cells, it is difficult to differentiate the cell–substrate interaction of cancer cells from that of normal cells using FN [15]. Therefore, challenges remain in the application of FN to cell–substrate interaction and further research is needed.

Optical techniques such as interference reflection microscopy (IRM) [16–18] and fluorescent microscopy (FM) [4–6] [9] were developed for cell–substrate interaction measurements. However, several issues still remain to be resolved in these optical techniques. For example, multiple spurious reflections and poor contrast can affect the result of IRM, and the addition of fluorescent conjugates in the FM method can possibly affect the original property of the living cells due to the molecular effect [19]. At the same time, a label-free characterization technique has been reported in which living cells are cultured on wrinkled or elastic micropillar substrates and the traction force of the cells are determined from the substrate deformation with the help of optical microscopy [14] [20,21]. More recently, a similar technique using silicon nanowire has been proposed to quantify the difference in traction force between normal cells and cancer cells by determining the deformation of a silicon nanowire detected by a scanning electron microscope [8]. In both of these techniques, the Young's modulus of the substrate/silicon nanowire used must be known in advance. However, it is difficult to obtain a uniform Young's module over the entire substrate

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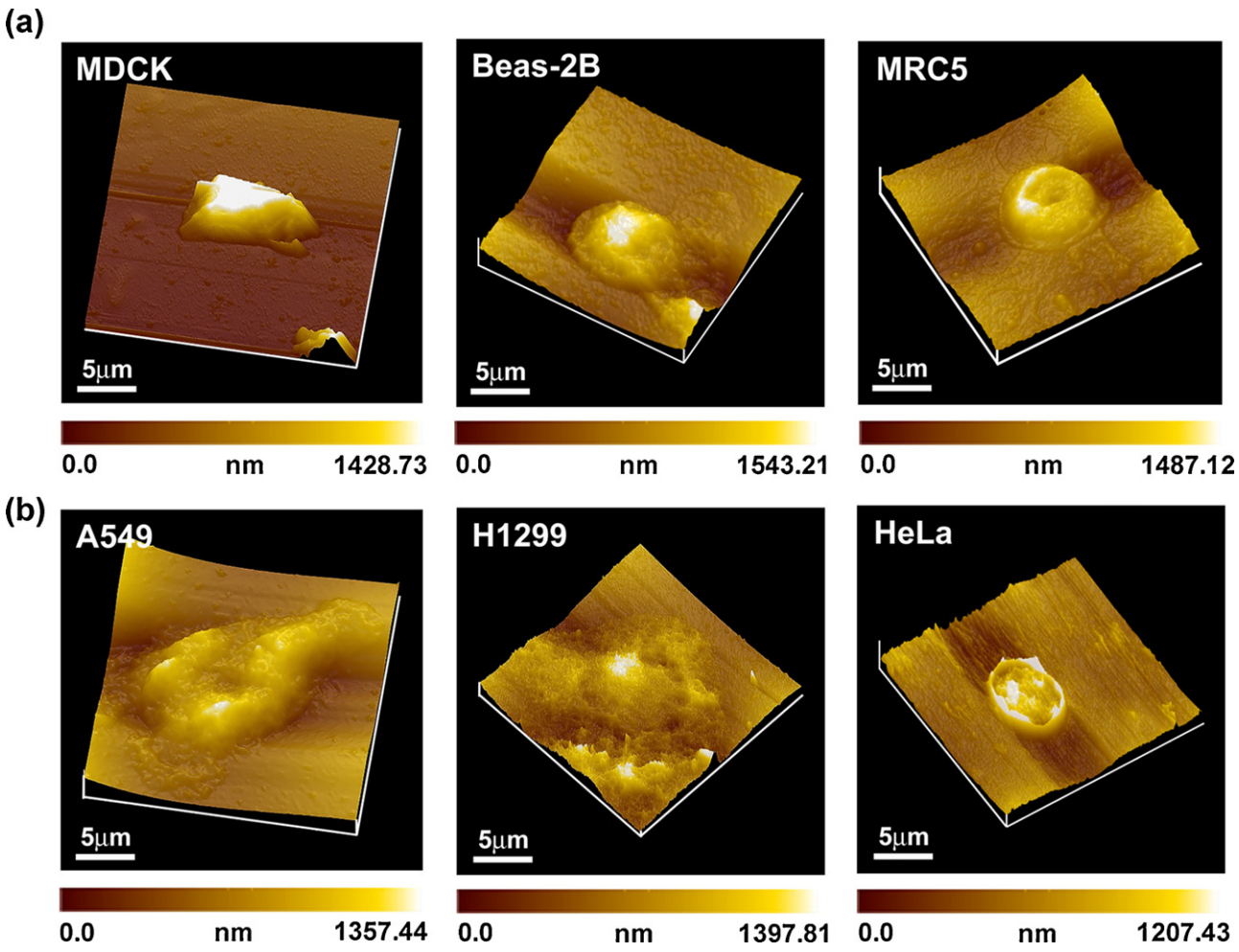

Fig. 1. AFM surface morphology images of (a) normal cells MDCK, Beas-2B and MRC5, and (b) cancer cells A549, H1299, and HeLa cultivated for 24 h on a γ-APTES modified SiO_2 surface, respectively, before they were removed.

or for every silicon nanowire. With its nanoscale detection ability, atomic force microscopy (AFM) is also being used to detect the cell–substrate adhesion strength [22–24], in which an AFM cantilevered tip with the tested cell attached interacts with a functionalized tissue or another cell surface at a given location on the substrate. The deflection of the cantilever tip caused by the force of the interaction between the attached cell and the functionalized tissue is then determined. However, none of these above mentioned techniques has demonstrated the ability to detect the evolvement of the cell–substrate interaction over time.

In this study we cultivated the living cells on a γ-APTES modified SiO_2 surface over different periods of time. The reason that we used γ-APTES is that cells can have better attachment and higher survival rate when cultured on a substrate surface containing amine (NH_2) group [25], and the γ-APTES can provide rich NH_2 bonds on its surface [26,27]. The cell–substrate interaction was then determined by measuring the post-cell-removal surface topography profile using an AFM. This could be achieved because the traction force exerted by the cell on the substrate leaves an imprint on the substrate surface. The imprint left by each cell after its specific culturing time correlates to the cell traction force. Consequently, the imprint for each different cell culturing time reveals the evolvement of the cell–substrate interaction over a specific time. In addition, different cells will have different imprints due to their different traction forces in nature. Since the measurement is performed after the cells are removed, the issue of operating the AFM tip under a liquid environment can be avoided [28,29].

2. Methods

2.1. Si substrate preparation

A p-type (1 0 0) silicon wafer with a 2 μm-thick SiO_2 surface layer was used as the supporting substrate in this work. A 1% ethanol solution of γ-APTES was then spin-coated onto the SiO_2 surface and cured at 120 °C for 5 min on a hot plate. The final thickness of the γ-APTES layer was determined to be about 550 nm by ellipsometry. After coating with γ-APTES, the Si wafer was subjected to sterilization in an autoclave sterilizer at 110 °C for 90 min in vacuum. The Si wafer was then divided into 24 pieces and placed in a 24-well culture plate for cell cultivation.

2.2. Cell culture and removal

Madin–Darby canine kidney (MDCK) epithelial cells, human cervical cancer cell HeLa, and human lung adenocarcinoma cancer cells A549 and H1299 were immersed in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum supplemented with 10 mg/mL penicillin/streptomycin and 2 mL glutamine, while human bronchial epithelial cell Beas-2B was immersed in a commercial RPMI1640 medium (Invitrogen; U.S.A.), and human lung fibroblasts cell MRC5 in a 5 mL. Eagle's Basal Medium (BME) containing 10% fetal buffer solution (FBS) supplemented with non-essential amino acid with 2 mM L-glutamine (Life Technologies, Inc., Frederick, MD). MDCK is a common