

Influence of structured wafer surfaces on the characteristics of Caco-2 cells

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Abstract

Currently there is an increasing demand for high-throughput methods to identify and to verify the potential of new drug candidates. Cell-based microelectronic biosensors might be powerful tools for rapid screening assays. However, reliable cultivation of cells is influenced by the material characteristics and the surface topography of the wafers serving as growth supports. In order to investigate the influence of micropatterned structures on cell viability, Caco-2 cells were seeded on silicon wafers featuring trench/mesa patterns obtained by lithography and reactive ion etching. Besides determination of the cell growth pattern by electron microscopic inspection, the adherence of cells on different patterned silicon wafers and the formation of the tight junctional network was investigated. Microstructured trench/mesa patterns, especially their lateral distances, remarkably influenced the adhesion and proliferation behavior of Caco-2 cells. Lateral distances below the average cell diameter were easily overgrown by the cells, whereas dimensions above the average cell diameter increasingly limited cell proliferation. Notably increased cell growth was observed using trenches with a width of 10–20 μm and a trench depth of around 35 μm . All in all, the results of this study might improve the production of microstructured biosensors and open up new perspectives concerning the combination of biosensors and microfluidic systems.

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1. Introduction

To date there is an increasing demand for rapid screening of new drug candidates. Recently, research has recognized cell-based assays as powerful tools not only for screening the efficacy but also for elucidation of the biopharmaceutical characteristics of the drug of interest. That way, animal experiments may be reduced in favor of cell-based assays, which are less time-consuming, less expensive, and without controversy regarding ethical considerations [1]. However, cell culture requires work-intensive handling, restricting its routine applicability for high-throughput screening.

This limitation may be overcome by adapting cell-based microelectronic biosensors for this purpose. The versatility of bioelectronics for the screening of cells has been demonstrated in several studies: the response of cells to toxic substances was successfully investigated by electrical impedance measurements [2], the metabolic activity during cultivation was monitored by an amperometric sensor system [3], and the influence of gold-patterned silicon surfaces was investigated in terms of on-cell patterning [4].

Nevertheless, the microelectronic monitoring of living cells has to meet specific requirements. In contrast to conventional tissue culture materials, microelectronic devices are typically based on inorganic surfaces. Consequently, the biocompatibility of the microelectronic material used

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plays an important role for successful cell cultivation. At this, gold, silicon, and silicon oxide revealed an adequate biocompatibility, while several metals such as copper and tungsten have been proven less suitable [5].

Another crucial issue when working with adherent cells is to provide good attachment facilities on the wafers. It is known that cells can adhere to entities like trenches or columns which are within the size of the cell [6]. Moreover, rat dermal fibroblasts were shown to follow the contours of microgrooves on polystyrene [7] and human fibroblasts were observed to alter their shape and orientation depending on the topography of micro-machined titanium surfaces [8]. However, there is less information on the response of human cells to structures in the range between 500 nm and 20 μm , which is a widely used dimension for microelectronic interfaces including electronic biochips.

Proper adhesion facilities are of great importance, particularly when working with microfluidic systems due to the induced shear stress. Microfluidic systems provide a well-established basis to miniaturize analytical devices for biological applications. Smaller analytical platforms mean low consumption of reagent which results in low cost and less expenditure of time [9]. Moreover, the feasibility of microfluidic devices for real-time monitoring of concentration-dependent biological responses was demonstrated [10].

Caco-2 cells are a widely accepted model for the human small intestinal epithelium to characterize intestinal drug absorption [11,12] and hence the Caco-2 cell line is an approved model for the Biopharmaceutics Classification System (BCS) [13]. The Caco-2 cell model was established by J. Fogh in 1974 and derived from the human colon adenocarcinoma of a 72-year-old Caucasian man [14]. Despite their colonic origin the cells form monolayers that undergo complete and terminal differentiation forming a columnar absorptive epithelium with tight junctions similar to that of the human small intestine [15]. Due to their widespread application, new high-throughput screening systems employing Caco-2 cell-based biosensors would be of high interest and potential.

The aim of this work was to investigate the behavior of cells on structured wafer surfaces and to identify the influence of surface patterns on cell attachment. For this purpose, silicon wafers were patterned by lithography to generate structures with dimensions above and below the average cell diameter. Caco-2 monolayers were grown on such trench-mesa patterns with lateral distance variations in the range between 2 and 60 μm and characterized microscopically concerning adhesion, cell growth and the integrity of the cell layer. Furthermore, the influence of the microstructures on the morphological differentiation was examined by immunofluorescent staining techniques. The results of this study should offer decisive basic information for the design of microstructured interfaces and perspectives for an expedient method to screen new drug candidates on human cells.

2. Materials and methods

2.1. Materials

2.1.1. Microstructured materials

As cell culture substrates p-doped (100) silicon wafers (single side polished) were used. The 6" wafers were broken into 20 \times 20 mm pieces for individual processing in different batches. Prior to patterning the channel structures, the samples were cleaned with acetone and isopropanol. After processing the trench structures the 20 \times 20 mm pieces were cut to identical 4.00 \times 4.00 mm units with a wafer saw with a 60 μm wide diamond cutting blade to provide identical growth areas for cell culture experiments. Gases for etching of the trenches were SF₆ (Linde, purity 5.0) and oxygen (Air Liquide, purity 5.0).

2.1.2. Cell culture materials

The CyQuant™ cell proliferation assay kit was obtained from Molecular Probes (Eugene, OR). CellLytic™ Cell Lysis Reagent (Molecular Probes, Invitrogen), albumin from bovine serum, and propidium iodide were purchased from Sigma (St. Louis, MO, USA). Purified mouse anti-ZO-1 antibody was obtained from BD Biosciences (San Jose, CA, USA). Goat-anti-mouse immunoglobulins/FITC were purchased from DAKO (Vienna, Austria). FluorSave™ was obtained from Calbiochem (Darmstadt, Germany). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Processing of microstructured materials

2.2.1. Mask design

Masks were fabricated with an optical pattern generator using designs developed with a commercial computer-aided-design (CAD) program. Five-inch photomask blanks (Hoya) consisting of glass substrates with a thin, reflective metal coating were used. Trench/mesa designs of 6/12, 8/14, 12/18, 16/22, 20/26, and 30/36 μm were chosen for photo-resist patterning.

2.2.2. Pattern design

Microstructures were fabricated by optical lithography using a AZ 5214 photo-resist (Microchemicals, Ulm, Germany) which was deposited by spin coating at 3500 rpm to form a resist layer of 1.5 μm in thickness. Exposure was performed with a MJB3 mask aligner (Karl Süss, Germany) equipped with a 200 W lamp. The resist was developed with a metal ion free developer AZ 726 (Microchemicals, Ulm, Germany). The photo-resist served as a polymer mask for etching of the trench structures.

2.2.3. Trench fabrication

The three-dimensional trenches in the silicon substrates were obtained by reactive ion etching (RIE) with a reactive plasma etcher (Oxford Instruments, Plasmalab System 100)

ID	Title	Pages
2368	Influence of structured wafer surfaces on the characteristics of Caco-2 cells	10

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