Cell Growth on Prestructured Microelectronic Materials

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Bioelectronic transducers offer powerful new analytical tools with major applications in medicine, pharmaceutical research, environmental diagnostics and the food and processing industries. The interface between inorganic materials and living cells is crucial for the operation of biosensors. The viability and the adhesion of colon carcinoma cells (Caco-2) was tested on an assortment of commonly used metals, dielectrics and semiconductor materials. Growth inhibiting materials such as copper and blank gallium arsenide have been idientified as well as highly biocompatible materials such as silicon, silicon nitride, chromium and gold. In addition, the growth on the metal-dielectric boundary was performed and neither the sub-200 nm height step nor the change of the material showed to affect the cell growth. Several materials have been successfully tested to facilitate the growth of cell structures. The results allow a versatile application for microelectrode arrays.

Keywords: direct write deposition, focused ion beam, FIB, 3-dimensional, chemical composition

Introduction

Methods for the investigation of living cells have historically been of significant importance for research in neuroscience and cell biology. For cell-based biosensors, the signal is originating from the presence or the metabolism of the living cell. Classical measurement techniques utilize the patch-clamp method where the single cell is contacted with a micropipet containing the electrode. For the high-throughput testing of drugs, electrical methods are most promising, as the characterization of a large amount of cells may be performed simultaneously [1]. During the last decade, the research on cell-based biosensors has experienced increased scientific activity [2], [3]. While a variety of different measurement techniques exist, microelectrode arrays [4] provide a simple interface for monitoring the electrical activity and impedance characteristics of populations of cultured cells over extended periods. Wide ranging applications of this emerging technology can be expected not only to the detection of chemical and biological warfare agents, but also to the testing of new pharmaceuticals and the monitoring of medical parameters [9]. Various metals, dielectrics and semiconductor materials have been tested as substrates for culturing cells. Also a composite substrate consisting of two materials — a dielectric and a metal simultaneously present on the surface — has been investigated. This work clarifies the suitability of microelectronic materials for sensors monitoring the activity of living cells.

Experimental

For this study, sample chips of 5x5 mm size were fabricated out of different materials. The pure semiconductor materials tested during this study were silicon (Si), Germanium (Ge) and gallium arsenide (GaAs). As insulating materials dielectrics such as silicon oxide (SiO_2), silicon nitride (Si_3N_4) and polymers such as polymethylmetacrylate (PMMA) or the photosensitive resist recipes maN410 and maP were used. The metals Al, Ti , Au, Cr, Pt, W and Cu were separately deposited as a thin film on silicon wafers. Standard glass slides of sodium potassium silicates were processed as reference material.

The prepatterned surfaces were fabricated by sequentially depositing a continuous Au layer and a silicon nitride layer on top of a Si-wafer. A silicon nitride grating with a periodicity in the 50-µm range was fabricated by local etching. The resulting patterned surface displayed lines of Au and Si_3N_4 in a regular arrangement.

Human colon carcinoma cells (Caco-2) were selected as an exemplary cell culture. The Caco-2 cells were obtained from the American Type culture Collection (Rockville, MD, USA). The cells were cultured in RPMI-1640 cell culture medium supplemented with 10% fetal calf serum, 4 mM glutamine and 150 µg/ml gentamycin. The cell cultures were grown in microtiter plates at 37 °C in a humidified 5% CO_2 / 95% air atmosphere for a period of 10 to 14 days. The progress of the cell growth was repeatedly controlled by visual inspection. For inspection, the substrates with a Caco-2 tissue attached to the surface were placed under a microscope to evaluate the viability on the substrate.

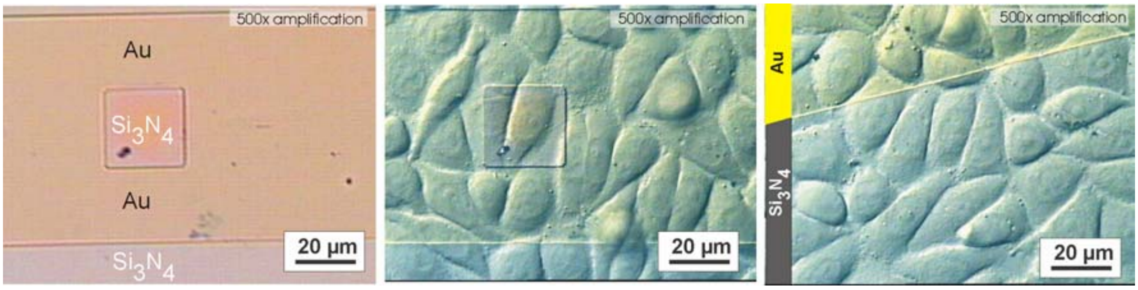
Results

The first efforts were directed at clarifying the biocompatibility of semiconductor materials themselves. Caco-2 cells were cultivated on top of a microelectronic substrate. A dense coverage with cells on the surface was obtained with silicon and germanium, while only few cells were found on the gallium arsenide surface. Dielectric interfaces to cells are relevant for capacitive biosensors. Homogeneous cell coverage was achieved with silicon nitride, deposited by plasma enhanced chemical vapor deposition, and silicon oxide generated by thermal oxidation of silicon. With thermal silicon oxide, the cells appear rather round-shaped suggesting a not so good adhesion. The polymer polymethylmetacrylate is not considered an ideal growth substrate for biological materials. For biosensors interacting with the cells over electrodes, the interface to the cell is a conductive material.

A homogeneous surface coverage of Caco-2 cells was found on chromium and gold surfaces. With tungsten and titanium, a low cell coverage around 50% was obtained. On aluminum, cells also appear to be healthy and the shape suggests a sufficient attachment to the surface. However, experimental results concluded that copper intoxicates the cells and is a totally unsuitable material.

Microelectronic biosensors are constructed as integrated circuits acting as signal transducers for biological systems. Such microelectronic systems usually consist of several material layers and may have metallic surface areas such as microelectrodes next to dielectric areas acting as passivation for the circuitry. A prestructured sample with gold and silicon nitride structures coexisting on the surface was fabricated (Fig. 1 (a)). The Caco-2 cells cultured on this composite surface displayed a dense tissue with the cells grown together so continuously that the entire surface was covered (Fig. 1 (b)). The shape of the cells suggests a good adhesion on the entire surface. It is concluded that both materials together establish a biocompatible surface.

A close examination of the sharp boundary between the gold structure and the silicon nitride area has been performed (Fig. 1 (c)). The cells have grown over this rim totally unaffected. Even the 200 nm height difference at the interface between the gold layer and the silicon nitride layer left the growth totally unaffected. The cell growth showed no orientation to the border between these materials. It can be assumed that the cell activity remains totally unchanged on microelectrode circuits utilizing gold and silicon nitride.



(a) (b) (c)

Fig. 1: Optical images (500x magnified) of a prestructured microelectronic sample with (a) the original surface with gold and silicon nitride structures, (b) a Caco-2 cell layer grown over the surface, (c) another sample spot with cells growing over the edge between the different materials.

This result opens the path for the application of this material system for the development of cell-based biosensors to assess the vital parameters of these cells.

Conclusion

In this study, the biocompatibility of materials frequently used in microelectronics was determined by an examination of Caco-2 cells cultivated on various surfaces. The feasibility to grow cells on prestructured surfaces with conductive and insulating materials has been demonstrated. It has been demonstrated that the cell growth remains unaffected by structuring of the surface. On prestructured composite surfaces with a metal and dielectric present in direct vicinity, cell growth was observed over the interface between those materials.

This initial study has provided reliable experimental data on the biocompatibility of microelectronic materials and shines an optimistic light on the future development of microelectronic biosensing devices.

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