Development of a Lab-on-a-Chip for the Characterization of Human Cells

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Abstract

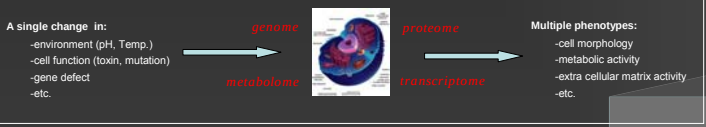
Microfabricated biochips are developed to continuously monitor cell behavior in a non-invasive manner. In the presented work we describe the novel application of cellular dielectric spectroscopy (CDS) using contact-less micro-dielectric sensors for quantitative cell analysis. The cell chip consists of a polymeric microfluidic (PDMS) system bonded to a glass wafer containing the high-density interdigitated capacitors (µIDC). The objective of the developed biochip is to monitor real-time cellular phenotype dynamics under varying conditions. The lab-on-a-chip is designed to continuously assess cell viability, reproduction and metabolic activity over long periods of time using different sensors on a common chip platform. The µIDCs are isolated by a 300 nm multi-passivation layer of defined dielectric property that provides stable, robust and non-drifting measurement conditions. The presented work addresses aspects of chip design, fluidic flow profiles and sensor characterization as well as sensor modification and biocompatibility tests using HeLa and Endothelial cells.

Introduction

In the past decade the miniaturization of analytical techniques by means of MEMS technology has become a dominant trend in research. The creation of microanalytical systems, such as biochips, have demonstrated the ability to provide quantitative data in real-time and with high sensitivity. Microfluidic biochips or lab-on-a-chip systems are vital for biological analysis because they allow spatial and temporal control of growth conditions. Monitoring cell behavior under varying conditions and understanding genotype-phenotype interactions in the context of a living cell is expected to have a considerable impact on medicine. The principle behind cell analysis is that a cellular phenotype represents the expression of a genotype, thus revealing gene function and its interaction with the environment. However, to gain a deeper biological understanding of cells, it is necessary to first make progress in experimental devices, as well as computational and analytical methods.

Monitoring Cellular Dynamics

Monitoring cellular phenotypes may ultimately reveal gene function and its interaction with the environment. The objective of the biochip is therefore to detect in real-time cellular phenotype dynamics under varying environmental conditions. The lab-on-a-chip is designed to continuously assess cell viability, reproduction and metabolic activity over long periods of time using multiple sensors on a common chip platform. In addition, the integrated fluidic and heating systems allow controlled manipulation of living cells adhered to modified/activated chip surfaces that are comparable to biological niches. Furthermore, the chip contains an integrated reference arm providing a low-noise detection environment by eliminating background signals and interferences.



Development of a Cell-Chip

Lab-on-a-Chip Design

1. Micro-electromechanical systems (MEMS) Technology
   - Two-component chip using different materials (glass & polymer)
2. Multiparameter detection platform
   - High-density interdigitated capacitors (µIDC) and band electrodes
   - Optical window for microscopy
   - Integrated reference sensors
3. Integrated microfluidic system
   - Three input reservoirs (flow rate & shear stress control)
4. Heating system and pumping station
   - Maintaining constant temperature over long periods of time
   - Three individually addressable fluid reservoirs
5. Continuous & long-term viability study

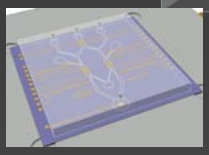


Figure 1: Microfluidic system showing AgCl precipitation using three laminar flows of 10 mM $AgNO_3$ and 100 mM KCl.

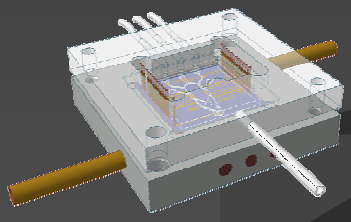


Figure 2: Design drawing of the microchip comprising of a glass bottom and a polymer (PDMS) cover and fixture.

Principle of Cellular Dielectric Spectroscopy using µIDC

In the context of cell analysis, high-density microfabricated interdigitated capacitors (µIDC) are ideally suited as sensors due their large active areas and flexible geometry that allow the creation of designs capable of covering the entire proliferation chamber. Another benefit of µIDES is its ability to tune electric field distribution by controlling the space ratio between fingers, as well as the width and thickness of individual fingers. For instance, calculations (conformal mapping technique) of electric field distribution in the presence of SiN/SOG passivation layers using air and DI water data indicated that 50%, 95% and 99% of the electric field is located within a height of 3.13, 5.51 and 8.1 µm, respectively. In other words, dielectric changes occurring e.g. at 8.1 µm distance from the sensor surface contribute only 1% to the overall signal while the 300 nm thick multi-passivation layer is responsible for a 9 % sensitivity loss. Since sensitivity decreases exponentially with distance, thin passivation layers of well-defined dielectric properties are a crucial component of a high sensitivity sensor.

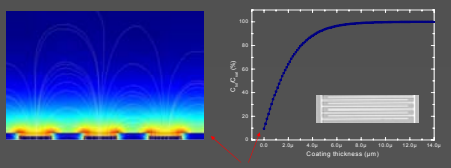


Figure 3: FEMlab simulation of dielectric displacement using the present sensor geometry of 200 fingers with 5 x 5 µm width and gap. Calculation of electric field distribution using conformal mapping technique.

Dielectric spectroscopy makes use of the electrical properties of cells exposed to a radio-frequency electrical field. The non-conducting nature of the cell plasma membrane allows a build up of charge thus acting as tiny capacitors. The principle of this approach is to agitate the nanoliter reactors with an alternating signal (ac) of small magnitude (±15 mV) and to observe the way in which the system follows the perturbation at steady state. Dielectric spectroscopy has already proven to be a useful technique for analyzing heterogeneous systems, especially biological cell suspensions and tissues because of its capability to measure non-invasively.

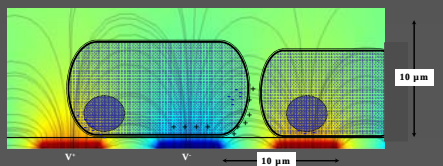


Figure 4: FEMlab simulation of electric potential distribution using the present sensor geometry.

Results and Discussion

Characterization of Sensor Passivation Strategies

An important aspect of the newly developed technology is the complete isolation of the high-density interdigitated electrode structures (µIDES) thus guaranteeing non-invasive monitoring conditions. However, dielectric permittivity (ε) of applied passivation materials can significantly influence electric field distribution and therefore it is important to first determine its effect on sensor behavior. Additionally, to increase sensor stability, multi-layer passivation strategies of varying thickness using SiNx and SOG are investigated using impedance spectroscopy. Phase angles (θ) of -84° using saline buffer indicated that the system behaves almost like an ideal capacitor (θ of -90°).

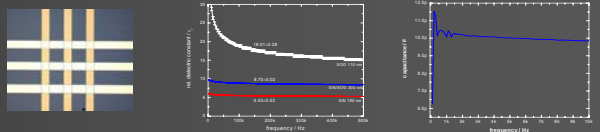


Figure 5: Crossed electrode systems used for capacitance spectroscopy to determine dielectric permittivity (ε) of SiN, SOG and SiN/SOG insulation layers. Frequencies above 3 kHz are required for stable background signals.

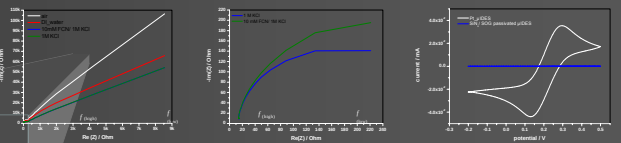


Figure 6: Impedance and CV signal comparison of µIDC sensors in the presence and absence of passivation layers.

Surface Modification of Microfluidics

In order to decrease fluid resistance of the polymeric microchannels and to create uniform fluidic conditions, both glass and polymer substrates are modified with aminopropyl-trisilane (APTS). Surface modification of PDMS further prevents unspecific adsorption of biomolecules. It is believed that the 50 to 70 nm diameter holes located throughout the SOG layer, as shown in both AFM images, are caused by solvent evaporation during the curing process at 425°C of the precursor substrates.

Figure 7: AFM images of APTS modified SOG surface. Pictures of contact angle measurements before and after APTS modification.

Micro-Dielectric Sensor Performance

The effect of temperature changes to the baseline is determined by stepwise increasing the chip temperature from RT to 37°C. A linear relationship between temperature increase and impedance signal decrease exhibited a 70 Ohm drop for every 1°C temperature increase. This is in accordance to literature, since the relative dielectric permittivity (ε) of water is inversely proportional to temperature (T) and conductivity (κ). Additionally, an increase in salt concentrations resulted in an impedance decreases of ~ 1 Ohm/ mM KCl at pH 7. As a result, precise control over the above parameters, as well as the presence of a reference micro-dielectric sensor, is necessary to compensate for fluctuations of temperature, pH and salt concentrations during cell analysis.

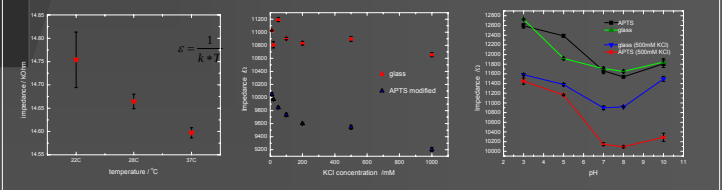
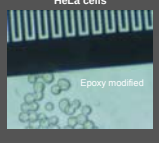


Figure 8: Dielectric sensor response to increases in Temperature, salt and pH.

Biocompatibility Testing

An important goal of the proposed research is to conduct measurements under physiological relevant conditions since a non-physiological environment can potentially lead to ambiguous cellular responses. One of the main challenges at this stage is the application of substrates compatible with biological samples to establish a basic methodology for mammalian cell cultures. Cell adhesion has been extensively studied by many researchers because cell adhesion to the extracellular matrix, other cells, or biomaterials is critical to cell functions. The first step is to conduct biocompatibility studies of various surface modification strategies using Endothelial cells.

HeLa cells



Endothelial Cells

ANNEX-V-FLUOS Bioassay

Modification	alive %	Apoptotic %	Necrotic %
SOG	48,59	38,20	12,69
SOG+APTS	61,84	21,54	15,72
SOG+APTS:MPTS (80:20)	69,01	23,36	7,03
SOG+APTS:MPTS (50:50)	65,73	25,44	8,34
SOG+APTS:MPTS (20:80)	65,71	23,20	10,36
SOG+MPTS	48,78	39,03	11,50
SOG+APTS+Gelatine	64	24,40	10,85

Figure 9: On-chip growth of Hela and Endothelial cells and biocompatibility tesing using various surface modifications.

Conclusion

Cells on Chip

- **New opportunities:**
  - Spatial and temporal control of cell growth and stimuli
  - Mimic complex biochemistries and geometries of the ECM using microfluidics
  - Integration of bioanalytical micro/nanosystems to create multifunctional platforms
  - Cell-based sensors with biochemical, biomedical and environmental functions
- **Highly integrated microdevices:**
  - Basic & applied biomedical and pharmaceutical research
  - Culturing cells in-vitro is one of the corner stones of modern biology
  - Robust instrumentation or portable point-of-care devices
  - Rapid information on cellular responses
  - Cost effective
  - Handling of nL volumes, power consumption, fabrication, ease of operation, packaging etc.