Combination of surface acoustic wave measurement and impedance spectroscopy for detection of cell adhesion process

G. Guhr, R. Brünig, and H. Schmidt
Dpt. of Magnetic and Acoustic Resonances, Leibniz Institute for Solid State and Materials Research Dresden
Dresden, Germany, Email: G.Guhr@ifw-dresden.de

M. Jäger and R. Poll
Institute of Biomedical Engineering, Dresden University of Technology, Dresden, Germany

M. Weihnacht
InnoXacs, Dippoldiswalde, Germany

***Abstract*— In this study, a new way of investigating a surface acoustic wave (SAW) based sensor device is reported. For that, SAW sensors with a fundamental mode of about 85 MHz and a metallization of gold were developed. Realized as one port resonators, these sensors allow the continuous detection of electric and viscoelastic properties of the same living cell culture in one measurement step. For obtaining the electric properties of the cells the sensors response in a low frequency range up to 10 MHz (impedance spectroscopy) can be investigated. For the detection of viscoelastic properties, the change of the sensors resonant frequency and its impedance are monitored. All signals are detected vs. time and can be compared face to face.**

I. INTRODUCTION

In biosensor applications even living cells can be used as sensing elements. Cells have a natural sensitivity to the surrounding biological processes. Thus, a great demand for the investigation and development of cell based biosensors exists. Such cell based biosensors are applicable in many fields ranging from environmental monitoring over pharmaceutical drug screening to tissue engineering. On one side there are invasive techniques like microneedles, micropipettes or atomic force microscopy under investigation [1] [2]. On the other side studies are reported using non invasive techniques like fluorescence microscopy, impedance spectroscopy (IS) and quartz-crystal microbalance (QCM) [3] [4]. Impedance spectroscopy detects electric properties like conductivity and capacity of the cell membrane. QCM measurements deliver information about viscoelastic properties.

In principle, SAW devices are comparable with thickness shear-mode resonators. In contrast to the bulk waves of TSM, the acoustic wave is kept on the surface of the SAW. Thus, compared to TSM, SAW sensors offer several advantages like smaller sample volumes, less responsibility to side effects like pressure changes or mounting forces. Oscillating at higher frequencies, SAW devices are smaller in size and reach higher sensitivities. A higher frequency leads to a smaller penetration depth of the acoustic wave into the fluid. In general SAW sensors were realized as so called delay lines. A transmitting interdigital transducer (IDT) creates a surface wave which is then damped in the delay line and received in a second IDT. The change of phase and oscillating amplitude is monitored. In this study, SAW devices realized as one port resonators are used. Such devices have one IDT and two reflectors on both sides. Thus, a standing wave is created and can be characterized by changes of resonant frequency, impedance, bandwidth and quality factor. However, a SAW resonator is affected by the mass shearing of the load and by electrical properties like dielectricity and conductivity of the surrounding media.

II. EXPERIMENTAL

The developed SAW devices were realized as one-port resonators made of 36°rot YX-LiTaO3. Thus, horizontal polarized standing shear waves are generated on the devices surface. The fundamental frequency of the SAW sensors is 85 MHz. The minimum of absolute value of impedance was detected as resonant frequency. For this study, the SAW sensors were fabricated with a double side mirror polished finish to allow microscopic observations during the measurements. For experimental investigations a special measurement chamber was developed allowing both, microscopic observation of the growing cell culture and the performance of impedance spectroscopy and acoustic measurements. It is made of a baseplate (cover glass) and a sealing of polydimethylsiloxane (PDMS). Such chambers

This work was supported by the German Federal Ministry of Education and Research (BMBF) under the grant 03IP610 (InnoProfile), by the Federal Ministry of Economics and Technology (BMWi) and the German Federation of Industrial Research Associations (AiF) "Otto von Guericke" (FV-No.: 14394 BR)

978-1-4244-3510-4/09/$25.00 ©2009 IEEE

cover an area of one square centimeter. Volumes of culture medium up to 1.4 ml are applicable. For continuous measurements the SAW cell culture chambers where mounted on the microscope including an incubator unit for temperature stabilization. For sterilization purposes the whole setup is autoclavable. Electrical measurements were carried out with a network analyzer (Agilent Technologies, Santa Clara, USA). Measurement programs to control the network analyzer and to store data were written in HP-VEE. The measurement points where taken at time steps between 1 and 3 min.

III. Results and Discussion

Due to the high sensitivity of SAW devices, much effort has to be made to get stable measurement signals. The temperature dependency of the SAW device, cell culture and medium was found as the main side effect. While an increase of temperature causes a decrease of resonant frequency, the viscosity of the medium is decreasing at the same time. In contrast a decrease of viscosity leads to an increase of the resonant frequency. But these effects are not balancing each other. To minimize these temperature dependent side effects the temperature of the measurement chamber was kept constant at 37.00 °C (+/- 0.01 °C). The SAW cell culture chambers and the cell suspension were brought to the final temperature one hour before start of measurement.

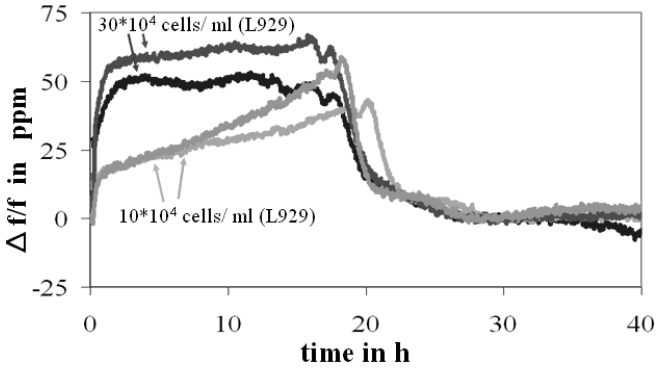

Fig. 1. Relative change of resonant frequency vs. time measured with 4 different SAW devices at one cell adhesion experiment. L9299 fibroblasts were seeded at t = 0 h in two different amounts.

Fig. 1 shows the relative change of resonant frequency of 4 SAW devices as measured within a cell adhesion experiment with L929 fibroblasts. The cells were seeded in amounts ranging between $10*10^4$ and $30*10^4$ cells/ ml. At both amounts of cells a rather steep increase of the resonant frequency and its impedance was detected in the first two hours. During this time the cells sediment to the sensors surface and immediately start to express their proteins of extracellular matrix (ECM). Thus, the cell adhesion process can take place. The ECM mainly consists of hyaluronic acid and proteins like collagen, fibronectin, laminin and elastin. Forming focal adhesion contacts the cells spread on the surface to increase their surface contact area. Independently of the seeded amount of cells these processes are finished after 2 h at all cell cultures. According to the amount of seeded cells, the change of resonant frequency was about 19 ppm at $10*10^4$ and 55 ppm at $30*10^4$ cells/ ml, respectively. After spreading the cells begin to reorganize their actin microfilaments and to move along the surface.

After the first two hours initial contact and spreading phase no more changes of resonant frequency was measured at the cell cultures with the higher seeded amount of cells. By optical observation we found dense populated cell cultures with an almost finished closed cell monolayer For that reason, no more changes of resonant frequency occur.

In contrast, resonant frequency and impedance of cell cultures with low amount of seeded cells are still increasing, but with a smaller slope. During this time the cells proliferate to form a closed monolayer.

When the medium is exhausted the cells start to die. Thus the resonant frequency and impedance are decreasing again. This effect appeared at all four cell cultures nearly at the same time. That means that the cultures with $10*10^4$ cells seeded had a higher consumption of growing media compared to the cultures with $30*10^4$ cells seeded. This can be explained with the different height of the cells metabolism. It is higher for cells being in their cell cycle (growth, proliferation) compared to cells being part of a closed monolayer.

In Fig. 2 the results of IS performed at a rather low frequency range, compared to the resonant frequency of the SAW device. The absolute values of impedance were normalized to the values at the start of the measurements (t = 0 h). At this frequency range the dielectric properties of the cell culture can be related to polarization effects in the so called β-dispersion [5]. The biggest changes were found at frequency ranges between 0.4 to 1 MHz. The lower charts in Fig. 3 represent the values of the maximum relative change of the absolute value of impedance as measured in the β-dispersion. The upper diagrams in Fig. 3 show the relative changes of resonant frequency and its impedance. All values were recorded simultaneously.

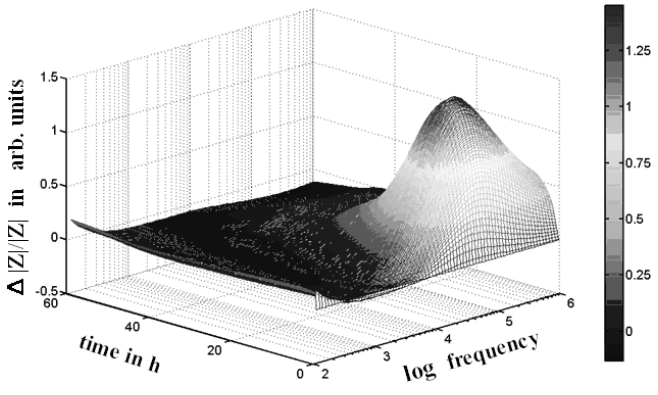

Fig. 2. Relative change of absolute value of impedance (|Z|) at low frequencies vs. time measured at the IDT electrodes of a SAW one port resonator during cell adhesion experiment performed with L929 fibroblasts ($10*10^4$ cells/ ml).

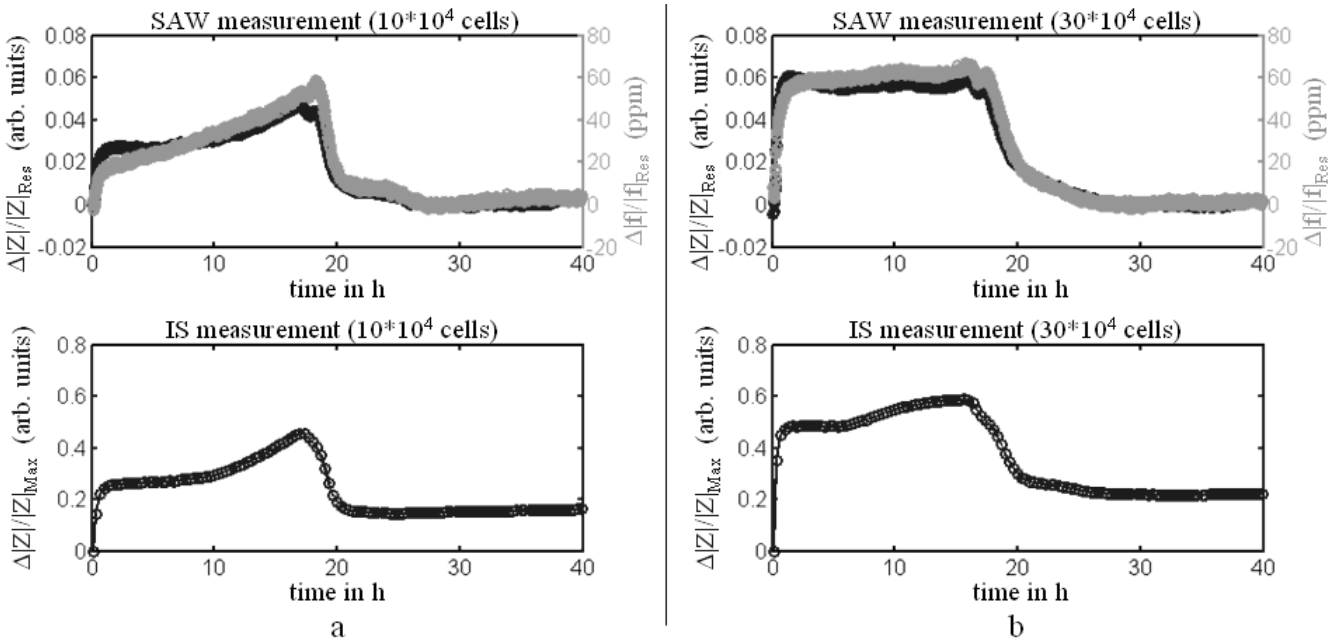

Fig. 3. Variation of amount of cells (L929) and comparison of the relative change of resonant frequency and its impedance (upper diagrams) and the relative change of |Z| at the particular frequency where the maximum of •|Z|/|Z| did occur (see for instance Fig. 2) vs. time.

The relative change of the IS-impedance is one magnitude higher, compared to the relative change of the impedance of the resonant frequency (black charts in Fig. 3). This observation gives a hint that the impedance changes in the β-dispersion do not overlap the behavior of the resonant frequency. For the cell culture with $10*10^4$ cells the resonant frequency increases continuously after the 2 h of initial contact and spreading phase. This can be related to the increase of ECM formed by the moving cells on top of the sensors surface. In contrast, the IS-values start to increase first after 10 h. This might be a hint that the cells start to divide at this time. Impedance changes in the β-dispersion are related to the built up of charge at the cell membrane [6]. Therefore the number of cell bodies attached to the sensors surface should increase while the impedance is increasing. A similar observation can be made at the higher amount of seeded cells.

According to our studies there are at least 3 different explanations for the increase of both, resonant frequency and its impedance, respectively. One possibility is the formation of a thin layer as it appears while the cells express their proteins of ECM. Through acoustic wave modeling (see for instance [7]) was found, that if the shear elasticity and density of growing layers are small compared to the sensor substrate, always an increase of the wave propagation speed and thus an increase of the resonant frequency would occur. A change in the range of 35 ppm per nm layer thickness was calculated. That means, according to the measurement data, the thickness of the ECM would be in the order of 2 nm. Another explanation is the influence of a decreasing permittivity of the surrounding fluid-like media. Besides, a change of the acoustic reflection coefficient of the SAW resonators electrode can also enhance the resonant frequency. Finally, also a superposing mixture of all these effects must be taken into account.

Summarizing can be said that more experiments are needed separately to investigate these influences. The combination of impedance spectroscopy and SAW measurements offer enhanced information of cell growth processes within one measurement.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Lorenz from the Institute of Biomedical Engineering, for providing and preparing the cell cultures. The authors would like to thank the colleagues from the electronic department and from the machine shop for the help in preparing the experimental setup.

REFERENCES

[1] S. Felder and E.L. Elson. Journal of Cell Biology. 111 (1990) 2512-2526.

[2] C.M. Franz, A. Taubenberger, P.-H. Puech, and D.J. Müller. Science STKE (2007) 406, l5.

[3] R. Ehret, W. Baumann, M. Brischwein, A. Schwinde, K. Stegbauer, and B. Wolf: Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures. Biosensors and Bioelectronics 12 (1997) 29-41.

[4] C.G. Marxer, M.C. Coen, T. Greber, U.F. Greber, and L. Schlapbach: Cell spreading on quartz crystal microbalance elicits positive frequency shifts indicative of viscosity changes. Anal Bioanal Chem (2003) 578-586.

[5] H.P. Schwan, Electrical properties of tissue and cell suspensions, Adv. Biol. Med. Phys. 1957;5:147-209.

[6] G. Cevc, Membrane electrostatics, Biochimica et Biophysica Acta 1990;1331-3:311-382.

[7] A.N. Darinskii, M. Weihnacht, Acoustic waves guided by a fluid layer on a piezoelectric substrate, J App Phys 104 2008;5: 54904/1-9.