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A new tool to assess mechanical and dielectric properties of tissues

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Abstract—**In this paper a new tool to assess viscoelastic and dielectric properties of biological samples is presented. Shear horizontal polarized surface acoustic waves (SH-SAW) are used to detect the viscoelastic properties of the extracellular matrix (ECM) expelled by adhering fibroblast cell cultures. Therefore a one-port SAW resonator, with a fundamental mode of 85 MHz was developed. Its electrode structure can be used simultaneously to detect the dielectric behavior of the whole system by impedance spectroscopy (IS) while the frequency ranges from kHz to MHz. The applicability of the combination of both methods appearing as new tool is exemplarily shown by cell adhesion experiments performed with L929 and NIH-3T3 fibroblasts.**

I. Introduction

The method presented here combines two well known measurement techniques, IS and SAW. Both techniques offer high accuracy and reproducibility suitable for biological research.

Research including impedance measurements of biological systems has been performed for over 100 years. That cells are surrounded by a small non-conducting layer was discovered in 1910 [1]. Well known work on the general interpretation of dielectric spectra was performed by Cole et al. (Cole-Cole-plot) [2]. Additional contributions to the investigation of cell cultures, tissue and cell suspensions were made by Schwan [3] and Giaever et al. [4] in the middle of the last century. The increasing number of publications in this field shows the great interest in this measurement technique [5-7].

In general, impedance spectroscopy is a non destructive method where the impedance spectrum over a wide frequency range is detected and interpreted. The permittivity of an adhering cell culture plotted vs. frequency shows four step changes, also called dispersions [3]. These dispersions can be attributed to frequency dependent polarization effects. The first step change occurs between 1 Hz and 5 kHz. It is called α-dispersion and was found to be due to the flow of ions along the cell surfaces. The next step change is the β-dispersion which is related to the increase of charge at the cell membrane [8]. The measurement system presented here deals with these two step changes.

SAW devices are widely used as frequency filters, ID tags and gas sensors. Their development started in the 1950's. Several approaches using SAW in biosensor applications have been made in the last fifteen years. For example the suitability to monitor DNA, enzyme and protein binding as well as antibody reactions have already been reported by several groups [9, 10]. The SAW method is also non destructive.

In principal, SH-SAW devices are comparable with thickness shear-mode resonators (TSM) also known as quartz crystal microbalance (QCM). 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 including smaller sample volumes, less susceptibility to experimental factors like pressure changes or mounting forces. Additionally, SAW devices can be made with a double side polished finish, allowing microscopic observations. 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. Therefore the acoustic wave is mainly influenced by material properties close to the sensor surface.

In many cases 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, the SAW devices where realized as one port resonators with one IDT and two reflectors. Thus, a standing wave is created and can be characterized by changes of resonant frequency, bandwidth or impedance. However the SAW resonator is sensitive to both changes of viscoelastic and dielectric properties of the surrounding media.

II. Experimental

A. Experimental Setup

The developed SAW devices were realized as one port resonators made of 36° YX-$LiTaO_3$, (*i.e.* $LiTaO_3$ cut parallel to the crystallographic x-axis but along a plane rotated 36° away from the xz plane about the x-axis). The propagation direction of the acoustic wave is along the x-axis. Thus, horizontal polarized standing shear waves are generated on the surface of the device. The fundamental frequency of the developed SAW devices is 85 MHz. The impedance minimum 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. Cell culture chambers consisting

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of a basis of cover glass and a seal made of polydimethylsiloxane (PDMS) were constructed and manufactured. These chambers cover an area of 1 cm^2. Volumes of culture medium up to 1.4 ml are applicable. For continuous measurements the SAW cell culture chambers were mounted on the microscope including an incubator unit for temperature stabilization. The whole setup can be sterilized in an autoclave. Electrical measurements were carried out with a network analyzer E5070B (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.

B. Investigated cell cultures

In this study, the adhesion behavior of murine fibroblasts L929 and the murine embryonic fibroblasts NIH-3T3 (CLS - Cell Lines Service, Eppelheim, Germany) were investigated. The cells were incubated at 37 °C, in 89 % Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 10 % fetal bovine serum and 1 % penicillin-streptomycin. For cell counting the Mühlbauer counting chamber was used giving a rough estimate of the concentration of cells inside the cell culture.

III. RESULTS

A. Measurement Protocol

Due to the high sensitivity of SAW devices, much effort has to be made to get stable measurement signals. The temperature dependency of the medium and the SAW device was found to be the main unwanted effect. An increase of temperature causes a decrease of resonant frequency of an unloaded sensor. But an increase of temperature also causes a decrease of viscosity of the sample fluid leading to an increase of resonant frequency of the sensor. The temperature of the measurement chamber was kept constant at 37.00 °C (+/- 0.01 °C) to avoid these temperature dependent secondary effects. The SAW cell culture chambers and the cell suspension were brought to the final temperature one hour before the start of the measurement.

B. Cell adhesion process measured with SAW devices

Fig. 1 shows the relative change of resonant frequency of 4 SAW devices as it was measured within a cell adhesion experiment with NIH-3T3 fibroblasts. The cells were seeded with an amount of $5*10^4$ cells/ ml at the start of the measurement (t = 0 h). During the first four hours a steep increase of the resonant frequency was measured. During this time the cells sediment to the sensor surface and immediately start to expel 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 out on the surface to increase their surface contact area. These processes are finished after 4 h when no further changes of resonant frequency occur. After the spreading out phase, the cells begin to reorganize their actin microfilaments in order to move along the sensor surface.

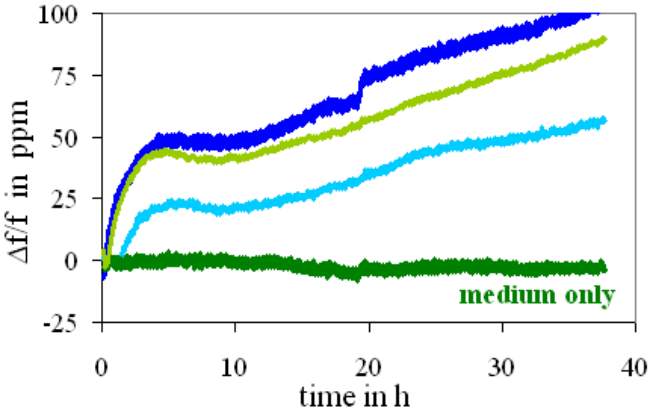

Fig. 1. Relative change of resonant frequency vs. time of 4 different SAW devices at one cell adhesion experiment performed with NIH-3T3 fibroblasts. Three sensors were loaded with cell suspension and one as reference with medium only (dark green chart).

After 10 h a further increase of resonant frequency was found in all three sensors loaded with the cells. At this time point the cells begin segmentation. This leads to an increase of the amount of cells in the measurement chamber. After 38 h an almost complete monolayer was observed by optical microscopy (Fig. 2a). At this time the measurement was stopped and the cells where treated with Wright's stain (Fig. 2b). This is a simple colorization test where adhered cells were fixed and colored in blue. Not adhered cells were flushed away during this procedure. With this test could be proven, that the cells adhere to the $LiTaO_3$ sensor surface. At the reference sensor loaded with medium no changes of the resonant frequency were measured.

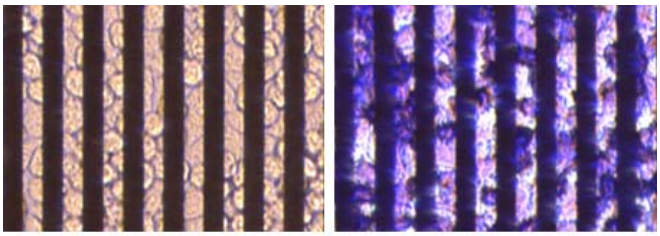

Fig. 2. Optical micrographs taken with an inverted microscope corresponding to the NIH-3T3 cell culture measured with a SAW sensor (bright green curve in fig. 1) after the end of the experiment. a) untreated state (the SAW electrodes can be seen as black lines). b) the same cell culture treated with Wright's stain (adhered cells were fixed and colorized, not adhered cells where flushed away during treatment). The dimension of the detail is 192 µm by 128 µm.

Generally, surface acoustic wave devices act as electromechanical transducers. Thus, the mechanical properties of the surrounding media affect the wave propagation on the sensor surface and can be related to changes of the resonant frequency. In fluids, the strongest influences came from the viscosity and partly from the elasticity. Without electrical isolation the permittivity of the samples might affect the resonant frequency.

The penetration depth δ of the acoustic wave into a fluid is limited to the value of the resonant frequency and the mechanical fluid properties viscosity and density. For SAW devices with a fundamental mode of 85 MHz, the penetration depth in aqueous media is less than 100 nm given by the respective viscosity-density-product. During the cell adhesion process, the medium that is in contact with the sensor surface at t = 0 h is replaced by a small layer of ECM topped with the layer of cells. While the spread cells have a height between 2 and 5 µm the thickness of the ECM lies in the nm range. Thus, the main impact on SAW propagation parameters during adhesion process will originate from the electrical and mechanical properties of cell layer and ECM and not from the surrounding medium.

In general, a decrease of the viscosity leads to an increase of resonant frequency and a decrease of the impedance of the resonance. In our cell adhesion experiments, an increase of both, resonant frequency and impedance of the resonance was always measured (impedance data comparable to those presented in upper diagrams, fig. 4). Consequently, these changes can not exclusively be explained with a change of the viscosity of the ECM.

Because of the complexity of the layered system of cells and ECM formed in the course of adhesion process several explanations for the observed change of SAW data with time are possible. The most notable increase of resonant frequency during cell adhesion can arise from the shear elasticity of ECM or of the cells as implied from acoustic wave modeling (details of the method used are given in [11]). In addition, the changes in the dielectric properties of the ECM/cell coating on the SAW resonator compared to the situation at the beginning of the measurement can affect the SAW resonant frequency in the observed manner (figs. 1 and 4). Besides, according to the working principle of SAW resonators, changes in the reflection coefficients due to cell adhesion and the resulting shift of the resonant frequency has to be taken into account. A mixture of all these effects can contribute to the experimental findings.

In summary, it can be said that more experiments are needed to investigate these influences separately. For instance, changes of the permittivity can be inhibited by preparing sensors with electrically shielded electrodes.

C. *Variation of the cell concentration*

In this experiment, the cell adhesion process of murine fibroblasts L929 with different cell concentrations was investigated. Therefore, a cell culture was grown, counted and diluted to $30*10^4$ cells/ ml. From this suspension, a 2nd dilution step down to $10*10^4$ cells/ ml was done. Afterwards, two sensors were loaded with the high cell concentration, two with the lower one.

In Fig. 3 and 4 the results of this experiment are shown. The sensors with the same concentration of cells brought similar results. For better presentation, only one result per group is presented.

1) *Impedance spectroscopy measurements*

The charts in Fig. 3 show the experimental results obtained with impedance spectroscopy measurements on a SAW device. The absolute value of impedance (|Z|) over a wide frequency range is recorded by using the SAW IDT electrodes. Afterwards, the relative change of the impedance $\Delta|Z|/|Z|_{Max}$ is calculated for each frequency point relative to the initial impedance values after seeding the cells. Finally, the frequency with the highest relative change is analyzed. The red dashed line in Fig. 3 indicates this frequency. According to [3] this frequency lies in the β-dispersion that in classical theory is related to the build up of charges in the cell membrane [12]. From these values the membrane capacitance of the particular type of cells and the membrane thickness can be calculated.

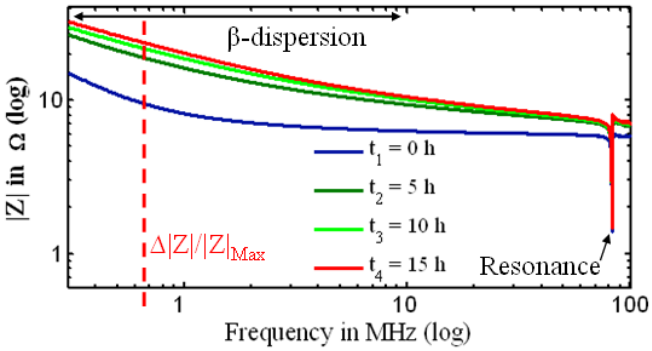

Fig. 3. Absolute value of impedance vs. frequency measured on a SAW IDT during a cell adhesion experiment (L929, $30*10^4$ cells/ ml). The frequency with the highest relative change of |Z| is indicated by the dashed red line. The change of the impedance at this frequency is plotted in the lower diagram of Fig. 4b.

The blue curve in Fig. 3 was recorded directly after loading the sensor with the cell suspension. Therefore it represents the impedance of the medium while the cells start to sediment. The other three charts show the impedance characteristics of the growing cell layer at different time points.

2) *SAW and IS measurement data in comparison*

The diagrams in Fig. 4a and b show the results of the cell adhesion experiment introduced in subsection III C. The values for the changes of the SAW and the impedance spectra were recorded simultaneously. After the loading, when the cells sediment to the sensor surface and start to express ECM followed by spreading and adhesion, all parameters are increasing. After 2 h this initial phase is finished. The cells are adhered. This initial change of the parameters can be correlated to the amount of seeded cells. For instance, the resonant frequency increases about 20 ppm at the lower cell concentration and about 60 ppm at the higher concentration of seeded cells.

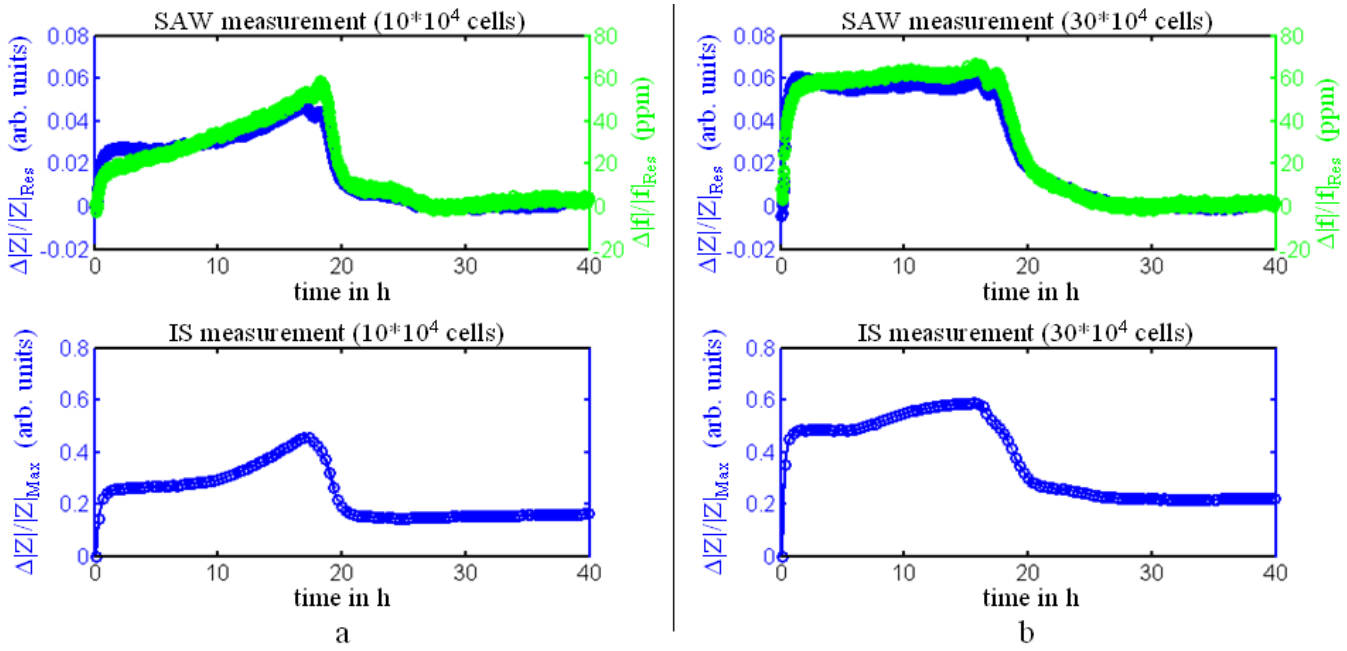

Fig. 4. Variation of the cell concentration (L929) and comparison of the relative change of resonant frequency and its impedance (upper diagrams) and the relative change of impedance at the frequency where the highest relative change of |Z| did occur vs. time.

After the initial phase, the monolayer is almost completely developed in the high concentration of seeded cells, as it can be seen from the constant resonant frequency in Fig. 4b. This implies that the whole sensor is covered with the ECM. In contrast, after 8 h, the impedance of the same cell culture starts to increase with a small slope. This might be caused by the division of some cells in order to complete the monolayer. After 18 h, the medium is exhausted and the cells start to die. The resonant frequency therefore decreases to its initial value and the impedance of the IS measurement reaches a new state slightly higher than the initial value. The most likely explanation is, that the viscosity of the exhausted medium is similar to its initial viscosity but the dielectric properties are different, changed through the metabolism of the cells.

The end of the initial sedimentation phase also can be found in the lower concentration of seeded cells after 2 h (Fig. 4a). Afterwards the resonant frequency starts to increase continuously. That could be caused by moving cells simultaneously expelling more proteins of ECM. As indicated by the IS measurement, the segmentation and proliferation of the cells starts after 8 to 10 h. The slope of the IS data for the higher cell concentration (Fig. 4b) is smaller than that for the low cell concentration (Fig. 4a). Therefore it is interpreted that the proportion of cells that divide is larger for the lower cell concentration. When the medium is exhausted, the cells also start to die causing a decrease of resonant frequency and impedance.

The relative change of the IS-impedance is one order of magnitude higher, compared to the change of the impedance of the resonant frequency (blue charts).

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REFERENCES

[1] R. Höber, Eine Methode, die elektrische Leitfähigkeit im Innern von Zellen zu messen, Arch Ges Physiol 1910;59:133-237.

[2] K.S. Cole, R.H. Cole, Dispersion and absorption in dielectrics. I. Alternating current characteristics, J Chem Phys 1941;51:9-341.

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

[4] I. Giaever, C.R. Keese, Monitoring fibroblast behavior in tissue culture with an app. el. field, Proc. Nat.Acad. Sci 1984;81:3761-3764.

[5] G.H. Markx, C.L. Davey, The dielectric properties of biological cells at radiofrequencies, Enzyme and Microbial Tech. 1999;25:161-171.

[6] P. Patel, G.H. Markx, Dielectric measurement of cell death, Enzyme and Microbial Technology 2008;43:463-470.

[7] G. Park, C.K. Choi, A.E. English, Tim.E. Sparer, Electrical impedance measurements predict cellular transformation, Cell Biology International 2009;33:429-433.

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

[9] K. Saha, F. Bender, A. Rasmusson, E. Gizeli, Probing of the Viscoelasticity and Mass of a Surface-Bound Protein Layer with an Acoustic Waveguide device, Langmuir 2003;19: 1304-1311.

[10] M.D. Schlensog, T.M.A. Gronewold, M. Tewes, M. Famulok, and E. Quandt, A Love-wave biosensor using nucleic acids as ligands, Sensors and Actuators B 2004;101:308-315.

[11] 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.

[12] H. Fricke, The electric capacity of suspensions with special reference to blood, J. Gen. Physiol 1925;9:137-152.