LABEL FREE BIO SENSING METHOD USING RADIO FREQUENCIES SPECTROSCOPY FOR CELL DETECTION AND DISCRIMINATION

Claire Dalmay, Arnaud Pothier, Pierre Blondy
XLIM – UMR 6172 Université de Limoges/CNRS, France

Fabrice Lalloue, Marie-Odile Jauberteau
Homéostasie cellulaire et Pathologies, Université de Limoges, France

Keywords: Bio sensor, Electrical bio-impedance, Microelectronics, RF planar devices.

Abstract: This paper presents an original label free bio sensing method allowing the study of electrical properties of human cells and so potentially cell identification and discrimination. The proposed bio sensor is based on a planar resonator operating at microwave frequencies, fabricated using a standard microelectronic process. As the result its microscopic sensitive areas allow an improved detection at the cell scale which represents a significant step in the study of many biological phenomenon. Thanks to a specific experimental protocol, we present in this paper a simple method allowing electrical parameters measurement on a small number of cells with a good accuracy.

1 INTRODUCTION

In recent years, biosensors known a great interest as there is an important need for tools that can quickly and accurately analyse biological elements like bio molecules or cells. Current optical and chemical bio detection techniques can effectively analyse biological systems but present some drawbacks ; especially their requirement of specific labels to enhance the signal generation. These labelled methods make the sample preparation more complex, expensive and time consuming. In addition, the sample can be largely chemically altered prior analysis. In the other way, electronic detection techniques are very interesting methods as they allow the development of label free methods (Kim *et al*. 2007). Thanks to microelectronic technology a significant improvement of electrical sensor detection performance can be expected since resulting miniaturized biosensors are now able to work at the cell scale.

In this paper, is presented an electric label free method allowing to evaluate cell inside medium permittivity and conductivity in the gigahertz frequency domain. Actually, these two specific parameters are influenced by the cell type and morphology but also by their physiological state.

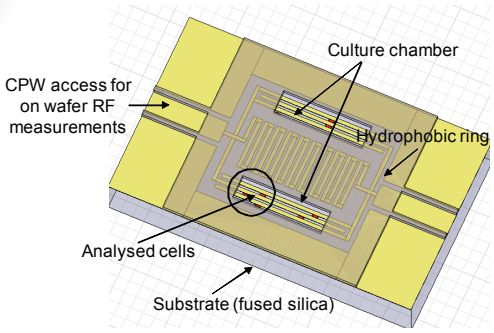

Figure 1: Schematic of the studied biosensor.

As example, tumorous cells are well known to present a larger conductivity and permittivity than normal cells (Blad and Baldetorp, 1996). Hence, individual cell electrical properties measurement represents a complementary tool allowing efficient cell identification.

Developed biosensors are actually based on a coplanar microwave resonator design (figure 1) able to operate at radio frequencies with a significant

Dalmay C., Pothier A., Blondy P., Lalloue F. and Jauberteau M. (2009).
LABEL FREE BIO SENSING METHOD USING RADIO FREQUENCIES SPECTROSCOPY FOR CELL DETECTION AND DISCRIMINATION.
In *Proceedings of the International Conference on Biomedical Electronics and Devices*, pages 3-6
DOI: 10.5220/0001122800030006

sensitivity to tiny concentrations of biological medium that interacts with the sensor. Moreover its planar configuration will make easier a coming integration in microsystems with microfluidic flow-through network enabling accurate cell sorting applications as example.

2 BIO DETECTION METHOD

2.1 Biosensor Design

In this study a resonant structure has been favoured because by nature much more sensitive to very small cell concentration in comparison with wide band device (Denef *et al*, 2004). But in the other way, available analysis spectrum will be limited to a narrow band around the sensor resonant frequency. Wider band investigation will so require fabricating several resonators with different resonant frequencies.

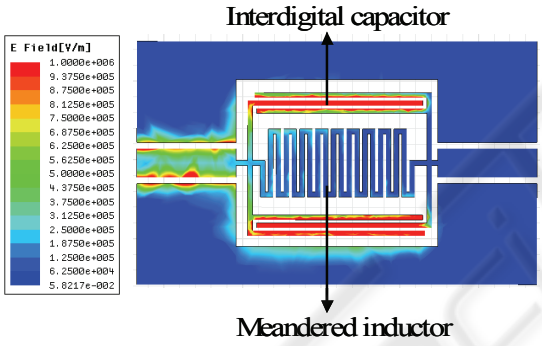

Figure 2: RF Electromagnetic field distribution plot at resonance frequency.

The developed micro biosensor has been designed as a coplanar RLC resonator made with a meandered inductor associated in parallel with an inter-digital capacitor. In our case, used resonators present a RF signal attenuation which becomes maximum at the resonant frequency. As shown on figure 2 at this frequency, the electromagnetic field distribution is strongly concentrate in the capacitive part of the device which represents the more interesting interaction area for a capacitive detection. Indeed, the introduction of any biological media even in very small concentration will meaningfully disturb the EM field distribution inducing a detectable shift in the measured resonance frequency of the sensor (figure 3). This frequency shift will be all the more significant if cells are located close to gaps between metallic lines where the electromagnetic field is strong.

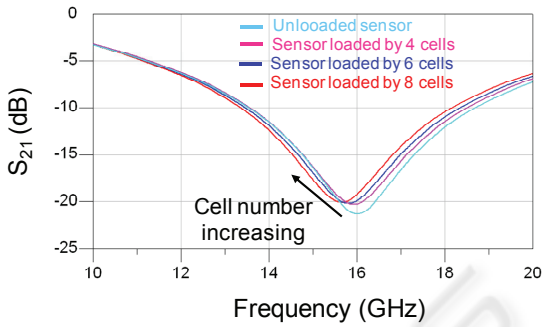

Figure 3: Electromagnetic simulation of the cell number influence on the sensor RF response:*S_{21} parameter relies on the RF signal attenuation through the resonator.*

Hence, the detection resonator performance strongly relies on the biosensor sensitivity capabilities where two parameters play a major role. First, interaction between the EM field and cells to be analysed must be maximized using an appropriated sensor design with gaps between metallic lines in the same order of magnitude of analysed cell sizes: in the present case considered gaps will be close to 10 µm. Then, a sufficient resonator unloaded quality factor (relative the resonator intrinsic loss) has to be also considered; as it controls how the resonant frequency pick will be narrow and so the sensor frequency sensitivity to a small frequency shift.

Once biological cells will be present on the sensor surface, both their location and their number will directly influence its response. As shown on figure 3, following our approach the detection of a low number of cells (at least less than ten) can be expected.

In the end, a microfluidic network will certainly be required but in order to demonstrate the sensor capability, we have chosen to develop a specific experimental protocol for instance, allowing an easier test procedure with the cost of the difficulty to work with real alive cells. This protocol will be presented in the following paragraphs.

2.2 Biosensor Fabrication Process

Micro sensors are fabricated using standard microelectronic process with biocompatible materials (figure 4).

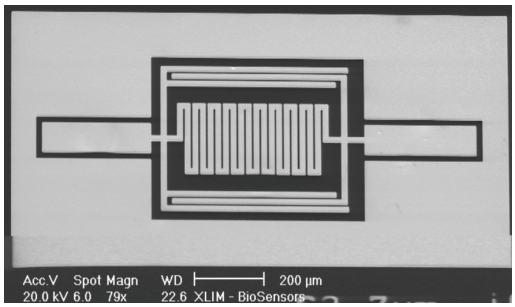

Figure 4: SEM photograph of the fabricated micro sensor.

A fused silica substrate has been preferred to classical silicon one especially for its lower loss properties in the RF frequency domain and also for its transparency that makes easier the observation of cells throw it. A classical photolithography allows to define thin gold lines which are next electroplating up to a thickness of 3 µm. Then a SU8 photoresist from Microchem is used to create a 20µm thick well localised culture micro-chamber on the sensor surface.

2.3 Experimental Protocol

During characterizations, we have to ensure the integrity of cells. Usually, a support biological media, in which cells could be protected, is required. As shown on figure 5, most of support medium commonly used in biology are aqueous saline solutions which present very strong losses for RF signal especially when the sensor is fully cover with it. Actually in this configuration, the biological media alters in a too important manner the RF performance of the device avoiding any accurate detection.

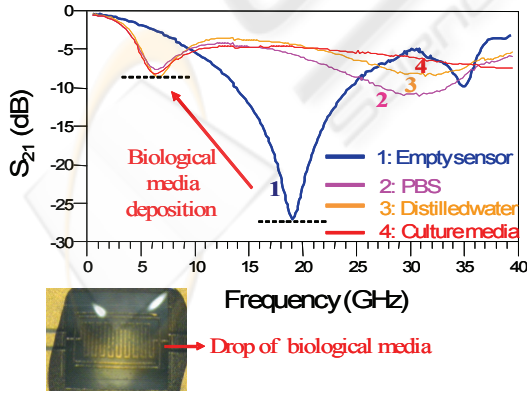

Figure 5: Influence of different biological support media 20nl drops on the sensor RF performances.

A first solution could be to limit used biological media to a very small volume, typically implying a microfluidic flow-through system.

Another alternative will be to perform analysis in a specific low loss support media, as done in previous work (Dalmay *et al*., 2008) using ficoll (a polymeric gel of sucrose). Hence, once dried, the ficoll drop allows to protect cells to be analysed in a low permittivity polymeric matrix with the cost of a aleatory cell location on the sensor surface and in the ficoll matrix that induces a significant error in their electrical parameter extraction. Consequently, there is a great interest in developing an experimental protocol without any biological support media.

Actually with the proposed approach in this paper, cells are directly grown on the sensor surface submerged in a classical culture media. Few days are required to allow a sufficient cell adhesion on the sensor surface. Then sensor are washed in deionised water following by paraformaldehyde 4 % bath (PFA) in order to definitively fixe cells and so to avoid cell degradation during the measurement sequence. Since most of cell adhesion occurs preferentially on the silica substrate than on gold lines or on SU8 resist, most of fixed cells are located only between gold lines in the culture micro-chamber (figure 1). Number of cell on the sensor are roughly controlled both with the cell concentration initially dispersed in culture media and the culture time.

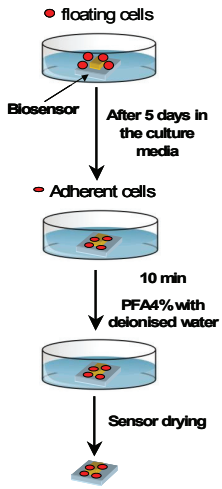

Figure 6: Experimental protocol process.

After PFA bath, cells are no longer alive, but their original form, their intracellular content and their electrical properties have been kept as in living conditions. Then sensor are again washed in deionised water and dried just before measurement.

3 EXPERIMENTAL RESULTS

All characterizations are performed using classical microwave measurement techniques with on wafer probing; as it allows a quick and a successive sensor measurements.

First, for each sensor, the transmitted microwave signal attenuation across the unloaded resonator is recorded using a calibrated vector network analyser. Then cell culture is performed; at the end, loaded sensors with fixed cells are measured following the same procedure than before cell growth. Hence, the induced resonator frequency shift value, related to the cells electrical properties can be extracted.

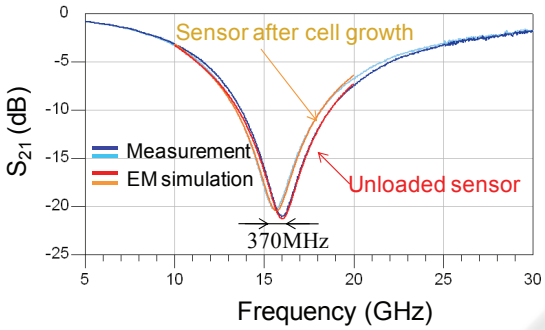

Figure 7: Biosensor measured response before and after the cell growth and simulated one.

Figure 7 shows results of experimentations with glial-cells-derived tumour glioblastoma coming from human nervous system cells. Used biosensors initially resonate at 16 GHz and shift down to 15.63 GHz when it is loaded with only 8 glial-cells (figure 8).

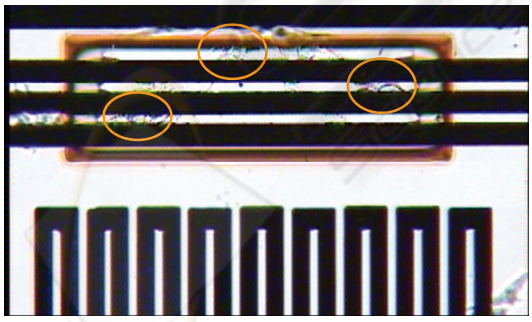

Figure 8: Photograph of the sensor after the cell growth.

Fullwave simulations, based on finite element method (HFSS from ANSOFT), are then used to extract individual cell electrical properties. Cells EM modelling is done assuming that they are homogenous, source-free and linear dielectric volume. Hence, on a narrow frequency bandwidth around the sensor resonant frequency, cell global permittivity and conductivity can be extracted with a good accuracy by fitting simulations data with measured one, as shown on figure 7.

Hence in the case of analysed glial-cells, we have obtained an effective permittivity value of 36 ± 1 while global conductivity has been estimated 0.100 ± 0.003 S/m at 16 GHz and 20°C. These results agree very well with previous analysis done with ficoll media (Dalmay *et al*., 2008) and can be compared to the effective permittivity of pure water which is closed to 45 at 20°C. Other characterizations are currently done with other cellular types, to demonstrate that it is possible with this approach to discriminate between different cell types.

4 CONCLUSIONS

An original label free bio-sensing approach for cellular analysis at radio frequencies has been demonstrated. Thanks to their sub millimetric size, used sensors are able to work at the cell scale with a very limited number of cells and can potentially be a novel promising tool for cell discrimination. Further work is ongoing to evaluate experimentally the minimum number of cell analysis achievable and to improve the sensor design and experimental process for one single cell analysis.

REFERENCES

Young-Il Kim, Yunkwon Park, Hong Koo Baik, 2007. “Development of LC resonator for label-free biomolecule detection”, Sensors and Actuators A.

B. Blad, and B. Baldetorp, 1996. “Impedance spectra of tumour tissue in comparison with normal tissue; a possible clinical application for electrical impedance tomography”, Physiol.Meas., vol. 17, pp. 105- 115.

T. W. Athey, M. A. Stuchly, S. S. Stuchly, 1982. “Measurement of radio frequency permittivity of biological tissues with an open-ended coaxial line : Part I,” IEEE Trans. Microwave Theory Tech,. vol. 82, pp. 82-86.

N. Denef , L. Moreno-Hagelsieb, G. Laurent, R. Pampina, B. Foultier, J. Remacle, D. Flandre, 2004. “RF detection of DNA based on CMOS inductive and capacitive sensors”, EUMW Conference Digest, pp. 669-672.

C. Dalmay, 2008. “Label free biosensors for human cell characterization using radio and microwave frequencies”, IEEE MTT-S International Microwave Symposium Digest, IMS 2008.