Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Cell Investigation by Terahertz BioMEMS

A. Treizebré, B. Bocquet, D. Legrand, J. Mazurier

Abstract—**Quite recently, it was found that metal wires can effectively guide terahertz radiation. We report in this communication an original planar excitation of surface wave on a single wire transmission line. This configuration is well suited for the design of THz BioMEMS dedicated to cell investigation. We show that we can deal with a micrometer spatial resolution.**

Index Terms—**Biological micro-electromechanical systems (BioMEMS), coplanar waveguide (CPW) transition, Goubau-Line (G-Line), Terahertz (THz), living cells.**

I. INTRODUCTION

The use of micro/nanotechnologies for the investigation in life science is a new key for a better knowledge of biological activities. We see an evolution of the biochips, dedicated in a first time to the studies on DNA, with a high socioeconomic impact. This effort in now followed on the development of protein biochips and the integration of mechanical or fluidic actuation directly inside the chips, and called BioMEMS (Biological Micro-ElectroMechanical Systems). The research activity in this field now concerns the investigation on cells, particularly for the knowledge of the informative assemblies and their transfers inside the cells.

On the other hand, dielectric spectroscopy is a widely used technique for the characterization of biological entities up to several Gigahertz (GHz) [1]. Note that this way allows removing the principal drawbacks of the electrochemical or optical investigations which are probably the alteration of the biological activity by chemical reaction or by the fluorescent tags bounded on molecules. The interest in the direct measurements is still reinforced by the fact that the low binding energies inside heavy molecules, like proteins, is now attainable. The photon energy level allowing this direct observation is in the TeraHertz (THz) electromagnetic spectrum. This bandwidth stayed for a long time unexploited is now usable due to the development of integrated sources and detectors.
Most of THz investigations on biological entities are done in free space which is a good technique for the spectroscopy of gas or solid phase. Unfortunately, the investigation on cells requires working on liquid phase. This trend is difficult due to the very high absorption of THz waves in aqueous solutions, except if we work on very small volumes. The development of BioMEMS brings us interesting solutions by combining microfluidic circuits and THz planar waveguides [2]. Note that the propagation of the THz waves is possible with a one conductor waveguide. We have designed and proved a good propagation with a planar wire, with the main advantage of a possible use in integrated chips. Moreover, we have demonstrated that a strong narrowing of the line's width to several hundred of nanometers maintained a good propagation, with a micrometer scale resolution [3].
We apply and describe in this paper, the investigation on Chinese Hamster Ovary (CHO) cells for studying the influence of the lactoferrin on the information transfers through the cell membrane. We have demonstrated recently the nucleoline, a nuclear protein, has been recognized as a binding site of lactoferrin on the membrane cells [4].

II. TECHNOLOGICAL CHALLENGE

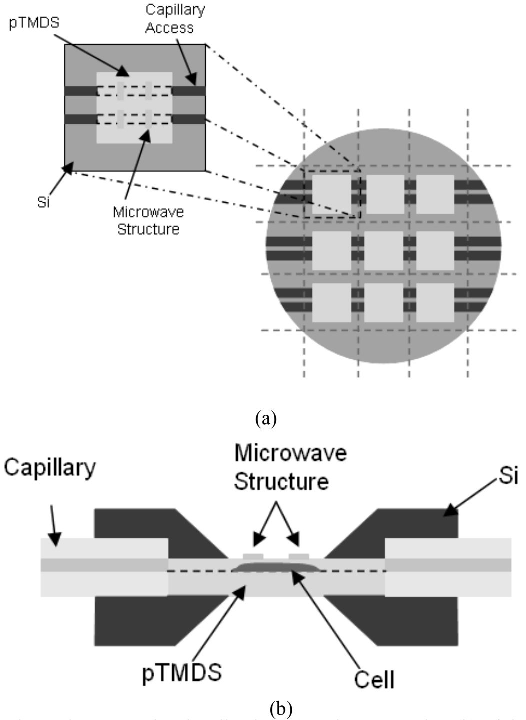
We developed a polymer on silicon mixed technology for the realization of BioMEMS. Silicon is the material used in micro-electronics. Its advantage is the integration of electronic components very closely to the detectors. The signal to noise ratio in the biological measurement is not always good, and it is important to treat the signal directly on the chip for reducing the noise information transmission. Moreover, the transduction of the biological phenomena in numerical data leads to directly exploitable information by the user. We have synthesized a new polymer with a "cold" plasma process and named pTMDS (Plasma Polymerized Tetra Methyl Disiloxane) [2]. There are very various characteristics, like its biocompatibility, essential for survival of the cell, its transparency to allow an optical observation during the insertion of the cells within BioMEMS, like its electromagnetic properties in terms of dielectric loss about 2.5dB/mm at 220 GHz, this is an

---

Manuscript received April 3, 2006.
Anthony Treizebré and B. Bocquet are with the Institute of Electronic, Microelectronic and Nanotechnology, DHS Dpt. Microsystems microfluidic and Terahertz Team, Lille University, France (e-mail: Bertrand.Bocquet@iemn.univ-lille1.fr Phone: +33(0)3-20-19-79-37
Joel Mazurier and Dominique Legrand are with the Unity of Structural Glycobiology and Functional, Lille University, France.

essential parameter in the development of THz BioMEMS [5].

The BioMEMS is composed of two parts: microfluidic part and microwave part (Fig.1a). In a first step, microfluidic circuits are constituted by microchannels. Their sizes are defined by the dimension of the living cells, CHO cells in our study. These cells have a mean diameter of 20µm in suspension and take a spindle shape of 50µm length, 20µm width and 10µm height. For that, we dig microchannels of 50µm of width by 50µm of depth for guaranteeing a good circulation of the nutritive fluid. The connection of the BioMEMS is carried out by using capillaries of 150µm of diameter with 50µm of internal diameter. The biological fluid as well as the cells will be injected by using microsyringes connected to the capillaries, and fitted with precise flow control. This flow can be decreased down to 1,6nl/mn and computations are in progress to confirm that very small flow intensity does not create stress on cells. The microwave part uses a new propagation mode around a central conductor. We have demonstrated recently that a thinning down of this wire decrease also the extension of the electric field [3]. This characteristic is a very good opportunity allowing a cell investigation with a micrometer spatial resolution. We explain in detailed this mode in the following part.



(a)

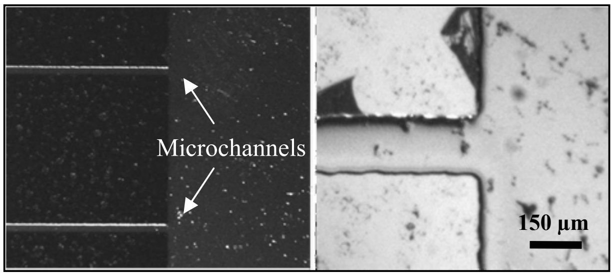
(b)

Fig1: Diagrammatic of realization (a) and cross-section (b) of the BioMEMS considered

The first step of the technological process (Fig.1b) consists with a silicon etching for fabricating the tank as well as the capillary access. The first wafer of silicon is etched by ICP (Inductively Coupled Plasma) dry etching with SF6 gas, the speed of etching is about 5µm/min. The mask used AZ4562 resin of 6µm thickness. The selectivity between silicon and the resin is 1:30 (Table.1). One comes to etch 150µm by Bosch (process with cycles of etching and passivity) (Fig.2a) with SF6 gas for etching and C4F8 for passivation. We obtain very good right blanks (Fig.2b).

Etch	Gas Flows (sccm)				Power (W)		Pressure (mTorr)	µm/min
	SF6	C4F8	CF4	O2	RIE	ICP		
Si	450	100		45		2500	30	0.4
ppTMDS			20	5	150	200	50	5

Table1: Various parameters of etching of Silicon and ppTMDS



(a)

(b)

Fig.2: Etching of the silicon micro channels by plasma ICP

The second step comes to fill this tank with the polymer pTMDS by a RPECVD (Remote Plasma Enhanced Chemical Vapor Deposition), also called “cold” plasma deposition [5]. We have optimized this process. The roughness is now 0,1µm peak to peak for a deposition of 30µm. We etch the microchannels in the pTMDS by ICP-RIE (Reactive Ion Etching) etching using plasma of CF4+O2 gas with aluminum hard mask of 0.5µm thickness. The speed of the polymer etching by this method is about 0.5µm/min (Table1).

On a second silicon wafer, we fabricate a polymer membrane which is used to close the microchannels. This membrane has 2µm thickness and it is realized in BCB (Benzocyclobuthene). We bound these two wafers with a small layer of BCB at a temperature of 230°C under a pressure of 2 bars during 30 min.

The last step is the realization of the measurement waveguides and wires by using the electronic aligner. We write directly the electronic resin PAMMA/EL13%. We evaporate Germanium (Ge) layer of thickness 50A for evacuate electron during the writing. The Ge layer is attack by hydrogen peroxide ½ and DI water ½ during 45 second. The revelation of the electronic writing is done with MIBK-IPA mixture concentrate to 1/3 during 2 min 30. We evaporate titanium layer of thickness 500A like a binding layer for the gold layer of thickness 2500A. The last step consists to do a lift-off of metal in acetone.

Another significant parameter is the immobilization of the cell within the channel. We chose an approach which used the hydrophilic-hydrophobic properties of our polymers. We have shown that pTMDS has a high hydrophobic property, quite different of the BCB which is hydrophilic.

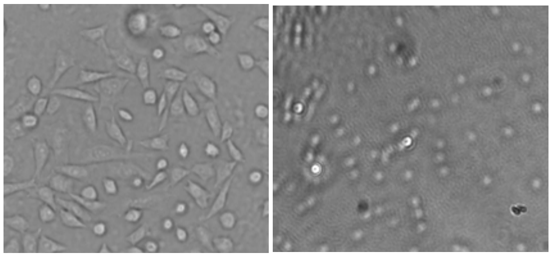

(a) (b)

Fig.3: Immobilization of cell on substrate of pTMDS (a) and substrate of BCB (b)

We show on the figure 3a that the cells have a good adherence on the BCB. It is not the case for the pTMDS (Fig.3b) where the cells keep a spherical form which means none adherence. Pads of BCB will be deposited at the bottom of the microchannels in pTMDS to immobilize the cells under the probes of measurements.

III. ELECTROMAGNETIC MICROSCOPY

The new development in THz rays show that low loss propagation around a conductor is possible. Electromagnetic surface wave modes have been studied more in details in the 50's by G. Goubau who has proposed a new kind of transmission based on a single wire. We have demonstrated that a thinning down of the line to some hundred nanometers lets a good transmission parameter. This design has a very high potential application in BioMEMS by the conception of THz probe down in the nanometer scale.

The key point for these modes remains their excitation and particularly, the excitation efficiency in the coupling with another waveguide topology. We develop an original solution based on a planar approach. In order to carry out on wafer-measurements by means of a Vectorial Network Analyser in the G-band, we have designed coplanar sections, thus to carry out a transition CPW-Goubau. If the excitation is a localized source, the radiating modes will be excited and the conversion efficiency between propagating CPW and Goubau line falls.

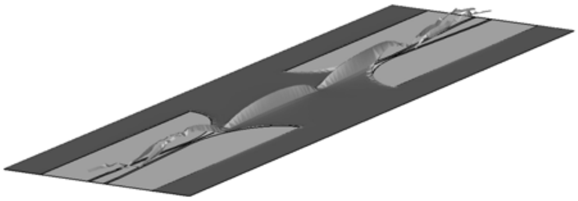


Fig.4: Result of simulation of the electromagnetic propagation wave along the structure

To validate this structure, we simulate it by using the commercial simulator "Microwave Studio" of Computer Simulation Technology CST®. This software makes it possible to simulate electromagnetic propagation structures 3D in the temporal field (Fig.4).

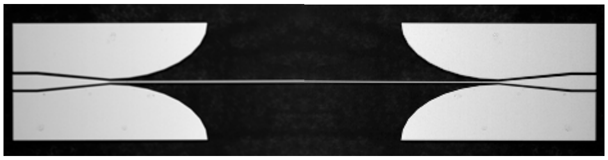


Fig.5: Realization of a structure made up of a line of Goubau of 1,5mm length with two coplanar-Goubau transition

The "classical" excitation of the Goubau line with horn antennas and a line going out from the central conductor [6], we can remark that our structure is a slice of it (Fig.5). We thus took a section of this one to carry out our planar excitation.

The measurements of these structures in the G-band present a flat transmission coefficient at a relatively high level: S_{21}=-6dB on a bandwidth of 140GHz (to 60GHz at 220GHz). From this study, we can conclude that the excitation efficiency is good.

As we have shown previously, characteristics of the Goubau modes are high confined spatial extension when the frequency increases and the wire diameter decreases. Our way is to design wires with nanometric sizes. We can obtain specific information on a part of a living cell for example, due to the high confinement of the electric field (Fig.6).

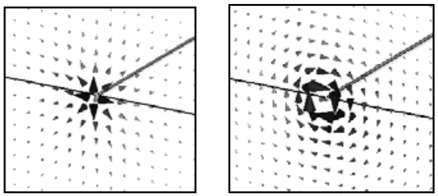
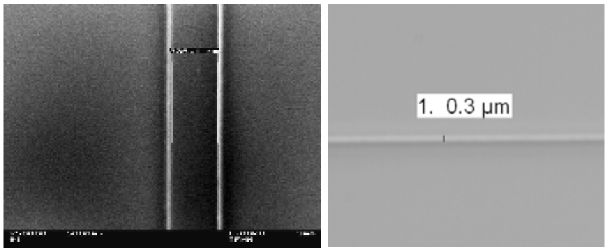

(a) (b)

Fig.6: Electric (a) and Magnetic (b) fields of Goubau mode around a metal strip of 1μm of large on a substrate.

We carry a wire of 300nm width to the electronic aligner on quartz substrate (Fig.7b).



(a) (b)

Fig.7: MEB photographs (a) and realization (b) of a line of nanometric resolution

IV. BIOLOGICAL INTEREST AND MEASUREMENTS

We propose to design, fabricate and test a BioMEMS dedicated to the electromagnetic spectroscopy of THz frequencies for the living cell analysis. The measurements are done with different type of cells for establishing the first dielectric spectroscopy database in this field. Moreover, the dynamical measurements of the internalization of informative molecules inside the cell membrane could bring complementary knowledge in cellular physiology. More precisely, the work is focused in the area of ligand-receptor interaction analysis, working with a well-characterized biological model (ligand: lactoferrin, receptors: nucleoline) available at the partners' laboratory. The purpose is to be able to assign, in real time, a specific crossing pathway to a ligand/receptor pair, without the use of molecular labels. The classification is based on changes in the electrical properties of the cell.

We propose to follow the various phases of molecular formation of assembly using models (lactoferrin, this receptor and co-receptor heparins sulphate and nucleoline) well characterized and controlled to follow their internalisation in a single cell. Thanks to the microfluidic system integrated into the BioMEMS, the protein ligands chosen for their different intracellular destiny (way of recycling, sees degradation, nuclear addressing) will be put in contact with the cell. The various stages of fixing, formation of the membrane complexes and intracellular traffic will be analyzed in real time by measuring their interaction with the electromagnetic waves. The spectra will be analyzed and compared with the results obtained from traditional techniques of microscopy.

V. CONCLUSION

The development of micro/nanotechnology is a chance for the biological science to improve dramatically its knowledge frontier. The research activity in this area combined different technological processes, new materials, extension of microsensors and so on. We demonstrate here that it is possible to gather microfluidic and electromagnetic circuits for designing very innovative microsystems. The electromagnetic propagation around a conductor, especially well fitted at the THz spectrum, allows investigation with a high spatial resolution, and consequently, with some applications in the cellular biology. We are going to show during the conference some realization of our BioMEMS and the first test and measurements on cells.

ACKNOWLEDGMENT

This work is supported by the National Research Agency funds PNANO n°05-0244-A3, the University Inter-disciplinary Program (PPF 2006 1803) and the National THz Network (GDR n°2897)

REFERENCES

[1] E.H. Grant, R.J. Sheppard, and G.P South, “Dielectric Behavior Biological molecules in Solution”, Clarendon Press, Oxford, 1978.

[2] B.Bocquet, N.E.Bourzgui, Y.Guhel, V.Mille, C.Vivien, P.Supiot, “Design of silicon-PPTMDS bio-MEMS by cold RPECVD”, Proc. Of SPIE Microfluidics, BioMEMS and Medical Microsystems II, 26-27 January 2004, Vol.5345, pp.118-129, San Jose, California USA.

[3] A.Treizebre, T.Akalin, B.Bocquet, “Planar excitation of Goubau Transmission Lines for THz BioMEMS”, IEEE Microwave and Wireless Component Letters, Vol.15, n°12, pp.886-888, 2005.

[4] F.R. Sallmann, S.B.Descamps, F.Pattus, V.Salmon, N.Branza, G.Spik and D.Legrand, “Porins OmpC an PhoE of Escherichia coli as Specific Cell-Surface Targets of Human Lactoferrin”, The Journal of Biological Chemistry, Vol.274, No.23, Issue of June 4, pp 16107-16114, 1999.

[5] V.Mille, N.E.Bouzgui, F.Medjdoub, B.Bocquet, “Design of silicon-pTMDS BioMEMS with millimetre and sub-millimeter waves transducers”, 34th European Microwave Conference, 11-15th October, pp.169-172, Amsterdam, 2004.

[6] G.Goubau, “Open wire lines”, IRE Trans. On Microwave Theory and Technique, vol. MTT-4, pp. 197-200, Oct. 1956.