



HAL
open science

RF sensor dedicated to the dielectric characterization of spheroids between 500 MHz and 20 GHz

Olivia Peytral-Rieu, David Dubuc, Katia Grenier

► To cite this version:

Olivia Peytral-Rieu, David Dubuc, Katia Grenier. RF sensor dedicated to the dielectric characterization of spheroids between 500 MHz and 20 GHz. *International Journal of Microwave and Wireless Technologies*, 2023, 1 (Special Issue 10), pp.1643 - 1648. 10.1017/S1759078723000508 . hal-04091380

HAL Id: hal-04091380

<https://laas.hal.science/hal-04091380>

Submitted on 8 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RF sensor dedicated to the dielectric characterization of spheroids between 500 MHz and 20 GHz

Olivia Peytral-Rieu^a, David Dubuc^a and Katia Grenier^b

^a LAAS-CNRS, Université de Toulouse, CNRS, UPS, Toulouse, France

^b LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France

To achieve better accuracy in their investigations, biologists have recently been using three-dimensional models as intermediates between 2D cell culture and the *in vivo* study of tissues. Some of these models are based on spheroids, which are cellular aggregates retaining many characteristics of *in vivo* behaviors while being still easy to use and implement in labs. To study such objects, multiple observation techniques can be used according to the objective of the study, including those using electromagnetic waves as non-destructive, non-invasive and label free analysis. Low frequencies (<1MHz) ones are currently under investigation as Electrochemical Impedance Spectroscopy. However, unlike microwaves (from 300MHz to 60GHz), they cannot penetrate the cell. Furthermore, most of the devices dedicated to microwave dielectric characterization are only focusing on cellular scales lower than spheroids (single cell, cell mats or suspensions) or on tissues and organs. In this article, a microwave spectroscopy device using a coplanar waveguide (CPW) adapted to the study of spheroids is presented with the dielectric characterization of fixed spheroids, with capacitance and conductance measurements from 0.5 to 20 GHz, aiming at filling the scale gap in the state of the art.

Keywords

Biosensor, Microwave, Electromagnetic waves, Dielectric characterization, Coplanar waveguide (CPW), Label-free analysis, Spheroids, Cells.

1. INTRODUCTION

As soon as cancers were discovered, it became clear to biologists that the use of *in vitro* models independently of *in vivo* analyses would be essential, given the complexity of the mechanisms involved in these diseases. 2D cell culture has been used extensively for its ease of manufacture and low cost. However, this type of model is far from the reality of *in vivo* systems. Therefore, 3D models of cell clusters in the form of spheres, also called spheroids, have been introduced as an intermediary between *in vivo* and 2D *in vitro* studies [1]. Indeed, spheroids are as easy and cheap to manufacture as 2D systems while having a complexity closer to physiological reality.

Numerous methods exist to analyse spheroids, the leading techniques being based on microscopy [2], [3]. In addition, electrical approaches are being studied, with a particular interest for

microsensors using the application of low-frequency electromagnetic waves [4]–[7]. Indeed, they have the advantages of being non-invasive, non-destructive, label-free and affordable. For example, impedancemetry, using waves with a maximum frequency of 1 MHz, makes it possible to study molecular exchanges with the extracellular medium and at the cell membrane surroundings [8].

Above a few hundred MHz, microwave dielectric spectroscopy involves the penetration of electromagnetic waves into cells through the cell membrane, opening up the possibility of analysing the intracellular content. Various devices exist to analyse cells in the microwave range, whether at the scale of the single cell, the cell suspension, the tissue or even the organ directly from the patient [9], [10]. However, for the intermediate spheroids scale, there is no device that allows their analysis in this frequency range. Thus, this article presents a radio frequency (RF) device designed for the analysis of spheroids of 300µm in diameter in the frequency range of 500 MHz to 20 GHz.

First the equipment and methods used will be detailed, including a description of the biological objects tested, the manufacture of the microwave sensor and a reminder of the electrical model used for data analysis, since it is described in [11]. Then, the first dielectric characterization of a spheroid in the frequency range 0.5-20 GHz will be presented. Finally, a conclusion proposing future perspectives will be given.

2. MATERIALS & METHODS

2.1. Materials & reagents

Formaldehyde (used with formalin solution, neutral buffered, 10%) is purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Gibco DMEM (high glucose, GlutaMAX supplement and pyruvate), fetal bovine serum (FBS) (HyClone), pen-strep solution (100X) (Gibco), 0.05% Trypsin-EDTA (HyClone), Gibco DPBS without Calcium, Magnesium (1X), Gibco Phosphate Buffered Saline 1X (PBS) and HEPES 1M (Gibco) are purchased from Thermo FisherScientific (Illkirch-Graffenstaden, France). The HepG2 cell line (catalog number HB8065) is purchased from the American Type Cell Collection (ATCC) (LGC Standards, Molsheim, France).

2.2. Cell culture

For our study, Human hepatoma cells (HepG2, passages 15-35) are cultured at 37°C/5% CO₂ in DMEM medium complemented with 10% FBS and 1% pen-strep solution. Cells are grown up to 70-80% confluence.

2.3. HepG2 spheroids formation

The spheroids formation protocol is illustrated in Fig. 1. The method we have chosen to make the spheroids is to use a mold made by Sigma, called Micro Tissue 3D Petri Dish size S, which is a 16 x 16 (256) cell array, sized to fit in plates containing 12 wells. 1% agarose is poured into these molds, left to cool, unmolded and then placed in six-well plates. The immobilization of this network of 256 wells at the bottom of the wells is done with agarose. Balancing the pH of the agarose is then necessary to find conventional cell culture conditions before placing the cells. It is carried out by putting culture medium for 45 min with a change of medium every 15 min. After this step, the micro-molds are ready to receive the cells.

The adherent cells cultured in flasks are passed through trypsin for 5 min, before undergoing centrifugation to re-suspend them at the concentration of 20 000cells/190µL. The cell suspension is, then, deposited in the micro-molds. The whole is finally left in culture for three days to form spheroids of approximately 300 µm in diameter (see Figure 1 for a detailed diagram of the protocol).

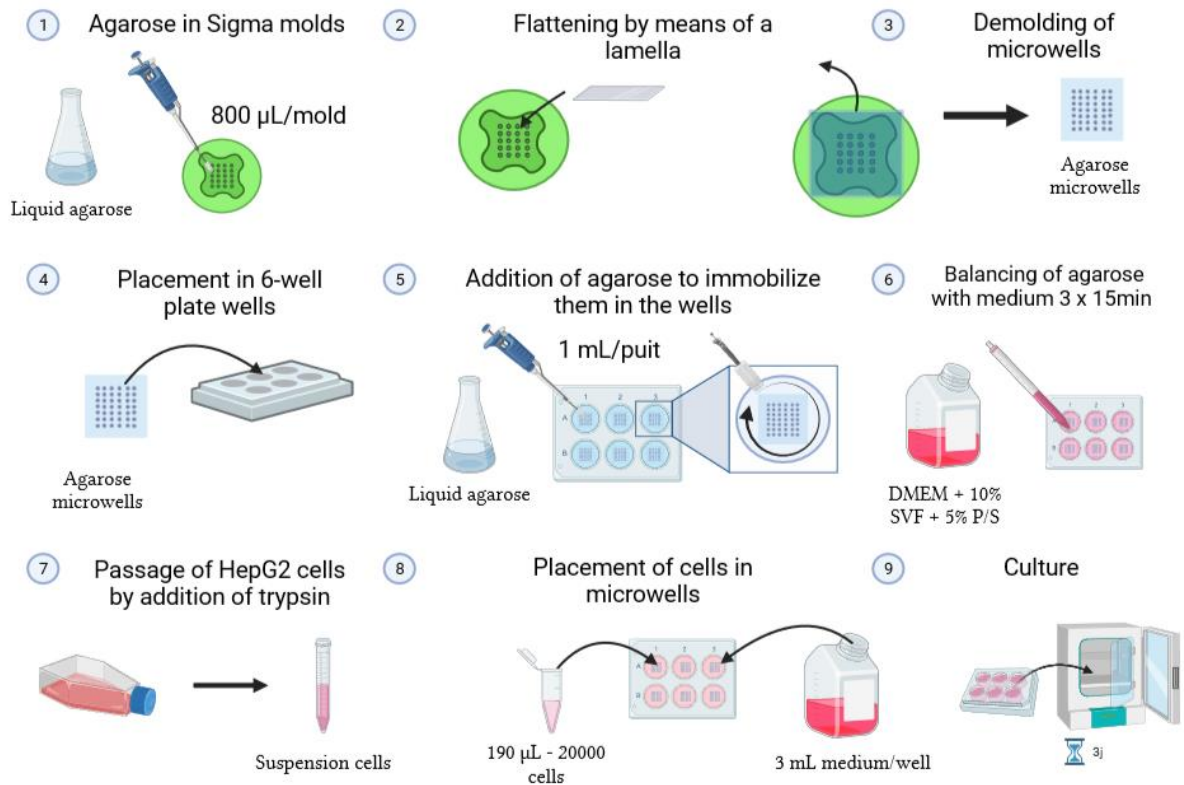


Figure 1: Experimental protocol for the manufacture of tumor spheroids

The spheroids are retrieved with a P1000 micropipette after the three days of culture and deposited in an Eppendorf for fixation or for individualization in 96-well plates depending on the experiments planned. In both cases, the spheroids are, at this step, placed in DMEM culture medium containing no SVF but P/S and 25mM HEPES (to buffer the pH of the medium at room temperature) to stop their growth. Individualized, experiments can be done on then up to three days after recovery of the molds. The fixation of the spheroids is carried out after their recovery in pure formalin for 2.5 hours at room temperature.

2.4. The developed sensor

2.4.1. Architecture

Figure 2 shows the developed biosensor. The biosensor consists of two distinct parts. A dielectric spectroscopy detection part with a microwave circuit involving of a coplanar waveguide of gold 300 nm thick on quartz substrate, with a capacitive gap in the middle of the structure on the central conductor (cf. Figure 2). Specifically, the RF accesses are implemented with a coplanar waveguide with a central conductor width of 130 µm, separated from the side grounds by a slot of 14 µm (cf. Figure 2 a). This coplanar configuration allows the placement of radio frequency (RF) GSG coplanar probes for characterization. The sensing area corresponds to the 10 µm wide capacitive gap in the centre of the structure and the central conductor, as visible in Figure 2 b).

A fluidic part, which includes a fluidic channel and a micro-tank and two lateral reservoirs. The geometry of this channel allows it to hold 30 µL of host medium (see Figure 2 d). This configuration is open, i.e. without a cover over the channel and reservoirs. The spheroid is then manually positioned

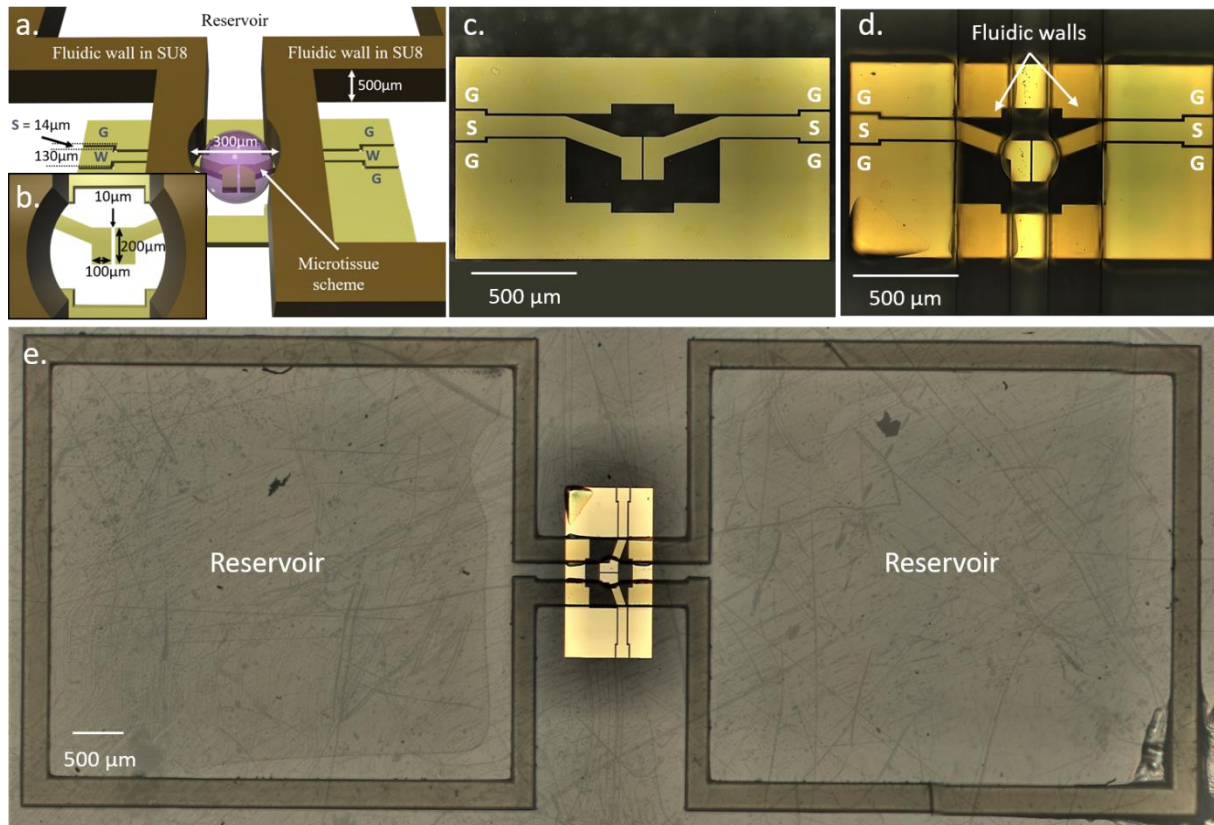


Figure 2: a) and b) Detailed diagram of the RF device with the schematic of a spheroid at its center in pink. a) Global view; W = Signal; S = Slit; G = Ground. b) Zoom in on the central part of the sensor with the dimensions of the electrodes and the capacitive gap. c), d) and e) Pictures taken with an HIROX microscope. c) Picture of the device only with the first metallization. d) Picture of the device after the second microfabrication step: the fluidic wall. e) Global picture of the microdevice.

above the capacitive gap by means of a 500 μm high fluidic system placed perpendicular to the RF waveguide. The fluidic walls terminate in reservoirs to keep the fixed spheroid in 30 μL of its host medium, typically Phosphate Buffer Saline (PBS), to later ensure the possibility to measure alive spheroids in future experiments.

2.4.2. Device micro-fabrication

The fabrication of the biosensor is composed of two main steps based on classical microfabrication processes resumed in the Figure 3.

The electromagnetic sensor is fabricated on a quartz wafer (Corning Eagle XG series) previously cleaned by two minutes in a piranha mixture (50/50v sulphuric acid H_2SO_4 hydrogen peroxide H_2O_2) followed by an oxygen plasma for fifteen minutes. The metallization is carried out using the lift-off technique. Indeed, a first photolithography of negative NLOF resin of 2.5 μm thickness is carried out to define the structure of the biosensor, then a metallization by evaporation of Titanium (Ti)/Gold (Au) is carried out. After metallization, a lift off in acetone for about one hour allows to remove the gold adhering to the NLOF photoresist and to obtain only the gold forming the desired structure. The waveguide obtained is composed of a thin layer of Ti, helping the adhesion of a metal on the substrate, followed by a 300 nm thick Au deposit. Figure 2 c) presents the obtained device. The second and final step corresponds to the elaboration of the fluidic walls and the two reservoirs placed on either side of the biosensor with a 500 μm thick SU8-3050 layer deposited by gravity and then exposed and developed [11], [12]. The gravity method consists of manually depositing 6g of SU8-3050 photoresist on the wafer, followed by a bake with a slow temperature ramp up to 95°C (then levelling off for 11h and naturally descending to room temperature) before carrying out the exposure at 365 nm with an energy of 3.6

J/cm², followed by the post exposure bake (with temperature ramp up to 95°C and a one hour plateau) and finally the development for about 45min. Figure 2 d) and e) present the obtained device.

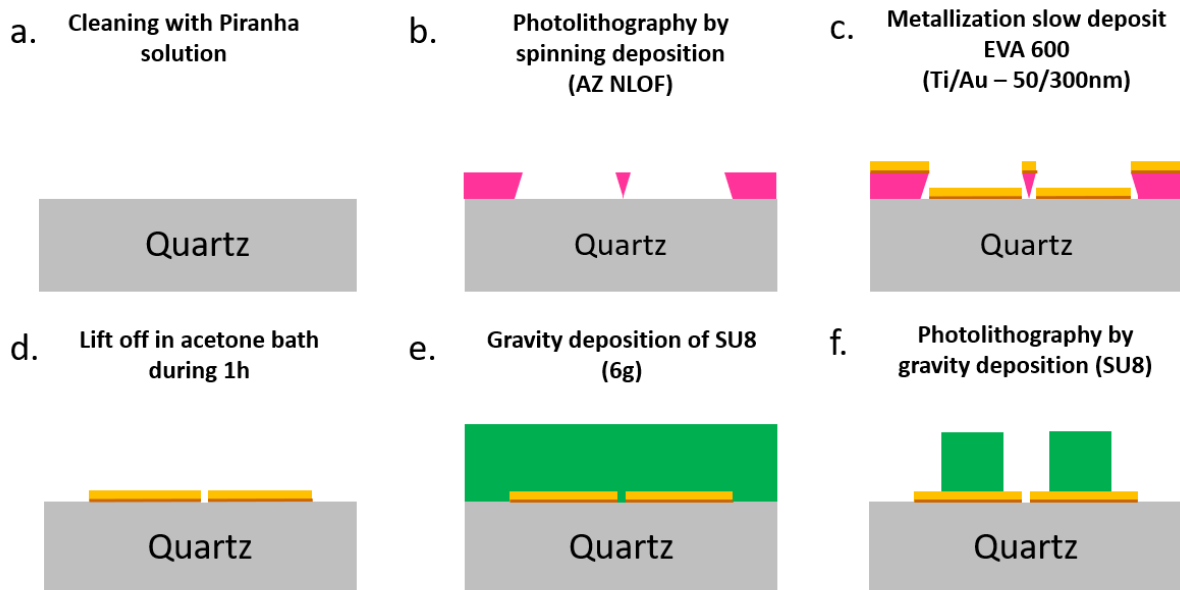


Figure 3: Diagram of the microfabrication of the biosensor. In pink, the representation of the photosensitive resin, in yellow the gold conductive layer, in brown the titanium layer allowing the adhesion to the quartz substrate. Finally, in green, the thickness of the SU 8 polymer. a) First cleaning step of the wafer. b) Result of the deposition and exposure of the 2.5 μ m NLOF resin. Ti/Au metal layer (50/300nm). d) Results obtained after a lift off of about one hour in an acetone bath. e) Results obtained after a lift off of about one hour in an acetone bath. f) Results obtained after a lift off of about one hour in an acetone bath. g) Results obtained after a lift off of about one hour in an acetone bath. e) Gravitational deposition of a thick layer of SU8. f) Fluid walls obtained after exposure and development of the SU8.

3. MODELLING AND EXPERIMENTAL PROTOCOL

The characterization of microwave components requires prior calibration of the two-port vector network analyser (VNA). A SOLT (Short, Open, Load, Thru) type calibration is performed with a Cascade Microtech calibration kit.

The test bench is presented in Figure 4 a). The probe station, shown on the right of Figure 4 a), is positioned on an anti-vibration table with the VNA at the rear. The positioning of the probes and the general control of the micro-device is done by means of an upright microscope, whose image is transcribed by a camera on a screen placed on the VNA.

After this calibration step, the device is connected to the VNA with two coplanar probes and cables. A first measurement of the S parameters is carried out while the sensor is unloaded. It allows to obtain the first measurement, which is necessary afterwards for data analysis, as developed in [11]. Next, the host medium alone is loaded into the device and measurements of the S parameters are performed for 25 min to check the measurement stability of the device before loading a biological object. The filling of the device is done by depositing 15 μ L of the host medium, PBS in our case, in each of the reservoirs and checking that no bubble forms on the detection area. Finally, the tested object available in culture medium is loaded into the middle tank. Just before manually positioning the object under study on the sensing zone using a micropipette, a measurement of the parameters S of the PBS host medium is performed. This measurement serves as a reference (giving C_{ref} and G_{ref}). Measurements of the S parameters of the spheroid (or of the polystyrene beads) are then carried out for 20min to extract its dielectric parameters.

In a few words, the data analysis is based on an electrical model allowing us to extract a capacitance C and a conductance G of the device under test (DUT). With a contrast method using a reference measurement, C_{ref} and G_{ref} , the specific influence of the studied object can be decorrelated from the culture medium in the form of ΔC and a ΔG calculated with equations (1) and (2) respectively. The raw data, C_{DUT} , G_{DUT} , C_{ref} and G_{ref} , and the standard deviations of the contrasts are given in [12] and [11] respectively.

$$\Delta C = C_{DUT} - C_{ref} \quad (1)$$

$$\Delta G = G_{DUT} - G_{ref} \quad (2)$$

4. RESULTS

In order to evaluate the stability of liquid measurements with our sensor, especially due to the open fluidic configuration, all PBS host medium measurements realized with different sensors and setup calibrations have been pooled and are presented in Figure 4 d) and e). The blue curve corresponds to the average response of PBS measurements. It is defined by taking the average response of each independent PBS measurements into account. Those ones are previously obtained by averaging the 25 measurements of a PBS solution achieved every minute during 24 minutes, while their reference corresponds to the first PBS measurement realized after the stabilization procedure done within the sensor. The capacitive and conductive contrasts obtained with PBS shown in Figure 4 d) and e) are extremely close to the zero level. Maximum deviations of - 1.39 fF and - 0.02 mS in absolute value are obtained, which demonstrate the high robustness and repeatability of the microwave measurements of this sensor.

Next, to validate the micro-device and its proper functioning, two polystyrene beads are first measured and then three spheroids fixed with Formalin are measured every minute for 25 min. Their capacitive and conductive contrasts in mean value over the measurement period are shown in Figure 4 d) and e). The used reference is a PBS measurement realized just before the microwave characterization of each individual sample (bead or spheroid). The responses obtained for the beads are represented by the curves in purple, while those of the spheroids are in green and orange respectively.

A large variation between the dielectric responses acquired for the reference liquid (PBS) and the beads is obtained and may be easily explained by the fact that the beads present a low permittivity close to 3, whereas the PBS reference liquid exhibits a permittivity close to the one of water, i.e. frequency dependent and around to 80 at 5 GHz. The extracted capacitive contrasts of polystyrene beads are located below - 60 fF around 2 GHz, while the conductive contrasts at 20 GHz are close to - 10 mS.

As far as the fixed spheroids are concerned, their capacitive and conductive contrasts are lower in absolute value compare to the polystyrene bead. The capacitive and the conductive contrasts of the fixed spheroids are indeed smaller than 20 fF around 2GHz and 2,5mS at 20GHz in absolute value respectively. These results are consistent since the permittivity of a spheroid is close to the reference liquid, PBS. This is all the more true that the spheroid has been fixed with formaldehyde, a treatment that causes permeabilization of the cell membrane. Thus, when stored in PBS, an equilibrium between the intra- and extra-cellular media happens, which decreases the dielectric contrasts in absolute value [13], [14]. Under these conditions, the measured contrasts are therefore lower in absolute value than

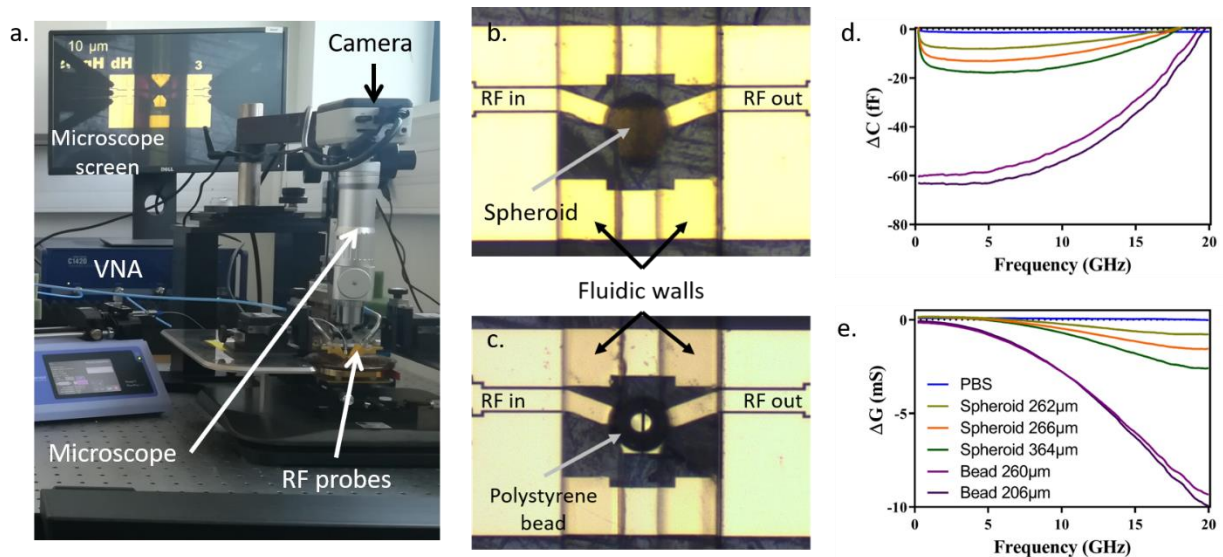


Figure 4: a) Presentation photo of the microwave test bench with the different elements. b) and c) Pictures of the device loaded with a spheroid and a polystyrene bead respectively. d) and e) Capacitive contrast ΔC and conductive contrast ΔG spectra obtained with the reference fluid in blue (PBS), polystyrene beads in green and fixed spheroid in red, purple and beige, respectively. The curves represent the average of measurements made over 25 minutes.

for a living spheroid, allowing a proof of concept with a biological object that is easier to manipulate but more difficult to detect. Such fixed spheroids are indeed easier to prepare, may be stored for weeks, and used at convenience for experiments.

Finally, each spheroid presents a specific response, notably due to differences in shape and size. Indeed, despite a method that allows the creation of many spheroids in a short time, it is difficult to obtain perfectly round spheroids having exactly the same diameter.

5. CONCLUSIONS

This article presents the first device allowing the dielectric characterization of spheroids and developed with its associated electrical model and characterized using first polystyrene beads and then formalin-fixed spheroids. The detailed protocol for the fabrication of the spheroids is also presented. The dielectric characterization results obtained are particularly promising for the use of microwave dielectric spectroscopy for the study of spheroids, since they allow the spheroids to be distinguished from each other despite the fact that they have undergone fixation, which induces very weak but nevertheless significantly observable capacitive and conductive contrasts with the host medium. These results show the feasibility of measuring spheroids in their culture medium by microwave dielectric spectroscopy, which had never been done before. The results of the measurement of polystyrene

beads and fixed micro-tissues are consistent and open new analysis perspectives to this intermediate biological model as the microwave sensing technique is non-invasive, non-destructive and label-free.

6. ACKNOWLEDGEMENTS

This work was partly supported by LAAS-CNRS micro and nanotechnologies platform member of the French RENATECH network.

7. REFERENCES

- [1] S. Nath et G. R. Devi, « Three-dimensional culture systems in cancer research: Focus on tumor spheroid model », *Pharmacol. Ther.*, vol. 163, p. 94-108, juill. 2016, doi: 10.1016/j.pharmthera.2016.03.013.
- [2] E. C. Costa, A. F. Moreira, D. de Melo-Diogo, V. M. Gaspar, M. P. Carvalho, et I. J. Correia, « 3D tumor spheroids: an overview on the tools and techniques used for their analysis », *Biotechnol. Adv.*, vol. 34, n° 8, p. 1427-1441, déc. 2016, doi: 10.1016/j.biotechadv.2016.11.002.
- [3] M. T. Santini et G. Rainaldi, « Three-Dimensional Spheroid Model in Tumor Biology », *Pathobiology*, vol. 67, n° 3, p. 148-157, 1999, doi: 10.1159/000028065.
- [4] F. A. Alexander, D. T. Price, et S. Bhansali, « From Cellular Cultures to Cellular Spheroids: Is Impedance Spectroscopy a Viable Tool for Monitoring Multicellular Spheroid (MCS) Drug Models? », *IEEE Rev. Biomed. Eng.*, vol. 6, p. 63-76, 2013, doi: 10.1109/RBME.2012.2222023.
- [5] K. Heileman, J. Daoud, et M. Tabrizian, « Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis », *Biosens. Bioelectron.*, vol. 49, p. 348-359, nov. 2013, doi: 10.1016/j.bios.2013.04.017.
- [6] P. Mehrotra, B. Chatterjee, et S. Sen, « EM-Wave Biosensors: A Review of RF, Microwave, mm-Wave and Optical Sensing », *Sensors*, vol. 19, n° 5, p. 1013, févr. 2019, doi: 10.3390/s19051013.
- [7] T. Gerasimenko *et al.*, « Impedance Spectroscopy as a Tool for Monitoring Performance in 3D Models of Epithelial Tissues », *Front. Bioeng. Biotechnol.*, vol. 7, p. 474, janv. 2020, doi: 10.3389/fbioe.2019.00474.
- [8] R. Pethig et D. B. Kell, « The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology », p. 38.
- [9] A. Tamra, M. Deburghgraeve, D. Dubuc, M.-P. Rols, et K. Grenier, « Microwave dielectric spectroscopy for single cell irreversible electroporation monitoring », in *2016 IEEE MTT-S International Microwave Symposium (IMS)*, San Francisco, CA, mai 2016, p. 1-4. doi: 10.1109/MWSYM.2016.7540429.
- [10] M. Lazebnik *et al.*, « A large-scale study of the ultrawideband microwave dielectric properties of normal, benign and malignant breast tissues obtained from cancer surgeries », *Phys. Med. Biol.*, vol. 52, n° 20, p. 6093-6115, oct. 2007, doi: 10.1088/0031-9155/52/20/002.
- [11] O. Peytral-Rieu, K. Grenier, et D. Dubuc, « Microwave Sensor Dedicated to the Determination of the Dielectric Properties of 3D Biological Models from 500MHz to 20GHz », in *2021 IEEE MTT-S International Microwave Symposium (IMS)*, Atlanta, GA, juin 2021, p. 4.
- [12] O. Peytral-Rieu, D. Dubuc, et K. Grenier, « Microwave Microfabricated Sensor Dedicated to the Dielectric Characterization of Biological Microtissues », in *2022 3rd URSI Atlantic and Asia Pacific Radio Science Meeting (AT-AP-RASC)*, Gran Canaria, Spain, mai 2022, p. 1-4. doi: 10.23919/AT-AP-RASC54737.2022.9814189.
- [13] F. Artis *et al.*, « Microwaving Biological Cells: Intracellular Analysis with Microwave Dielectric Spectroscopy », *IEEE Microw. Mag.*, vol. 16, n° 4, p. 87-96, mai 2015, doi: 10.1109/MMM.2015.2393997.
- [14] J. W. Chan, D. S. Taylor, et D. L. Thompson, « The effect of cell fixation on the discrimination of normal and leukemia cells with laser tweezers Raman spectroscopy », *Biopolymers*, vol. 91, n° 2, p. 132-139, févr. 2009, doi: 10.1002/bip.21094.

8. COMPETING INTERESTS

Competing interests: The author(s) declare none

9. BIOGRAPHIES



O. Peytral-Rieu received M.S. degree in translational medicinal chemistry from University of Montpellier, Montpellier, France, in 2019; Engineering Degree in chemistry, biology and health from the Engineering Chemistry School of Montpellier, Montpellier, France, in 2019. She received the Ph.D. degree in Electromagnetic, Hyper Frequency systems from the University of Toulouse, Toulouse, France, in 2022. She is currently a Post-Doctoral Fellow with the Laboratory of Analysis and Architecture of System, National Scientific Research Center, Toulouse. Her current research interests include the use of microwave based dielectric spectroscopy for the study and the characterization of microtissues.



David Dubuc received the Agregation degree from the Ecole Normale Supérieure de Cachan, Paris, France, in 1996, and the M.S. and Ph.D. degrees in electrical engineering from the University of Toulouse, Toulouse, France, in 1997 and 2001, respectively. From 2002 to 2013, he was an Associate Professor with the University of Toulouse, and a Researcher with the Laboratory of Analysis and Architecture of System of National Scientific Research Center, Toulouse, France. From 2007 to 2009, he was a Visiting Senior Researcher with the Laboratory of Integrated Micromechatronic Systems, CNRS/Institute of Industrial Science, The University of Tokyo, Tokyo, Japan. Since 2013, he has been a Professor with the University of Toulouse. His research interests include the development of microwave circuits integrated due to microtechnologies and their application to wireless telecommunication and biology.



Katia Grenier received the M.S. and Ph.D. degrees in electrical engineering from the University of Toulouse, Toulouse, France, in 1997 and 2000, respectively. She was engaged in microelectromechanical systems circuits on silicon. She was a Post-Doctoral Fellow with Agere Systems (Bell Labs), Murray Hill, NJ, USA. In 2001, she joined the Laboratory of Analysis and Architecture of System of the National Scientific Research Center CNRS (LAAS-CNRS), Toulouse, France. From 2007 to 2009, she was with the Laboratory for Integrated Micromechatronic Systems, CNRS/Institute of Industrial Science, The University of Tokyo, Tokyo, Japan, where she was engaged in launching research activities on microwave-based biosensors. Her research interests in LAAS-CNRS are currently focused on the development of fluidic-based microsystems, notably for biological and medical applications at the cellular and molecular levels. Dr. Grenier is a member of the IEEE MTT-10 Technical Committee on Biological Effect and Medical Applications of RF and Microwave of the IEEE Microwave Theory and Techniques Society.