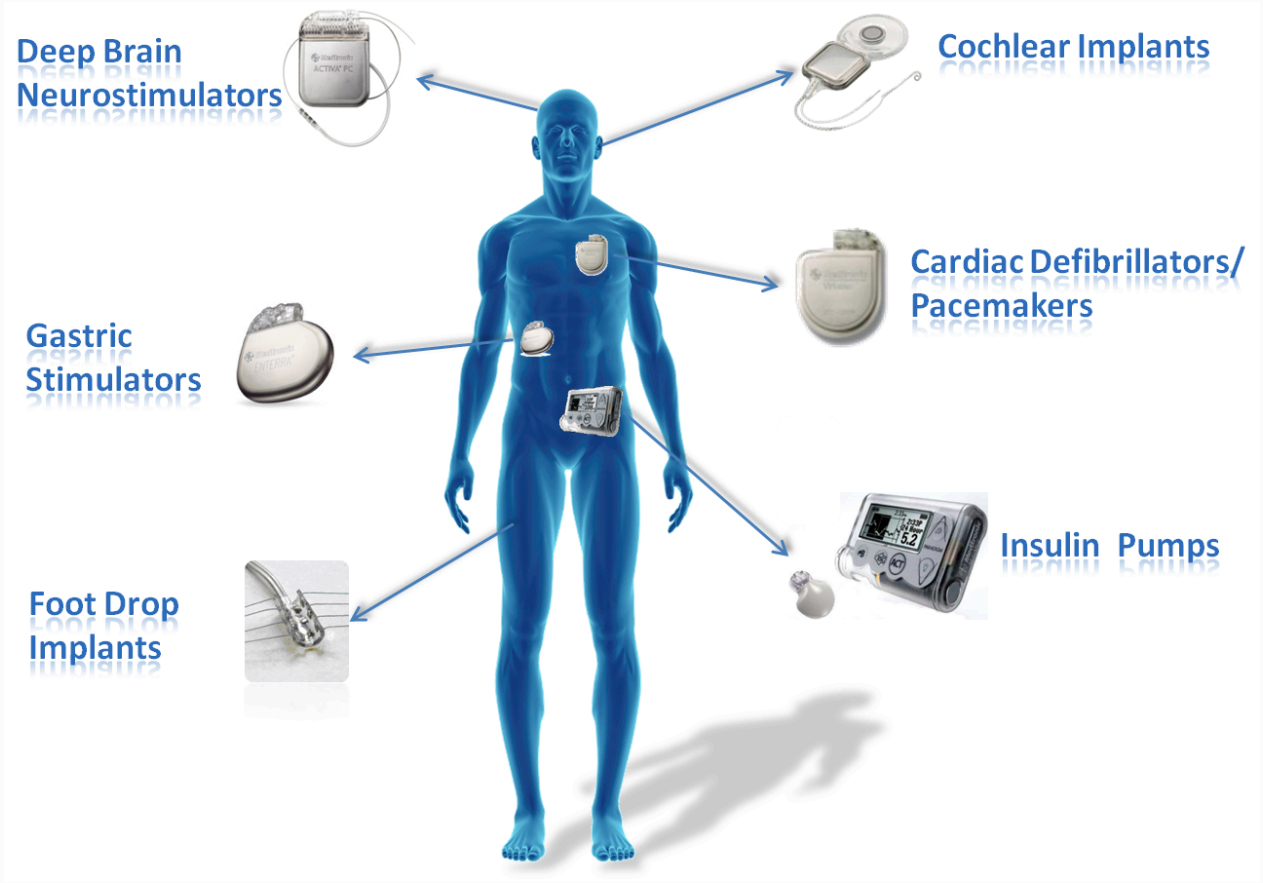
Toward An Embedded System For The In-Vitro Cell Proliferation Characterization By Impedance Spectroscopy

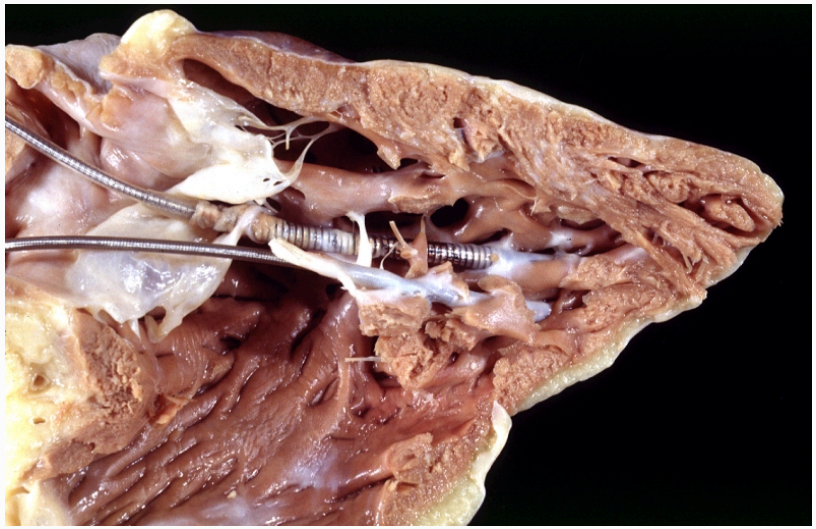
M. Terosiet[1], A. Histace[1], O. Romain[1], M. Boissière[2], E. Pauthe[2]

1 ETIS, ENSEA, University of Cergy-Pontoise, CNRS, Cergy, France
2 ERRMECe, Université de Cergy-Pontoise, Pontoise, France

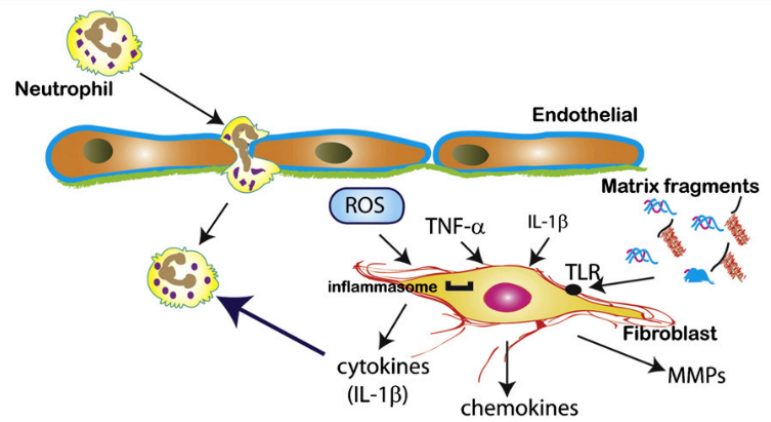
Implanted devices



Fibrosis



Right ventricular endocardial fibrosis due to implantable defibrillator



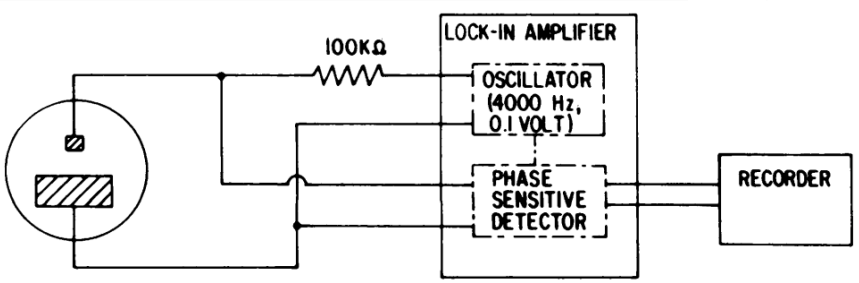
Fibroblasts :

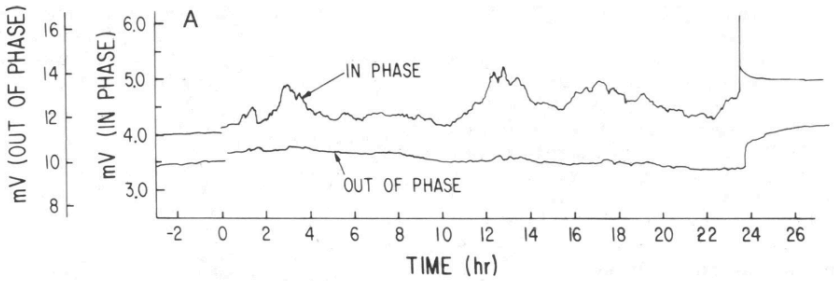
- ➔ serve as sentinel cell
- ➔ migrate and proliferate into the wound site
- ➔ initiate pro-inflammatory cascades
- ➔ activate the inflammasome

➔ **Good candidate for inflammatory response detection**

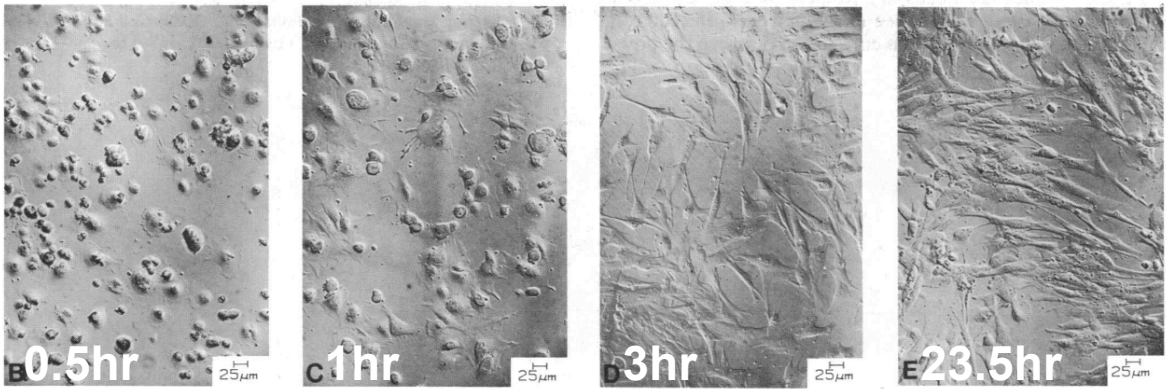
W. Chen and N.G. Frangogiannis, “Fibroblasts in post-infarction inflammation and cardiac repair”, BBA, 2013

First **E**lectric **C**ell-substrate **I**mpedance **S**ensing (ECIS) system



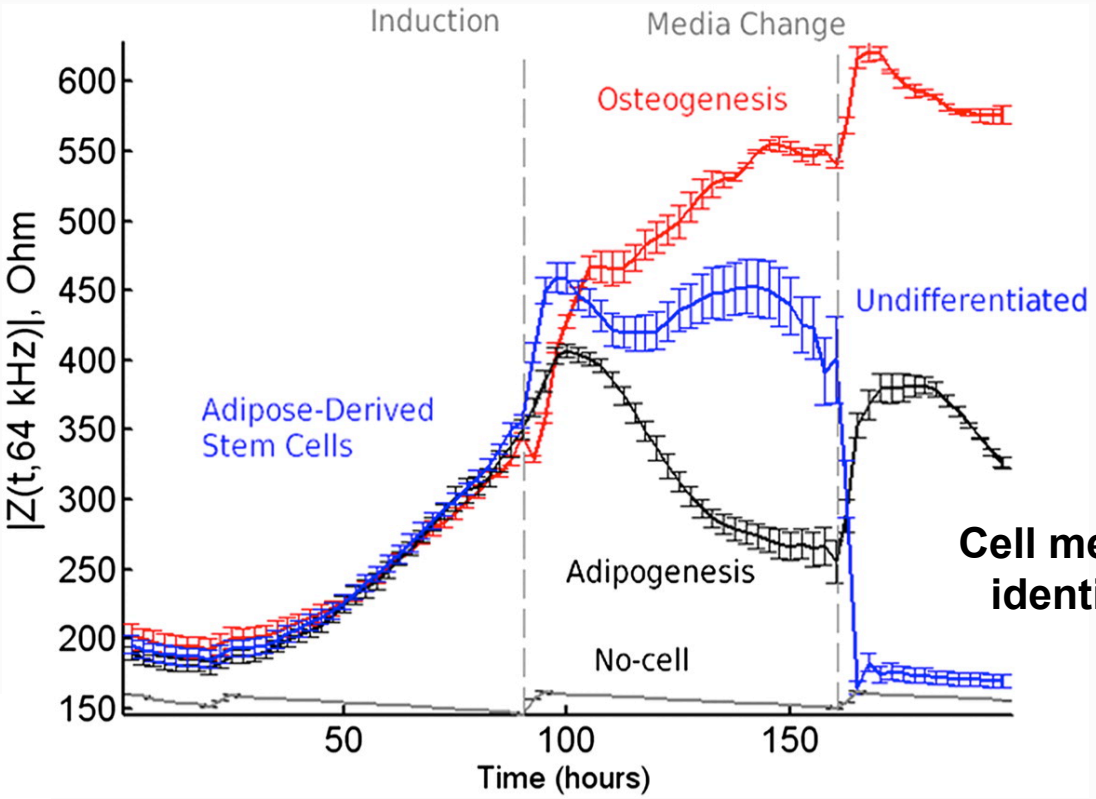


- ➔ The impedance increases as the cell density rises.
- ➔ The impedance falls as the cells round up and detach from the electrodes



I. Giaever and C.R. Keese, “Monitoring fibroblast behaviour in tissue culture with an applied electric field”, PNAS, 1984

Precedent for cell distinction by ECIS method



Cell membrane capacitance was identified as the main marker

P.O. Bagnaninchi and N. Drummond, “Real-time label-free monitoring of adipose-derived stem cell differentiation with electric cell-substrate impedance sensing”, PNAS, 2011

Our goal

To design an embedded system for the in-vitro cell characterization by using the ECIS technique

In-vitro in order to ease the exploration

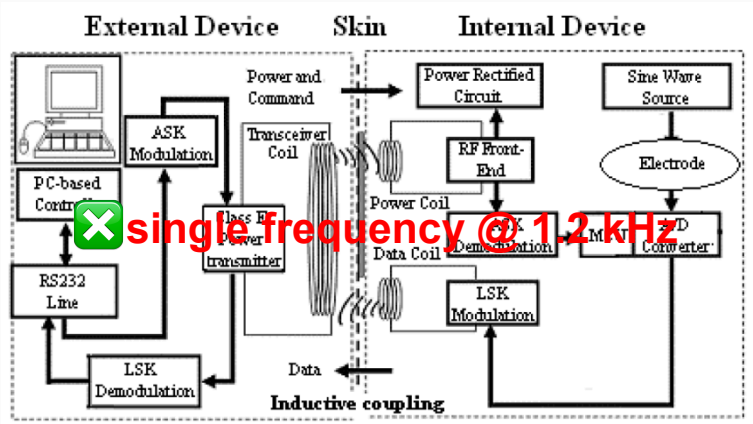
➔ To monitor cell proliferation/death (already established in literature)

➔ To determine electric parameters for cell distinction
- on a single frequency or within a particular frequency band ?
- by applying an additional DC electric field ?

➔ **Needs:**
- wide band frequency analysis capabilities
- wireless system for an arrangement in an incubator

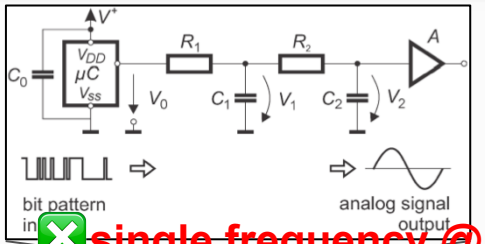
⇒ What are the existing solutions ?

Prior Work

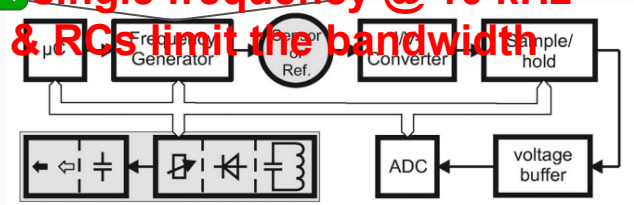


single frequency @ 1.2 kHz

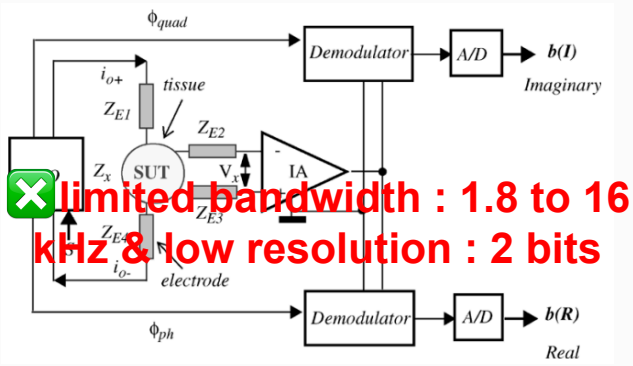
Y-T. Li, et al. "Development of Implantable Wireless Biomicrosystem for Measuring Electrode-Tissue Impedance", JMBE, 2005



single frequency @ 10 kHz & RCs limit the bandwidth



J. Wissenwasser, et al., "Signal Generator for Wireless Impedance Monitoring of Microbiological Systems", IEEE TIM, 2011



limited bandwidth : 1.8 to 16 kHz & low resolution : 2 bits

A. Yúfera, et al., "A Tissue Impedance Measurement Chip for Myocardial Ischemia Detection", IEEE TCASI, 2005.

⇒ No satisfying solution

System architecture

UART

MBED
LPC1768

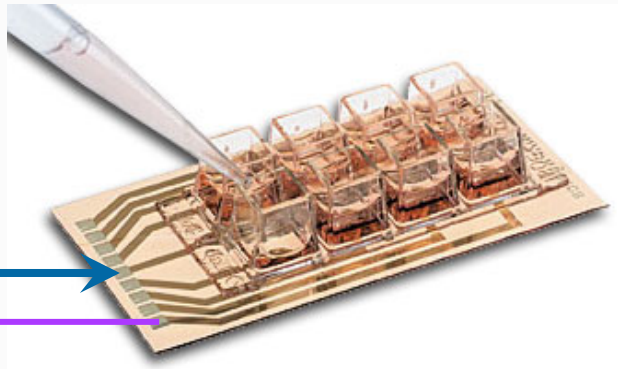
ARM
Processor

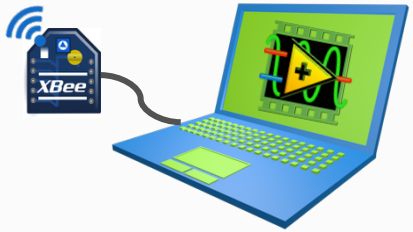
I2C

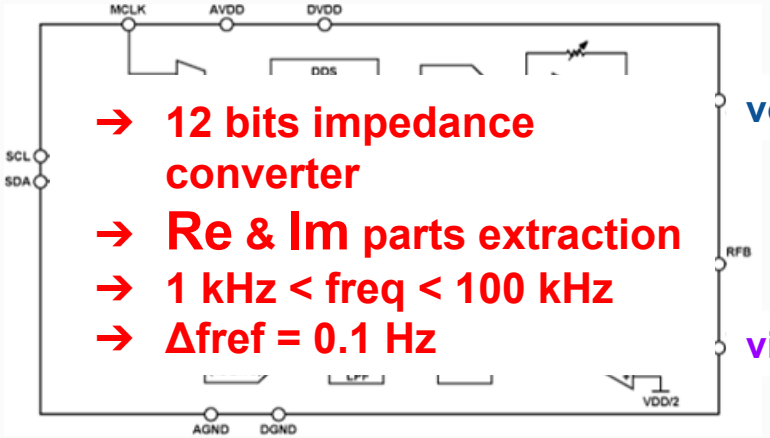
AD5933

excitation
(vout)

sensing
(vin)

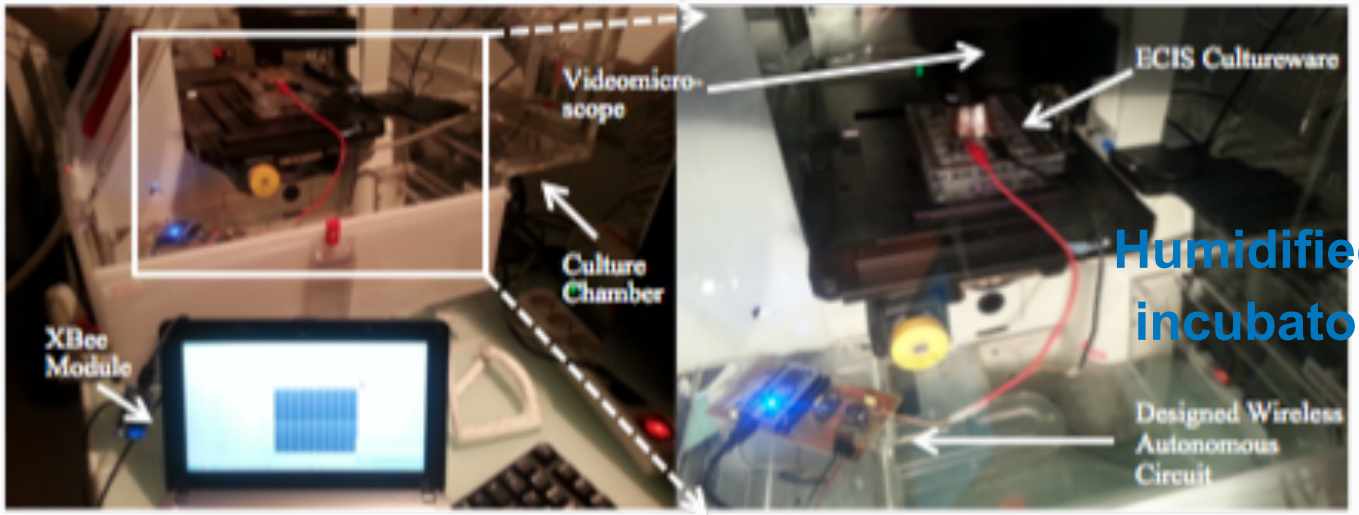




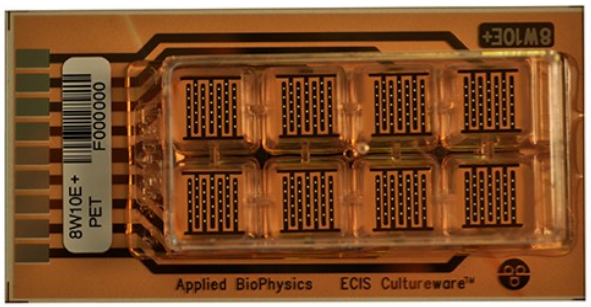


- ➔ 12 bits impedance converter
- ➔ Re & Im parts extraction
- ➔ 1 kHz < freq < 100 kHz
- ➔ Δfref = 0.1 Hz

Experimental setup



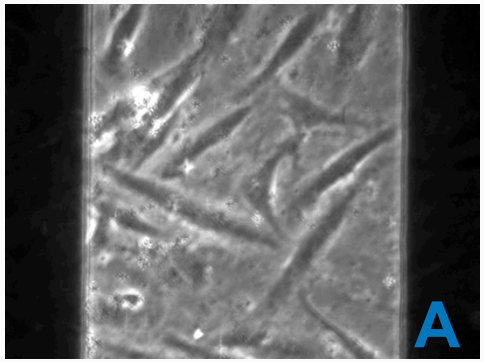
Humidified 5% CO_2 incubator at 37°C

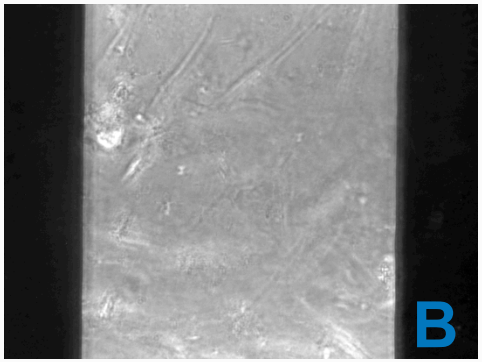


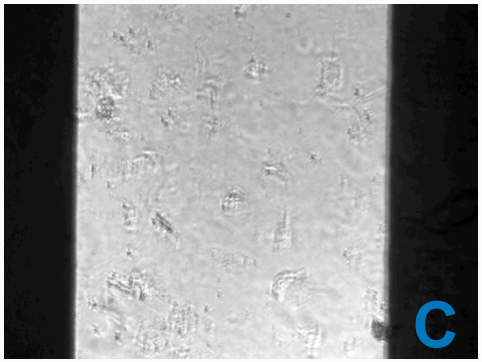
- ➔ Human fibroblast, from foreskin (FB-BJ, ATCC CRL 2522)
- ➔ Plated into wells at 5.10^4 cells/cm² density
- ➔ Subcultured at 70% confluent
- ➔ Cultured in DMEM high glucose medium supplemented with 10% (v/v) fetal bovine serum (FBS) and penicillin-streptomycin (100U/mL-100µg/mL)

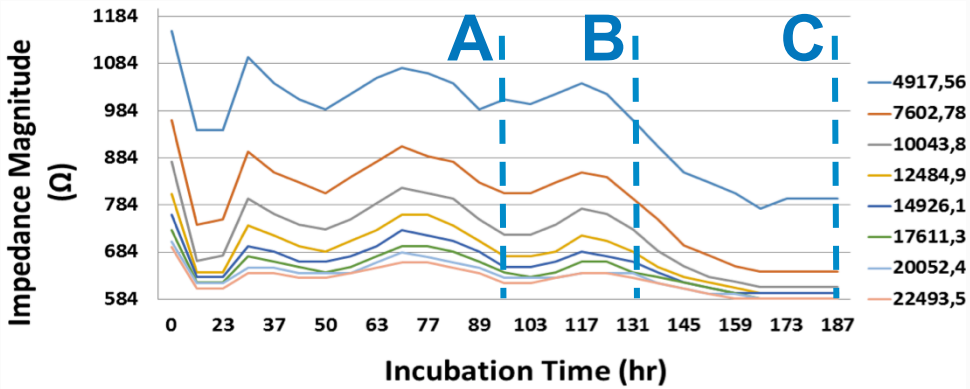
Measured impedance magnitude during the 8-days experimentation

Observed by light microscopy and photographed every 30 mins for the micromotion and comportment captures









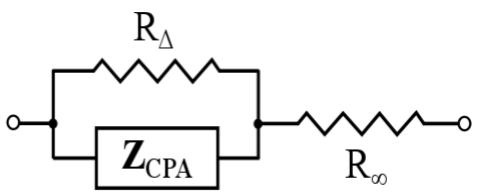
Medium was renewed after :

➔ 48 hours
➔ 90 hours

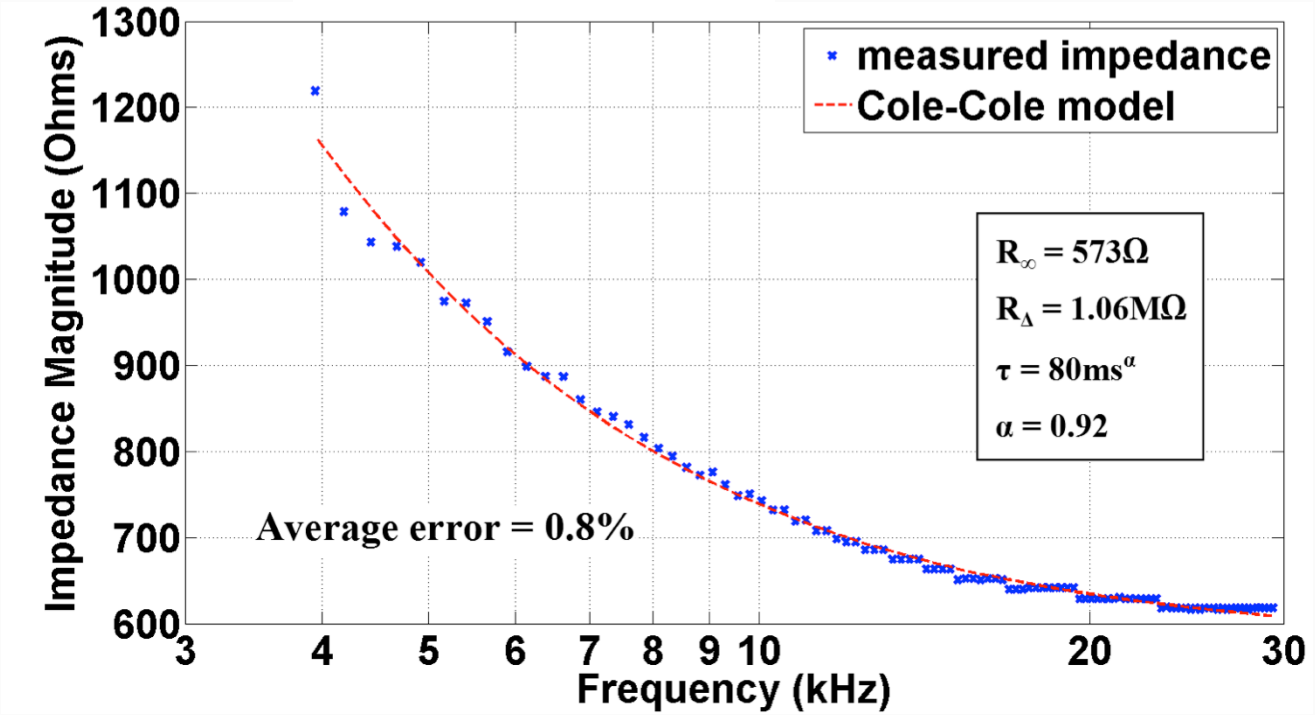
The impedance over the frequency band was measured every 7 mins

Measured impedance with respect to the Cole-Cole model

Cole-Cole Model :



$$Z = R_\infty + \frac{R_\Delta}{1 + (j\tau w)^\alpha}$$



Summary

➔ Immune system responses to the implanted devices can induce dysfunctions

➔ We have explored the possibility to monitor the fibrosis phenomenon by ECIS techniques

➔ A wireless system built around the AD5933 chip for large spectrum analysis and able to be arranged in a classic culture chamber is proposed

➔ Real-condition in-vitro experiments demonstrated its validity :

- agreement between measured impedance magnitude and cell behaviour
- average error of 0.8% on 8-days experiment with the Cole-Cole model

Perspectives

➔ To strengthen the experimental part so as to provide correlation between impedance measurement and usual biological markers of fibrosis :

- fibroblasts
- α-SMA
- collagen
- fibronectin
- ...

➔ Attention will be given to extend its use to in-vivo conditions

- Maximising the integration
- Minimizing the power consumption


BIOCAS
Biomedical Circuits and Systems Conference
2015

Thank you !


ETIS
ENSEA
UNIVERSITÉ de Cergy-Pontoise
cnrs