

Senzori electrochimici pentru evaluarea toxicitatii nanomaterialelor in vitro

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VI. Monitorizarea simultana a eliberării de superoxid si a aderenței celulare

VII. Concluzii



I. De ce metode alternative la testele pe animale?

- a.) din considerente etice (**54 milioane** vertebrate doar pt. REACH)
- b.) din cauza costurilor ridicate (**250 milioane – 1.2 miliarde \$** doar pt. testarea celor ~ 1000 nanoparticule deja comercializate) ¹
- c.) din cauza unui “throughput” limitat (**34-53 ani** doar pt. testarea celor ~ 1000 nanoparticule deja comercializate) ¹
- d.) din cauza predictivitatii limitate (**20-30%** din efectele adverse ale medicamentelor nu sunt detectate in testele preclinice) ^{2,3}

1. Choi, J.-Y *et al.* The impact of toxicity testing costs on nanomaterial regulation. *Environ. Sci. Technol.* (2009), 43, 3030-3034.

2. Seok, J. *et al.* Genomic responses in mouse models poorly mimic human inflammatory diseases. *PNAS* (2013). doi:10.1073/pnas.1222878110

3. Stevens, J. L., Future of toxicology – mechanisms of toxicity and drug safety: where do we go from here? *Chem. Res. Toxicol.* (2006)19, 1393-1401.



II. Alternativa la care lucram noi (pe scurt)



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Modele celulare (culturi 2D sau 3D, co-culturi, “organ-on-a-chip”, etc.)

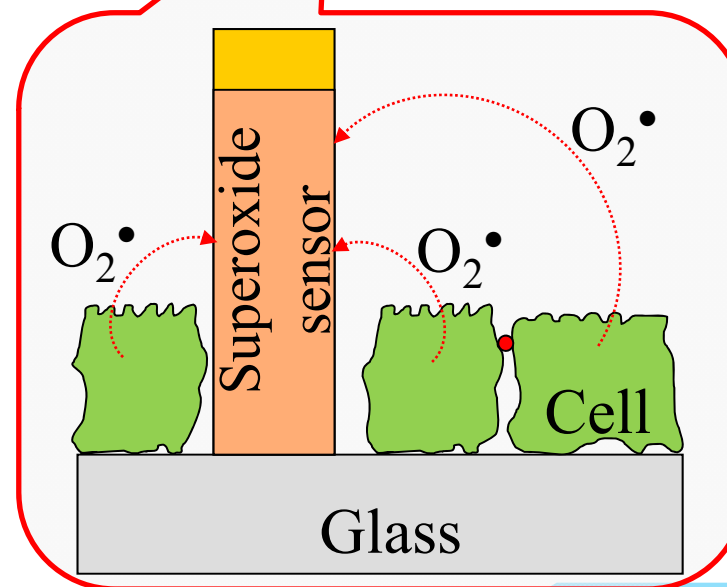
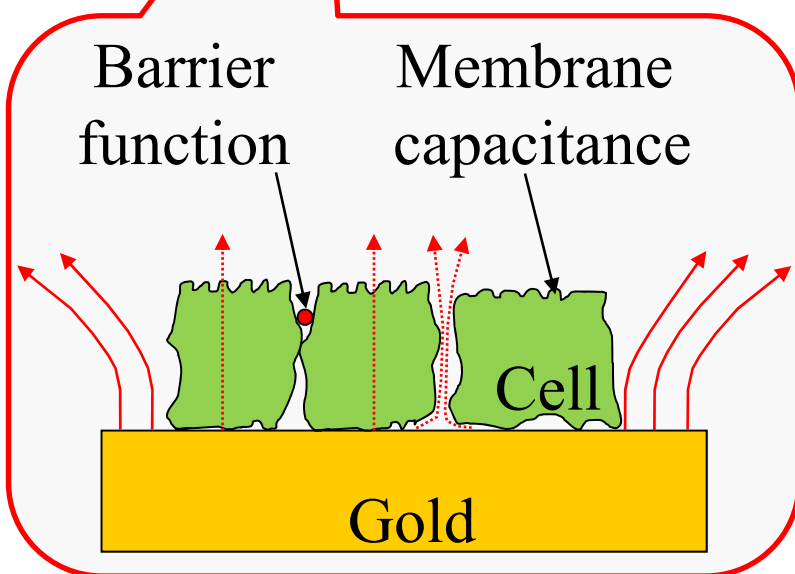
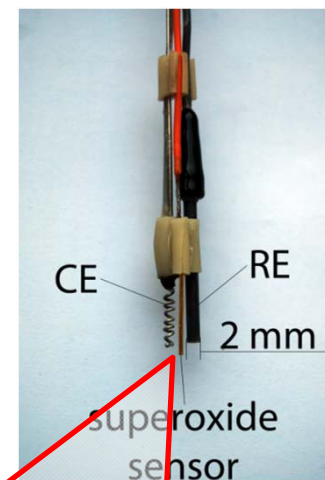
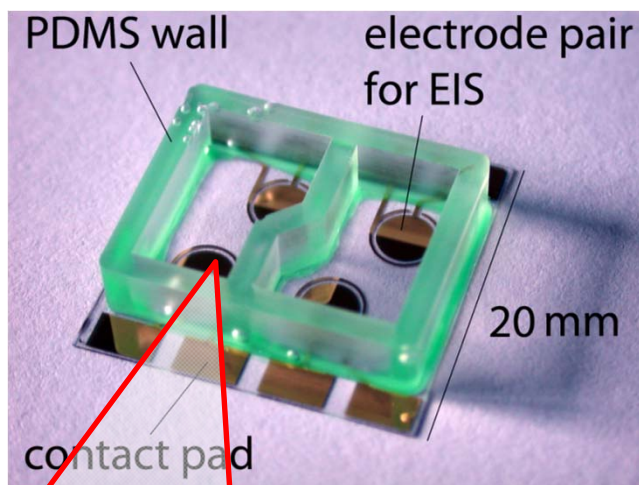


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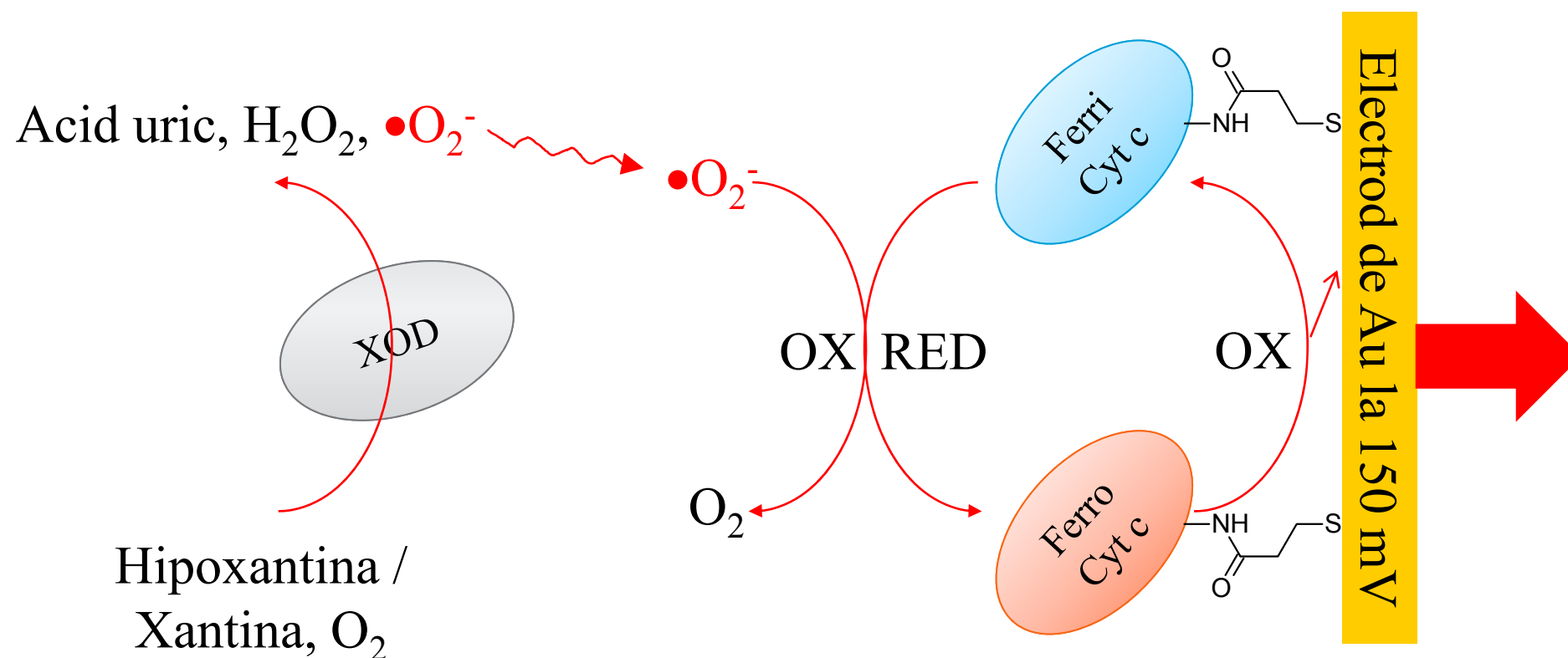
Metode analitice care permit monitorizarea continua si *in situ* a unor procese celulare



III. Sistem experimental



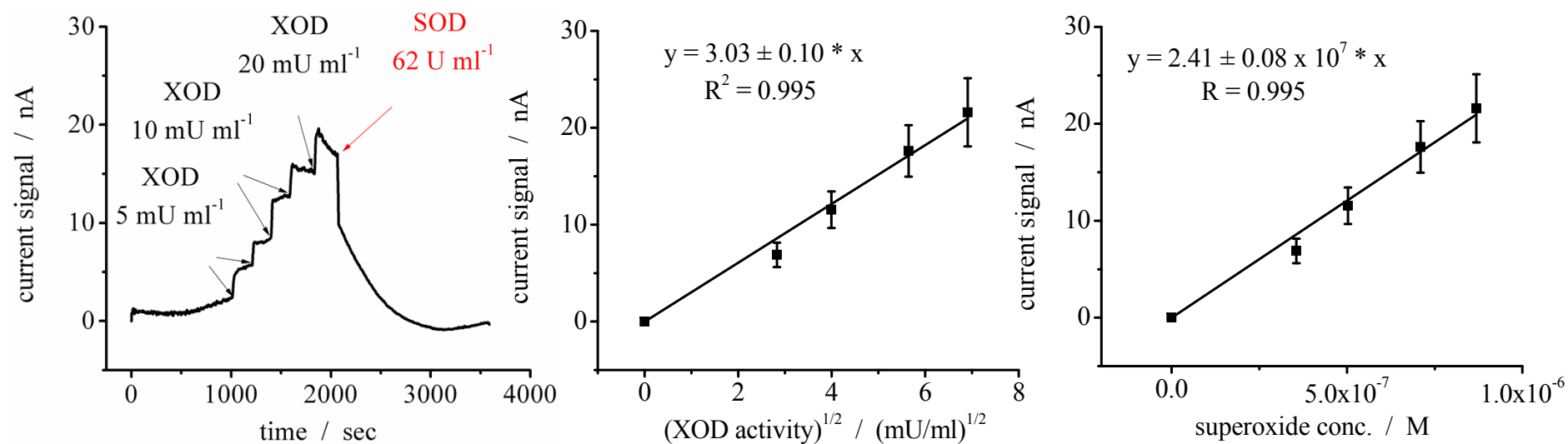
IV.1. Biosenzor pentru detectia superoxidului in spatiul extracelular



Gáspár, S., et al., *Electroanalysis* 25, 448–452 (2013).



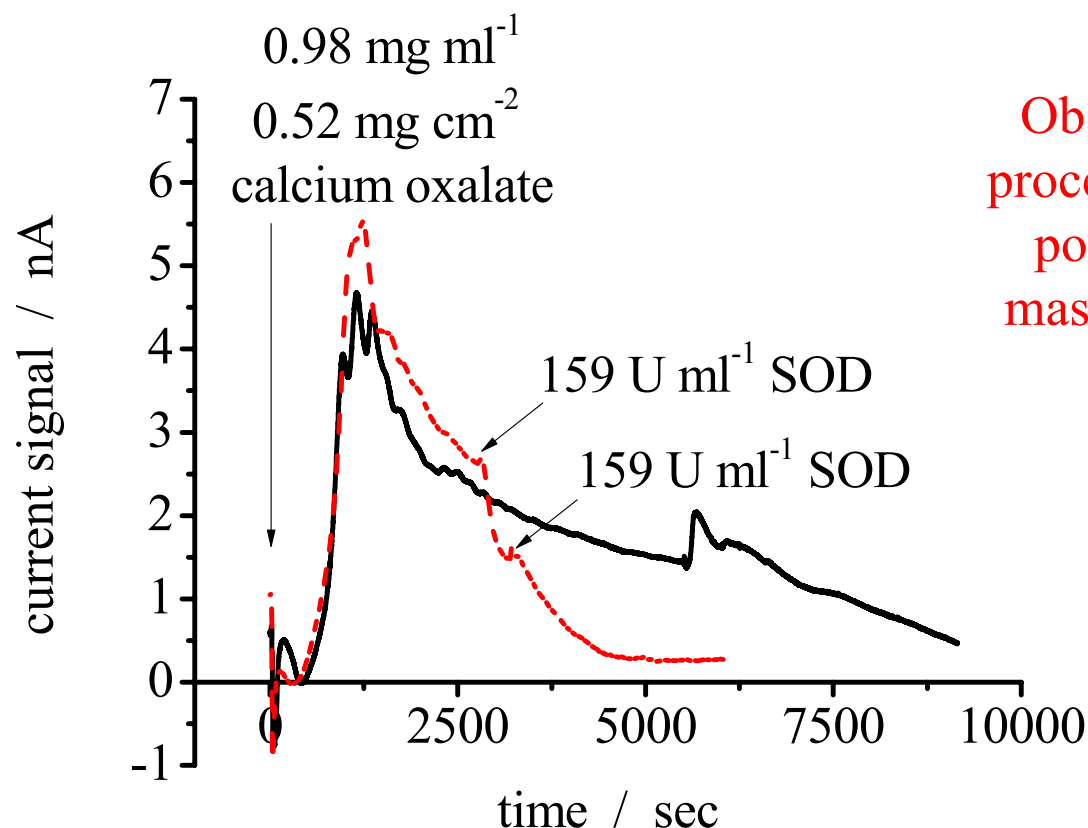
IV.2. Calibrarea biosenzorului pentru superoxid



Gáspár, S., Niculite, C., Cucu, D. & Marcu, I., *Biosensors and Bioelectronics* **25**, 1729–1734 (2010).



IV.3. Evolutia superoxidului in spatiul extracelular al celulelor renale incubate cu cristale de oxalat de calciu



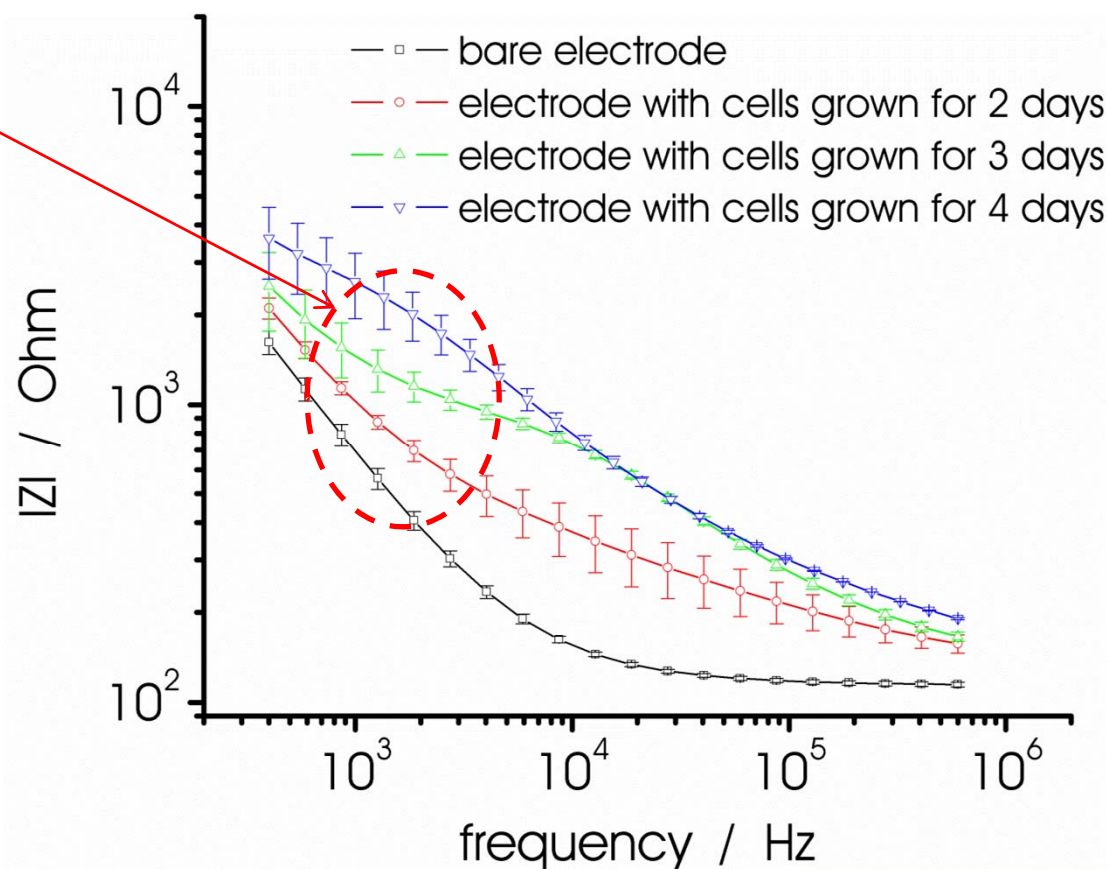
Obs.: Eliberarea de superoxid este un proces nemonoton si temporar. Astfel, el poate trece neobservat in cazul unor masuratori clasice (de tip “end point”).

Gáspár, S., Niculite, C., Cucu, D. & Marcu, I., *Biosensors and Bioelectronics* 25, 1729–1734 (2010).



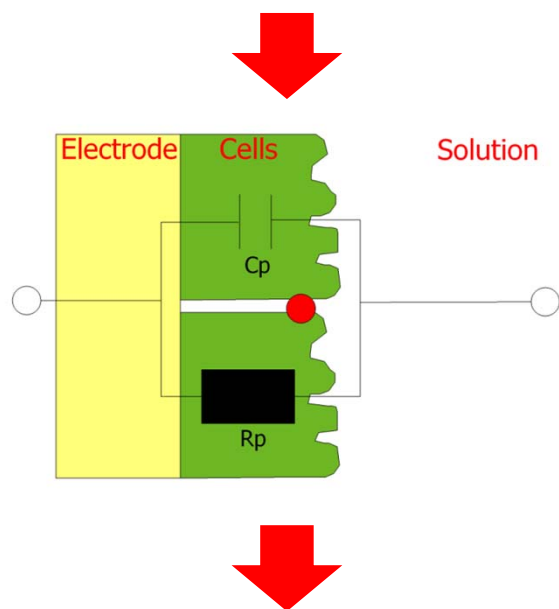
V.1. Impedanta electrochimica pentru monitorizarea aderentei celulare

Obs.: Se pot "observa" celule care cresc si adera pe electrod in jur de 1800 Hz!

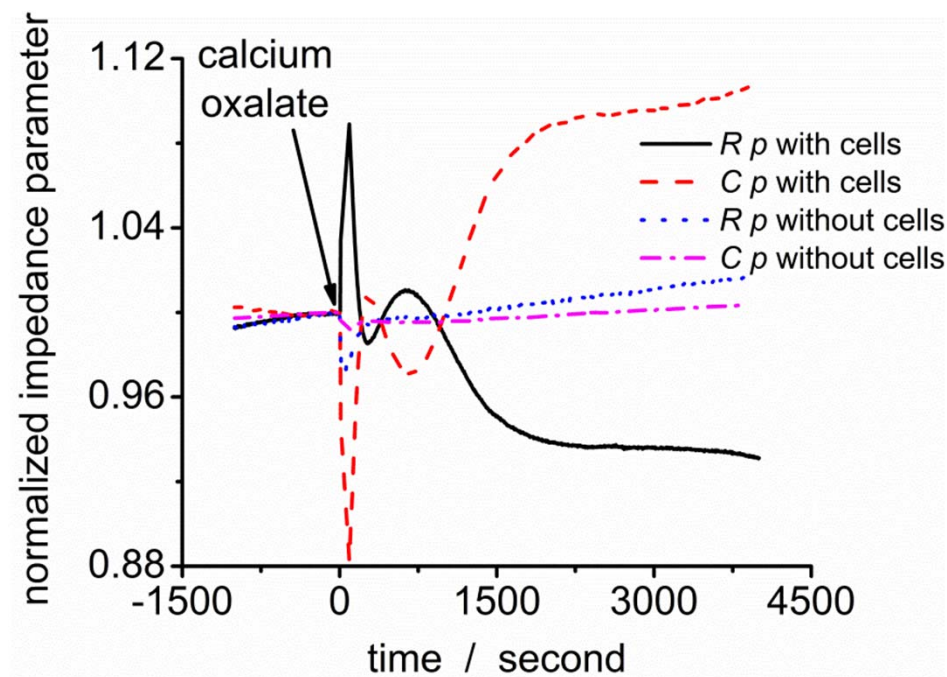


V.2. Impedanta electrochimica pentru monitorizarea aderentei celulare

$Z_{\text{electrod cu celule}}$ la 1800 Hz



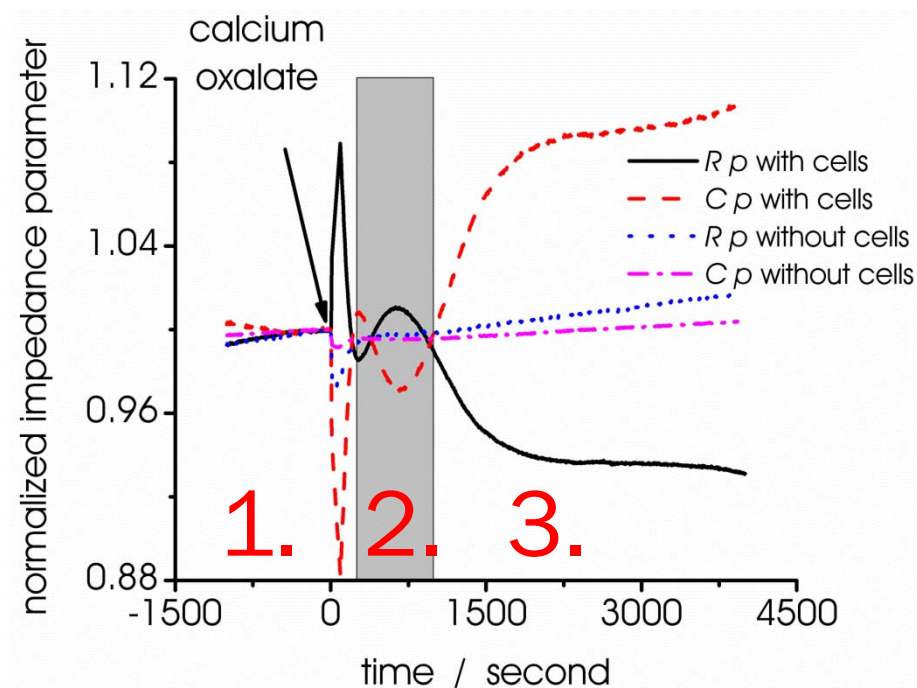
$R_p \sim$ rezistența transepitelială
 $C_p \sim$ capacitanta transepitelială



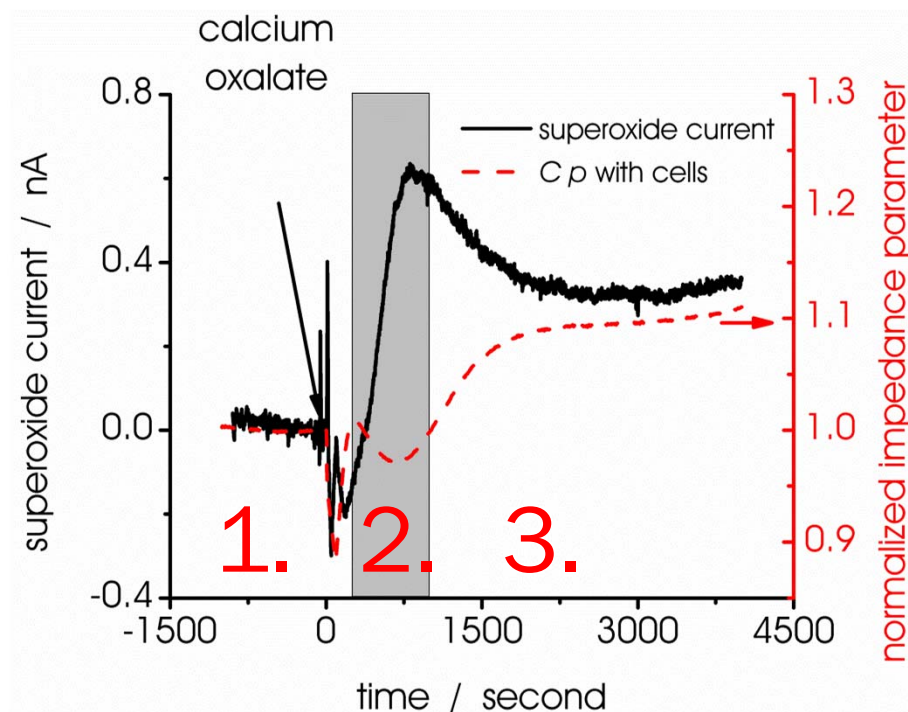
Gáspár, S. et al. , *Analytica Chimica Acta* **713**, 115– 120 (2011).

VI.1. Monitorizarea simultana a eliberarii de superoxid si a aderentei celulare

EIS



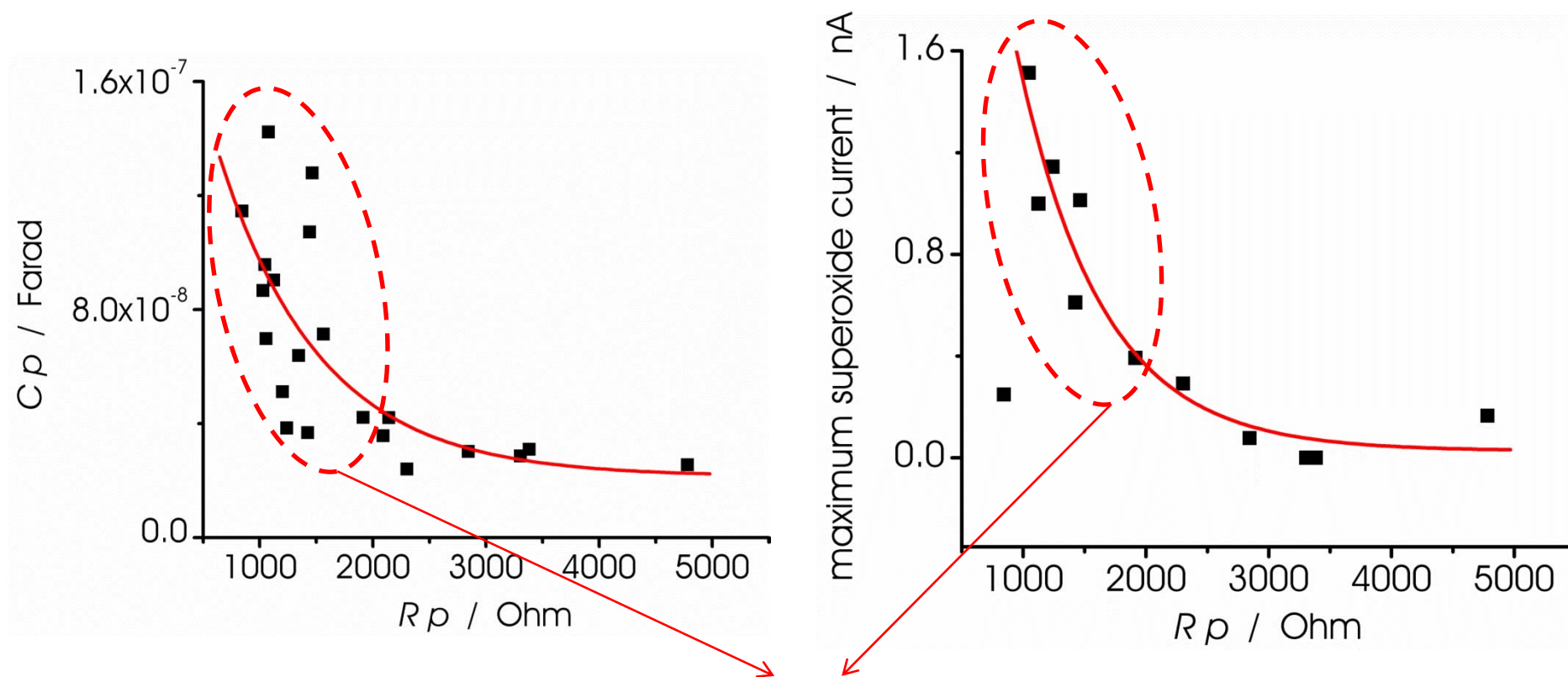
Amperometrie



Gáspár, S. et al. , *Analytica Chimica Acta* **713**, 115– 120 (2011).



VI.2. Efectul varstei celulelor asupra vulnerabilitatii acestora



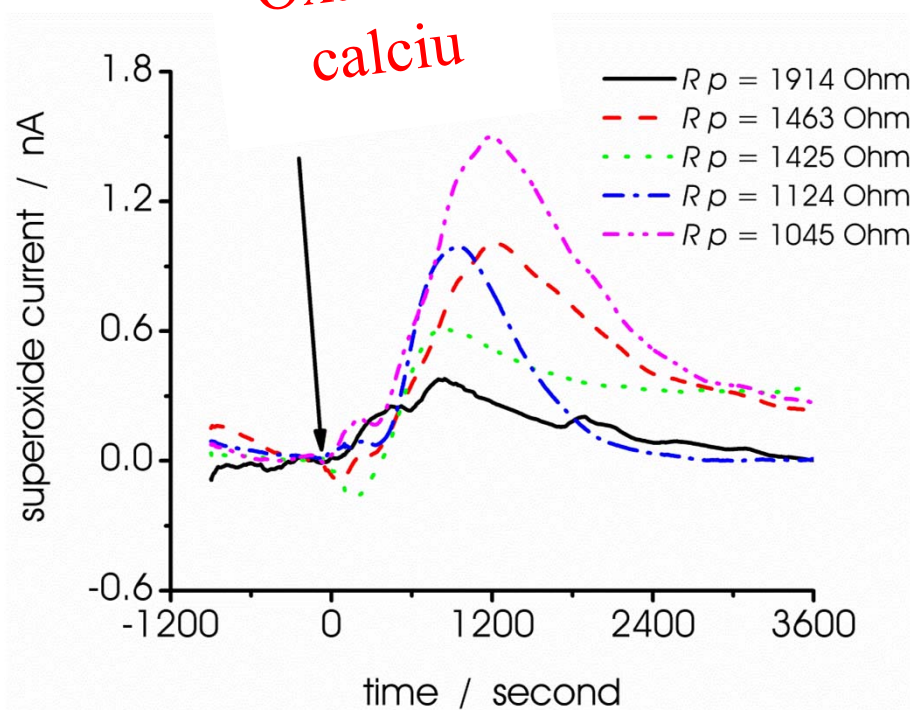
Obs.: Celulele “tinere” (fara jonctiuni stranse) sunt mai sensibile!

Gáspár, S. et al. , *Analytica Chimica Acta* **713**, 115– 120 (2011).

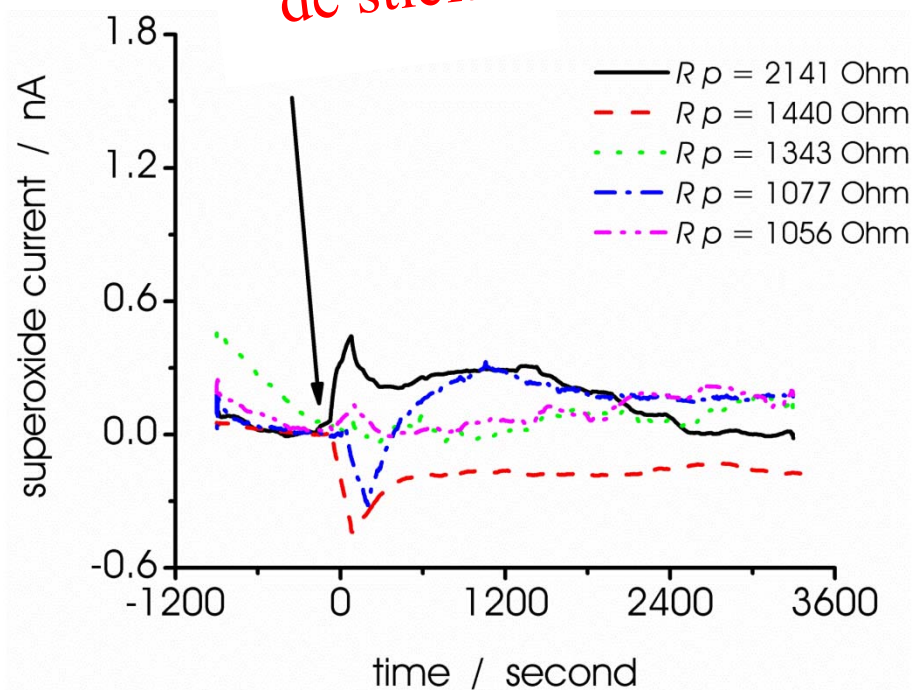


VI.3. Q:Stres mecanic sau chimic?

Oxalat de
calciu



Particule
de sticla



A: Stres mecanic + stres chimic

Gáspár, S. et al. , *Analytica Chimica Acta* **713**, 115– 120 (2011).

A 12-a ediție a Seminarului Național de Nanoștiință și Nanotehnologie,
Biblioteca Academiei Române, București



16 mai 2013

VII. CONCLUZII

- + Senzorii sunt reversibili (pot monitoriza atat productia cat si consumul de superoxid)
- + Marcarea celulelor nu este necesara
- + Sistem experimental simplu si usor de folosit
- + Rezolutie temporala excelenta (care permite observarea dinamicii proceselor)
- + Procesele studiate sunt ne-monotone si temporare si pot trece neobservate cand se folosesc metode clasice
- Sistemul poate fi utilizat doar la evaluarea toxicitatii acute (nu si a toxicitatii cronice)



MULTUMIRI



Dana
Cucu



Irene
Marcu



Sorin
David



Cristina
Polonschii



Cristina
Niculite



Dumitru
Bratu



Mihaela
Gheorghiu



Eugen
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Suport financiar: UEFISCDI

