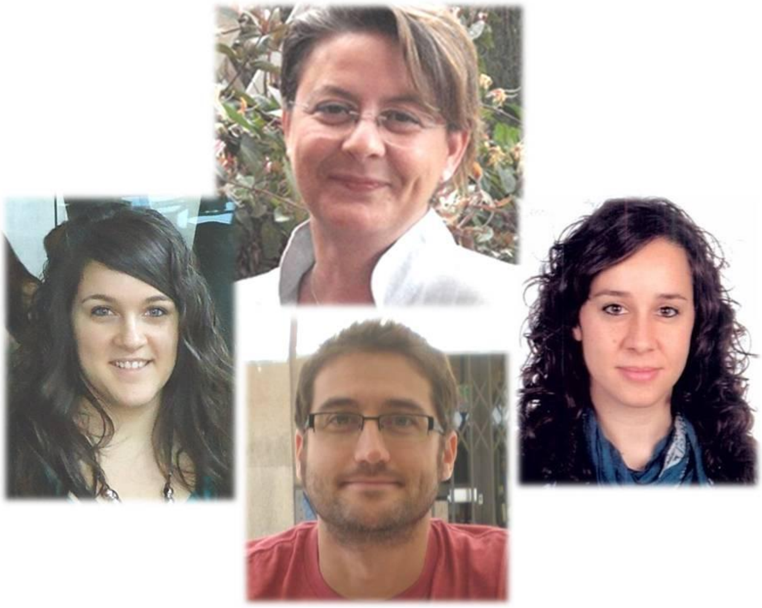
NANOSTRUCTURED ELECTROACTIVE MATERIALS FOR ELECTRODES IN CENTRAL NERVOUS SYSTEM (CNS) STIMULATION AND REPAIR

Maria Nieves Casañ-Pastor
Institut de Ciència de Materials de Barcelona ICMBA
M. Cristina Suñol Esquirol
Institut Investigacions Biomèdiques de BCN
Ann Marie Rajnicek
Institut of Medical Sciences Aberdeen, UK







1. Summary

Application of electric fields for tissue repair vs functional electrostimulation needs to rely on the use of adequate materials. To date functional electrostimulation of the nervous system is addressing symptom relief and is done at high frequency pulses to prevent overheating and damaging of tissue, and it is done with platinum-implanted electrodes. In that case, formation of radicals from water oxidation or oxygen reduction results in damage to the biological system.

This project attempted to develop new materials or coatings to facilitate a new path in which application of electric fields would not result in radical formation but rather, the mixed valence material as electrode would undergo an electron transfer.

Thus the project has focused on:

A) Development of new electroactive **materials**: Nanostructured intercalation mixed valence electrodes as "shock absorbers" in terms of chemistry and charge. Possible drug delivery during action. Self standing electrodes.

B) Development of **electrodes** based on proven biocompatible electroactive materials that allow appropriate electric field geometries to be imposed internally in the nervous systems. Brain and spinal cord electrodes will be self-standing in the long term, though with different geometries.

C) **Nanostructuring** of electrodes as hybrid systems should overcome the problems of independent pure materials, increasing electrode stability, charge capacity and a more flexible charge delivery through tunable interfaces. IrOx can be scaffolded with carbon nanotubes that will prevent delaminating for example. Interfaces will be tuned at the starting potential needed, by initial electrochemical doping. Adhesion on those interfaces may be modulated by such potential/ionic changes.

D) **Engineering** of final macroscopic shapes, as microelectrode arrays or as cylindrical pieces adjusted to spinal cord geometry.

E) **In vitro** evaluation of **electrodes** in the absence and in the presence of applied electric fields

2. Results

A short summary of achievements follows:

1) New hybrids of iridium oxide, ***IrOx, with nanocarbons***: **IrOx- carbon nanotubes**, **IrOx-graphite oxide, IrOx-graphene oxide and IrOx-pristine graphene** have been prepared as thin transparent **electrodes** and as thick, large-charge coatings. Aminoacid PEDOT-polypyrrole bilayer composites have also been prepared

IrOx has been proven to adhere to graphene and graphene oxide platelets, as well as graphite or carbon nanotubes. That induces a self-assembly in colloidal form, which facilitates the carbon electrodeposition driven by IrOx. The process works for all tested carbon materials, as reported previously for nanotubes. Conducting polymers have also been included in a trihybrid. The resulting electrochemical properties evidence a significant effect of carbon in charge storage capacity (see below) that is hindered if PEDOT-conducting polymer is also included in the hybrid.

Nanocarbons induce a nanostructure in the final material, as shown in the case of nanotubes below, rendering a more stable phase that can be cycled at least 100 times longer. In this example, **CNT act like steel wires in reinforced concrete**. As a result, the electrochemical properties are far more reversible and involve larger charge capacities

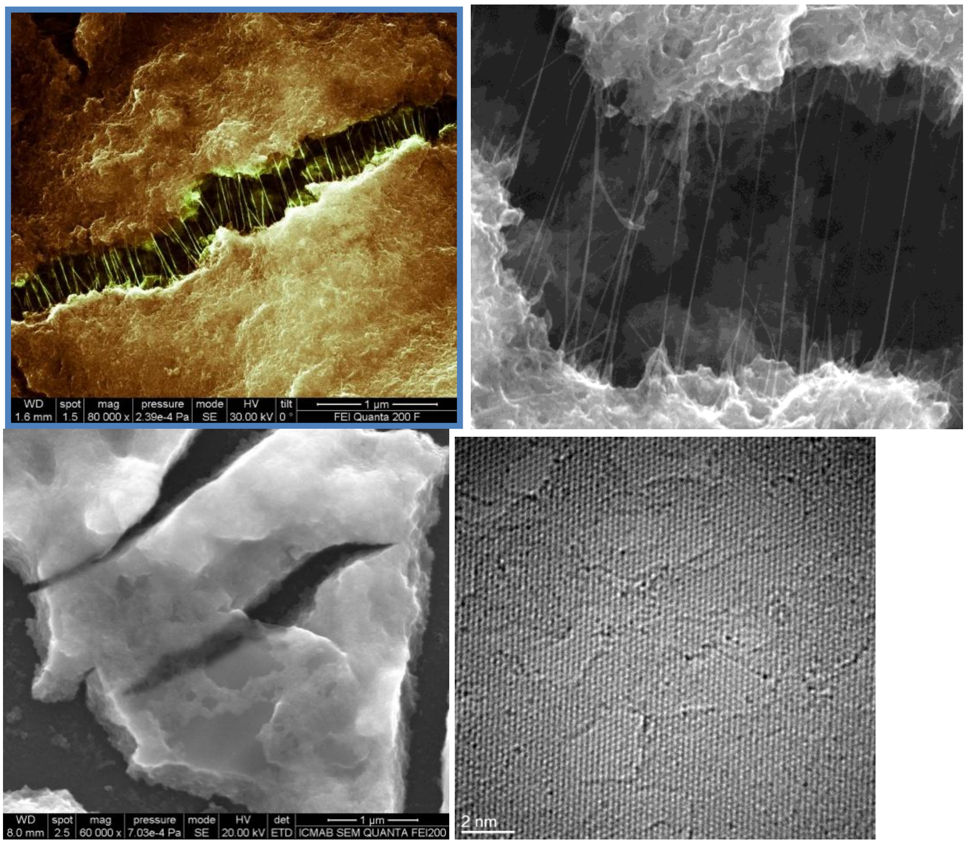


Figure 1. SEM images of IrOx-carbon nanotubes, of IrOx-carbon nanotubes-PEDOT showing the scaffold like nanostructure of the hybrids, and IrOx-graphene oxide milfeuille nanostructured material along with exfoliated graphene (HRTEM).

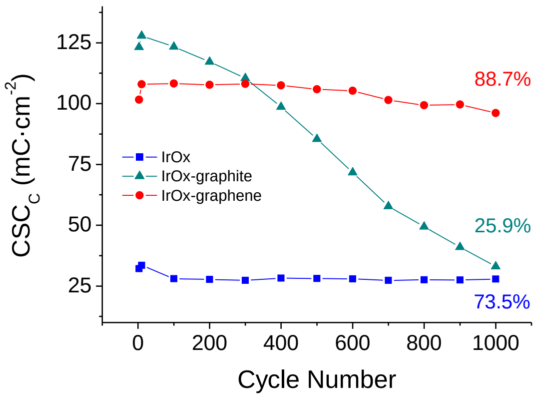
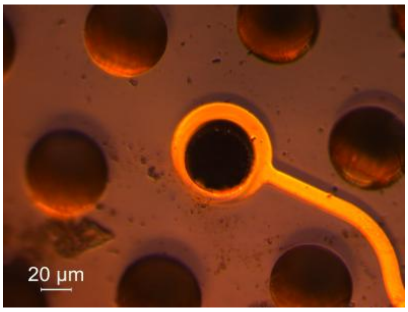


Figure 2. Charge storage capacity CSC, of graphite and graphene oxide-IrOx hybrids versus pure IrOx

Figure 2 show how graphene with large CSC maintains 90% of it after 1000 cycles, while graphite loses it abruptly at around 200.

On the other hand, **pristine graphene** obtained by electrochemical exfoliation, as reported by our group, has also been used to form **IrOx-graphene hybrids**, with significantly different results from those of graphene oxide. A significantly lower roughness yields a similar enhancement in charge capacity for the material. This fact shows that pristine graphene contributes more to the electrochemistry material than graphene oxide. All phases contain the same K/Ir ratio according to XPS spectra, as if all were identified as the iridium oxohydroxide described in former reports, **KzIrOx(OH)y.nH2O.** Only the one containing graphene oxide has extra oxygen, while the one obtained with pristine graphene does not have extra oxygen, which demonstrates that is undoped graphene.

2) All phases have been obtained as **coatings** on flat platinum electrodes of various geometries and sizes, and also over flexible platinized medical silicone and microelectrodes. All electrochemical properties are reproducible in all cases. All processes involve highly reproducible **electrodeposition** procedures and confirm that the materials could coat any commercially available electrode. Control of thickness and global CSC is possible in all hybrid materials.



3) Also for all cases, primary neuron culture has been performed (see Table 2) and compared with other materials. When the **carbon** component is **graphene** or graphene oxide, cell cultures evidence the same optimal compatibility that was shown previously for IrOx, IrOx-CNT and bilayers-aminoacid conducting polymers. Only one case proved toxic, when nitrurating graphene (not shown). And in the case of PEDOT, with no PSS surfactant, a very poor viability was found.

Table 2. Comparison of materials in terms of neuronal viability of cells deposited on top of nanostructured materials and grown in vitro up to 5 days.

	Nanostructured material	Neuronal viability. % of neurons (cells with tau labelling) with respect to control materials (borosilicate or IrOx)
Doped PEDOT monofilms	PEDOT-lysine 0.01 M	< 5%
	PEDOT-glutamine 0.01 M	< 5%
	PEDOT-glycine 0.1 M	< 5%
	PEDOT-glutamic acid 0.1 M	< 5%
	PEDOT- sodium glutamate 0.1 M	< 5%
	PEDOT-PSS	< 5%
Doped PPY monofilms	PPY-PSS	< 5%
	PPY-DBS	< 5%
Doped PEDOT/PPY bilayered films	PEDOT/PPY-glutamine 0.01 M	91 %
	PEDOT/PPY-glutamine 0.1 M	122 %
	PEDOT/PPY-lysine 0.01 M	97 %
	PEDOT/PPY-inosine	90 %
IrOx hybrids	IrOx-Carbon nanotubes (CNT)	90 %
	IrOx-PEDOT-CNT	< 5%
	IrOx-graphite oxide	112 %
	IrOx-graphene oxide	98 %
	IrOx-electrochemical graphene	94 %
Pt	Pt	< 5%

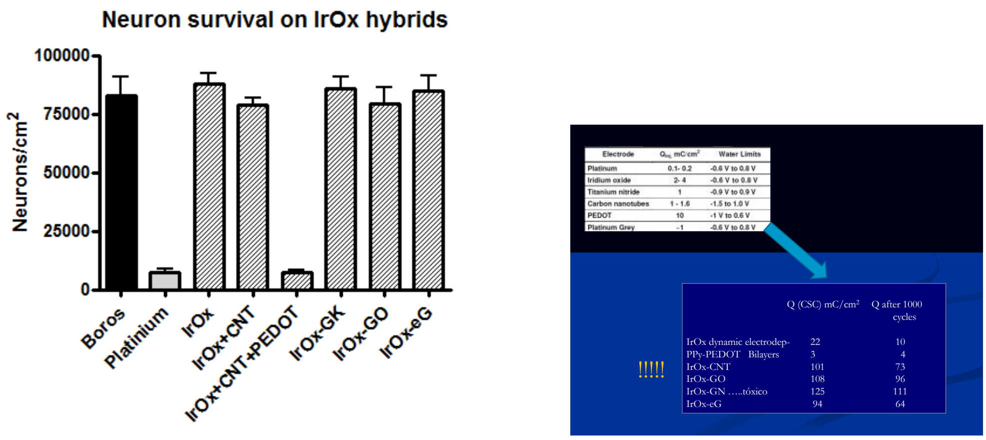


Figure 3. Cell culture quantification for carbon-IrOx hybrids and the one including polymer, as compared with borosilicate reference, platinum commercial electrodes and pure IrOx, along with charge capacity values attained

A comparison of the materials shown in previous reports and this last work period makes it possible to identify the optimal behaviour materials in terms of electrochemical properties and cell viability, previous to electric field application

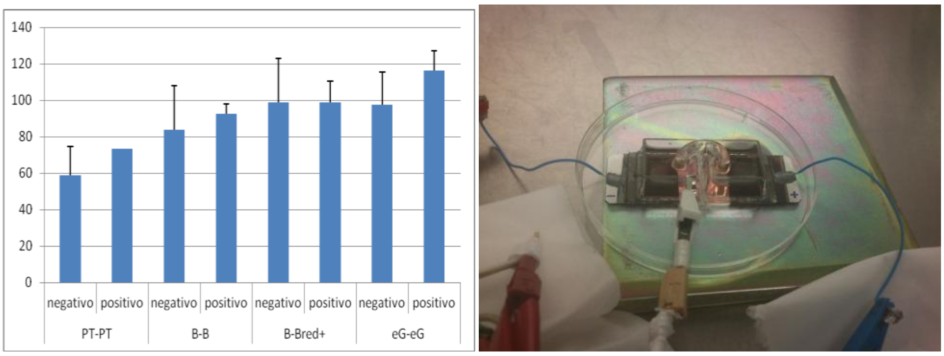
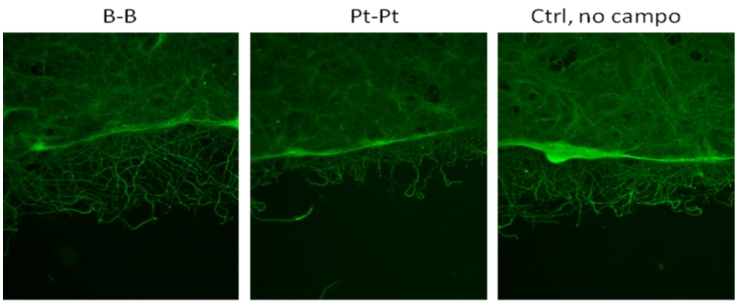


Figure 4. Representative cell scratch filling for several materials in anode and cathode

4) **An in vitro model, scratch cell culture, has been chosen to evaluate electrodes and electric field protocols** have been defined for electric field application with implanted electrodes. Comparison with no electric field and with standard platinum electrodes has been performed.

The comparison between charge capacities, as shown in part 1, yields an optimal hypothesis for highly compatible IrOx-carbon hybrids. Despite that, EF experiments also included polymer bilayers to consider the various types of materials, and it was accomplished with IrOx, IrOx-graphene, and PEDOT-ppy-lysine, using Glass and Pt references. Controlled current pulses or controlled potential pulses have been tested with limited charge, to prevent capacitive behaviour, and /or gas formation with pH changes. Cells decrease notably when CSC is surpassed. Astrocyte-neuron cocultures have also been developed as a future model.



3. Implications and relevance

New materials have been found that can be used safely in low-frequency electrostimulation in traumatic injuries, as coatings or in bulk form. They have been demonstrated to repair in vitro. A second stage in vivo is being planned

4. Literature derived and presentations

"Iridium Oxide sensor for biomedical applications. Case urea-urease in real urine samples" Elisabet Prats- Alfonso; Llibertat Abad; Nieves Casan- Pastor; Javier Gonzalo- Ruiz; Eva Baldrich, **Biosensors and Bioelectronics,** 39, **2013**, 163- 169 **SCI impact index: 5.437**

"Graded conducting titanium-iridium oxide coatings for bioelectrodes in neural systems"
A.M. Cruz, N. Casañ-Pastor. **Thin Solid Films**, 534, **2013**, 316–324, **SCI impact index: 1.97**

" Dynamic electrodeposition of aminoacid-polypyrrole on aminoacid-PEDOT substrates: Conducting polymer bilayers as electrodes in neural systems." J. Moral-Vico, N. M. Carretero, E. Perez, C. Suñol, M. Lichtenstein, N. Casañ-Pastor. **Electrochim. Acta** 111 **(2013),** 250-260
SCI Impact index: 3.90

"Nanocomposites of iridium oxide and conducting polymers as electroactive phases in biological media" J. Moral-Vico, S. Sánchez-Redondo, E. Perez, M. Lichtenstein, C. Suñol, N. Casañ-Pastor. **Acta Biomaterialia**, 10 (2014) 2177–2186 **SCI impact index: 5.684**

"IrOx-Carbon Nanotubes Hybrid:A Nanostructured Material for Electrodes with Increased Charge Capacity in Neural systems" Nina M. Carretero,† Mathieu P. Lichtenstein,‡ Estela Pérez,† Laura Cabana,† Cristina Suñol,‡,# Nieves Casañ-Pastor*,† **Acta Biomaterialia,** 10**, 2014,** 4548-4558**. SCI impact index: 5.684**

"*Enhanced Charge Capacity in Iridium Oxide-Graphene Oxide Hybrids"* N. M. Carretero†, M. P. Lichtenstein††, E. Pérez†, S. Sandoval†, G. Tobias†, C. Suñol††#, N. Casan-Pastor†* E**lectrochimica Acta**, 157, **2015**, 369-377 **SCI impact index: 4.086**

"*Coatings of Nanostructured Pristine Graphene-IrOx Hybrids for Neural Electrodes: Layered Stacking and the role of non-oxygenated Graphene*"
E. Pérez, M. P. Lichtenstein, C. Suñol, N. Casan-Pastor*
Materials Science & Engineering C, 55, **2015,** 218-226
SCI impact index: 2.736

PATENT FILED:
N. Casañ-Pastor, M. Lichtenstein, E. Pérez Soler, C. Suñol Esquirol, "PROCEDIMIENTO PARA LA IDENTIFICACIÓN DE ELECTRODOS ÚTILES PARA EL TRATAMIENTO DE LESIONES NEURONALES MEDIANTE UN MODELO DE LESIÓN IN VITRO Y PROTOCOLOS DE ACCIÓN DE CAMPO ELÉCTRICO. (Procedure for the identification of useful electrodes from the treatment of neuronal lesions using an in vitro model of lesion and electric field action protocols)
201531912, presented 24 Dec. 2015

Publications to be submitted as manuscripts in the coming months

"*Neuron Growth and Survival on Conductive Materials during electric field exposure*"
Z. Zao, J. Moral, N. Casañ-Pastor, C. McCaig, A. Rajnicek
Biomaterials, to be sent July 2016

"Iridium oxide hybrids containing carbon nanotubes and conducting polymers: A sequence of evaluation of electrodes for the neural system, and in vitro astrocytes-neuron co-culture cell model"
N. Carretero*, M. Lichtenstein*, J. Moral, E. Perez, C. Suñol*, N. Casan-Pastor*
Manuscript in preparation

"*Electric field protocols in Scratch model, using electroactive electrode materials*"
M. Lichtenstein, E. Perez, C. Suñol*, N. Casan-Pastor*

"Electrodeposition of iridium oxide hybrids in microelectrodes, electrochemical properties and sensitivity"
E. Perez, R. Villa, S. Falk, G. Gabriel, N. Casan-Pastor*

Invited talks

The collaborative work done has been the subject of:
invited talks at the Gordon research Conference in Bioelectrochemistry, (Ann Rajnicek)
invited seminar at Georgetown University, DC, USA (october 2012), (N. Casañ-Pastor)
invited seminar at IBEC (Barcelona) in April 2013, (N. Casañ-Pastor)

as well as the following

Congress Communications

Electrochemical Society, Hawaii, October 2012
J. Moral-Vico, N.M. Carretero, E. Perez, C. Suñol, N. Casañ-Pastor, "Electroactive materials in Biological systems"
oral communication

Meeting of the Spanish Neuroscience Society. Oviedo. September 25-25, 2013
M.P. Lichtenstein, J. Moral-Vico, N.M. Carretero, N. Casañ-Pastor, C. Suñol. Neural growth and functional development on new biocompatible hybrid materials.
Poster

TNT October 2014, Barcelona
"Electrochemical Graphene and graphene Ir hybrids"
N. Carretero, Mathieu Lichtenstein, Cristina Suñol, N. Casañ Pastor
Oral *Communication*

Graphene Symposium, ***Trobades Científiques de la Mediterrania Josep Miquel Vidal***,
Menorca October ***2014***
"Electrochemical Graphene and graphene Ir hybrids"
N. Carretero, E. Perez, M. Alanyologlu, Mathieu Lichtenstein, Cristina Suñol, N. Casañ Pastor
ORAL *Communication*

5th Zing Conferences on Bionanomaterials
Algarve, April 2015
"GLIAL AND NEURAL GROWTH AND FUNCTIONAL DEVELOPMENT ON NEW NANOSTRUCTURED IRIDIUM OXIDE HYBRID MATERIALS "
POSTER
M. Lichtenstein, E. Pérez, N. Carretero, N. Casañ-Pastor, C. Suñol

5th Zing Conference in Bionanomaterials
Algarve, April 2015
Nanostructured materials for neural electrodes:
Hybrids of iridium oxide with graphene, nanotubes or conducting polymers.
E.Perez[a], N. Carretero, M. Lichtenstein[b], C. Suñol[b], N. Casañ-Pastor[a*]
Oral *Communication*

5th Zing Conference in Bionanomaterials

Algarve, April 2015

IrOx nanoparticles driving graphene electrodeposition: Nanostructured coatings for neural bioelectrodes

Poster

*E. Pérez, M. Lichtenstein, C. Suñol, N. Casañ-Pastor**

Poster

ICREA Workshop on Graphene Biosensors

Barcelona, May 25-26, 2015

http://graphsense.icn2.cat/

Macro and microelectrodes based on iridium oxide-graphene hybrid nanostructured coatings

Estela Pérez, Sophia Falk, Rosa Villa, Gemma Gabriel Nieves Casañ-Pastor

Oral

HINTBCN: Scientific-workshop-on-biomedical-health-and-bio-related-applications-of-hybrid-materials

June 8-9, Barcelona

http://icmab.es/icmab-news/events/upcoming/262-june/1997-hintbcn-scientific-workshop-on-biomedical-health-and-bio-related-applications-of-hybrid-materials

Nanostructuring hybrid materials to be used as neural electrodes: Electrodeposition of Hybrids of IrOx-CNT, IrOx-graphene and IrOx-conducting polymers

N. Carretero, E. Perez, M. Lichtenstein, C. Suñol, N. Casañ-Pastor*

ORAL

HINTBCN: Scientific-workshop-on-biomedical-health-and-bio-related-applications-of-hybrid-materials

June 8-9, Barcelona

http://icmab.es/icmab-news/events/upcoming/262-june/1997-hintbcn-scientific-workshop-on-biomedical-health-and-bio-related-applications-of-hybrid-materials

Synthesis and characterization of a new pristine graphene-IrOx hybrid for neural bioelectrodes

E.Pérez, M. Lichtenstein, C. Suñol, N. Casañ-Pastor*

Poster

Accepted abstracts

Microscopy at the Frontiers of Science 2015 (MFS2015), EMS sponsored event

September 9-11, 2015, Porto University,

Portugal

STEM and HRTEM Analysis of IrOx-Graphene Nanoparticles: at the Root of Bioelectroactive Coatings for Neural Electrodes.

R. Fernández-Pacheco, R. Arenal, E. Pérez, F. Sandiumenge and N. Casañ-Pastor

Latest Advances on Carbon Nanomaterials for Biomedical Applications" (NANOBIOAPP2015)

Sept 8, 2015, Barcelona

"Pristine graphene versus Graphene Oxide in IrOx-Hybrids. Enhanced charge capacity in Coatings for Neural Electrodes"

E.Pérez, M. Lichtenstein, C. Suñol, N. Casañ-Pastor*