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Graphene Oxide: a potential scaffold for promoting cell differentiation.

G. Fioravanti*, E. Benedetti, M. Catanesi, F. Perrozzi, G. Panella, F. Angelucci, L. Ottaviano, R. Ippoliti and A. Cimini

***Department of Physical and Chemical Sciences, University of L'Aquila**
giulia.fioravanti@univaq.it

Outline

- Introduction on toxicity of graphene-based 2D materials
- The importance of new biomaterials that support neurons growth and differentiation
- SHSY5Y on Graphene Oxide (GO) film: effect of the thermal reduction
- SHSY5Y with GO hydrogel (+ Peroxiredoxin)

First of all…

The prospective use of graphene-based materials in a biological context requires a detailed comprehension of the **toxicity** of these materials (*in vitro* and *in vivo* cytotoxicity and/or genotoxicity).

Safe or Toxic? It depends… toxic and nontoxic effects were simultaneously observed…

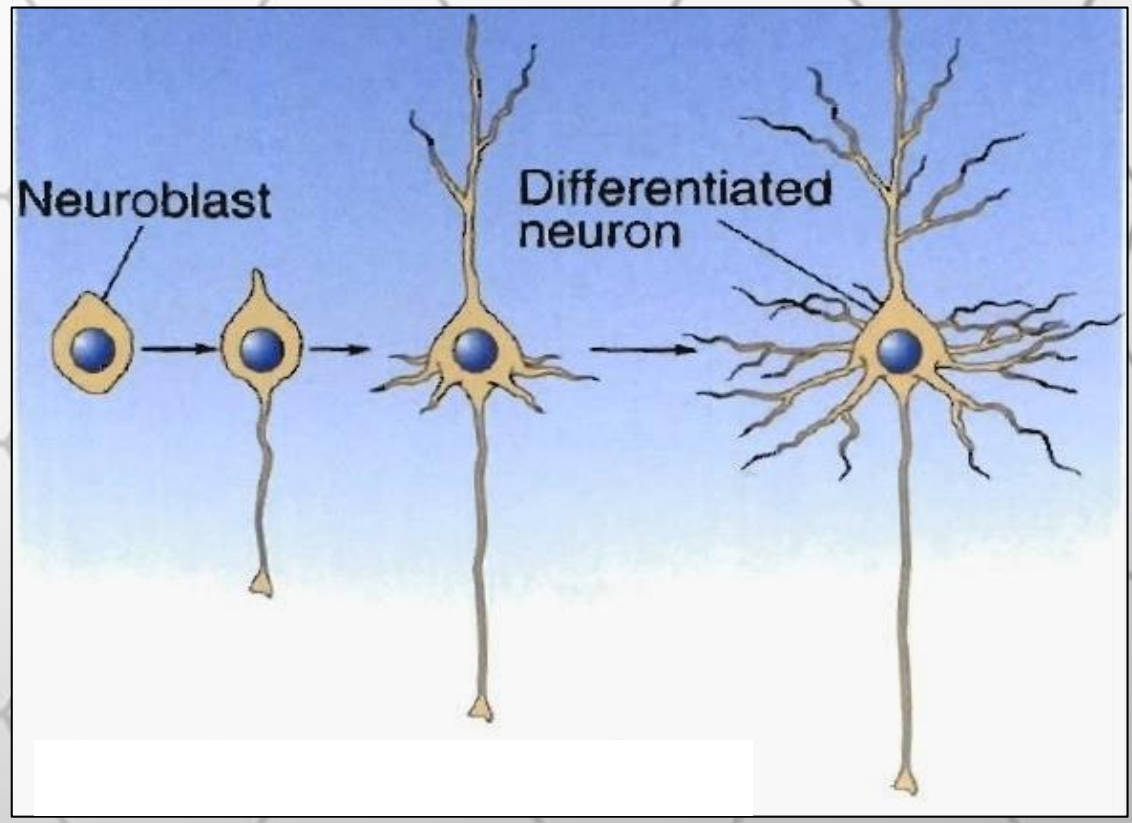
The biological responses certainly vary depending on the **number of layers, lateral size, stiffness, hydrophobicity, surface functionalization, dose administered, and purity of the material.**

Moreover, the **type of cells**, the **doses** (concentration range goes from 0.01 to 1000 mg/mL), the **times** of incubation and, in the case of animal experiments, the **routes of administration**.

The importance of neurons growth/differentiation

- **From Neuroblasts to differentiated Neurons**
- **Use of new bio-materials to promote cell adhesion, growth and differentiation**

Due to the inability of the nervous system to regenerate, biomaterials that support neurons growth and differentiation are of great interest for neurodegenerative disease, especially in cases of damages to the central and peripheral nervous system.



System studied: SHSY5Y on GO/rGO film

- **SHSY5Y**, human neuroblastoma cell, a well known model used to study neuronal differentiation and disease in vitro.
- **GO and rGO film**, with negligible toxicity (stable coating).
- Effect of the different chemical composition of GO/rGO film on the cell differentiation.

See for example:

- *Adv. Healthcare Mater.* **2015**, *4*, 1451–1468
- *J. Microbiol. Biotechnol.* **2013**, *23(2)*, 274–277
- *Nanoscale*, **2012**, *4*, 3861-3866

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Effect of “dispersed” graphene oxide on undifferentiated and retinoic acid-differentiated SH-SY5Y cells line.

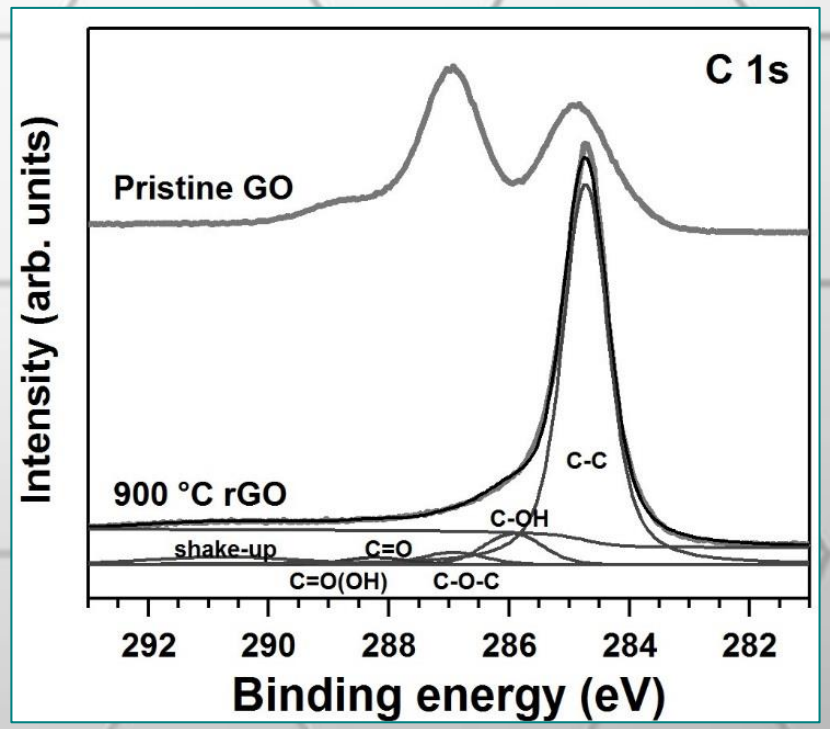
- Dose-dependent toxicity
- GO could assist RA to enhance differentiation of SH-SY5Y cells (at low concentration same as control)

From previous study: effect of reduction T on GO film

Experimental: GO films were prepared by **spin coating** (thickness 30 nm) and then **reduced** at different temperatures in **UHV** (Ultra High Vacuum).

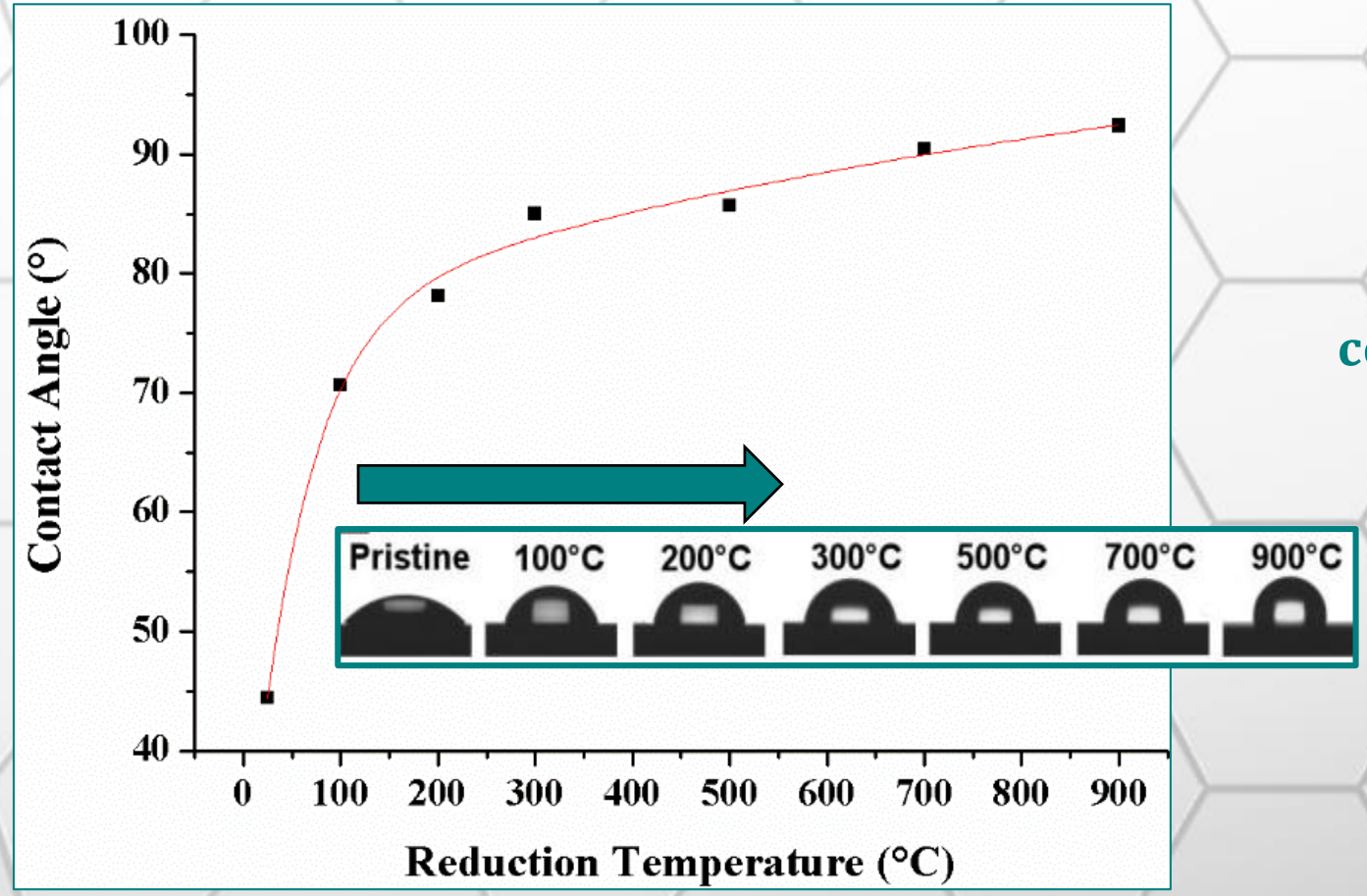
The thickness of 30 nm of the GO and rGO films is enough to avoid wetting transparency effects.

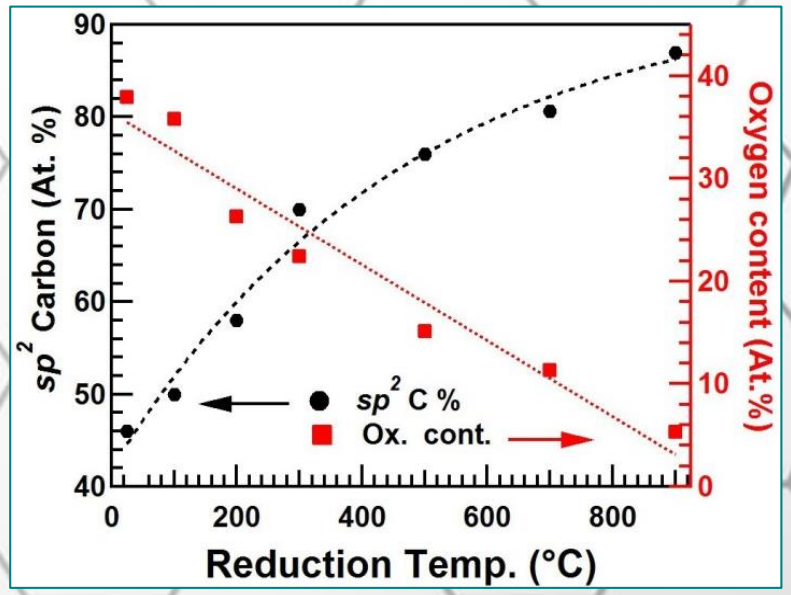
XPS C 1s spectra of pristine GO and 900 °C rGO



Carbon **2014,** 77, 473-480

Water Contact Angle





$$\cos(\theta) = Csp^2 \cos(\theta_G) + (1 - Csp^2)\cos(\theta_{Ox})$$

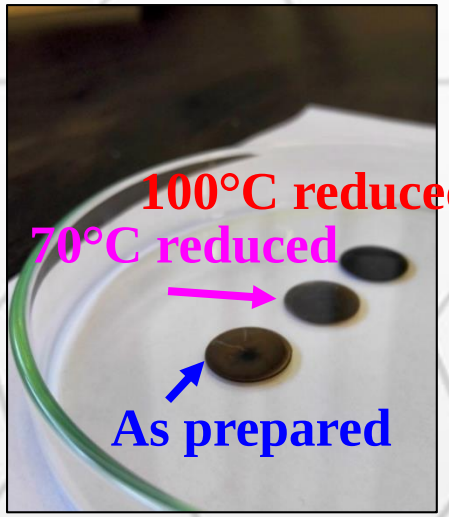
The CA can be correlated with the chemical evolution analyzed by XPS technique.

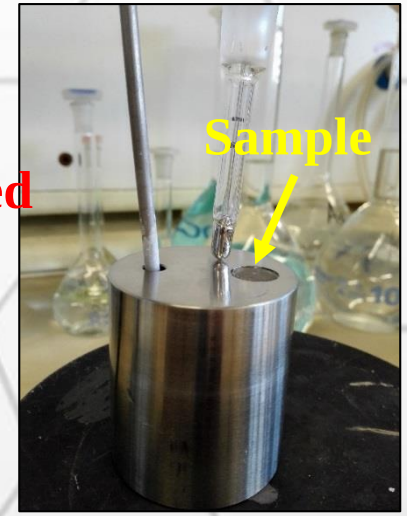
Oxygen total content and sp^2 carbon atoms percentage evolution as a function of the reduction temperature.

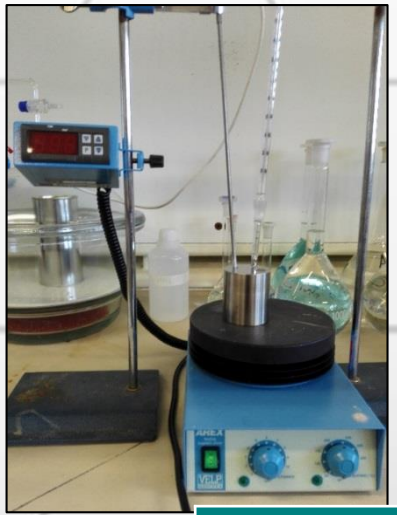
Carbon **2014**, 77, 473-480

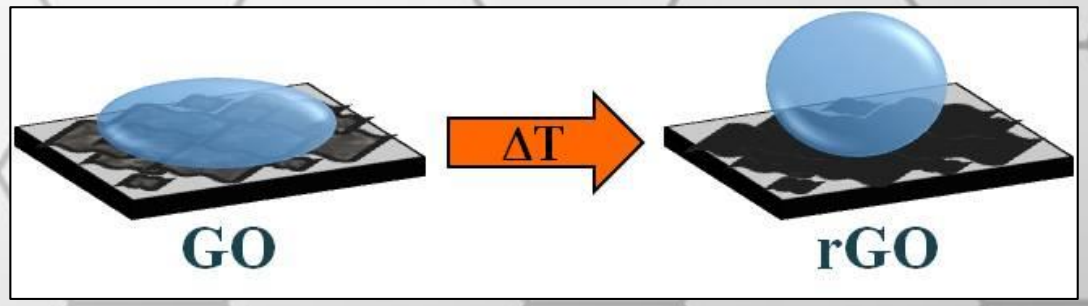
GO/rGO film

Experimental: GO films were prepared by **drop casting** and then **reduced** at different temperatures in air. The thickness of the GO and rGO films (μm) is enough to avoid wetting transparency effects.





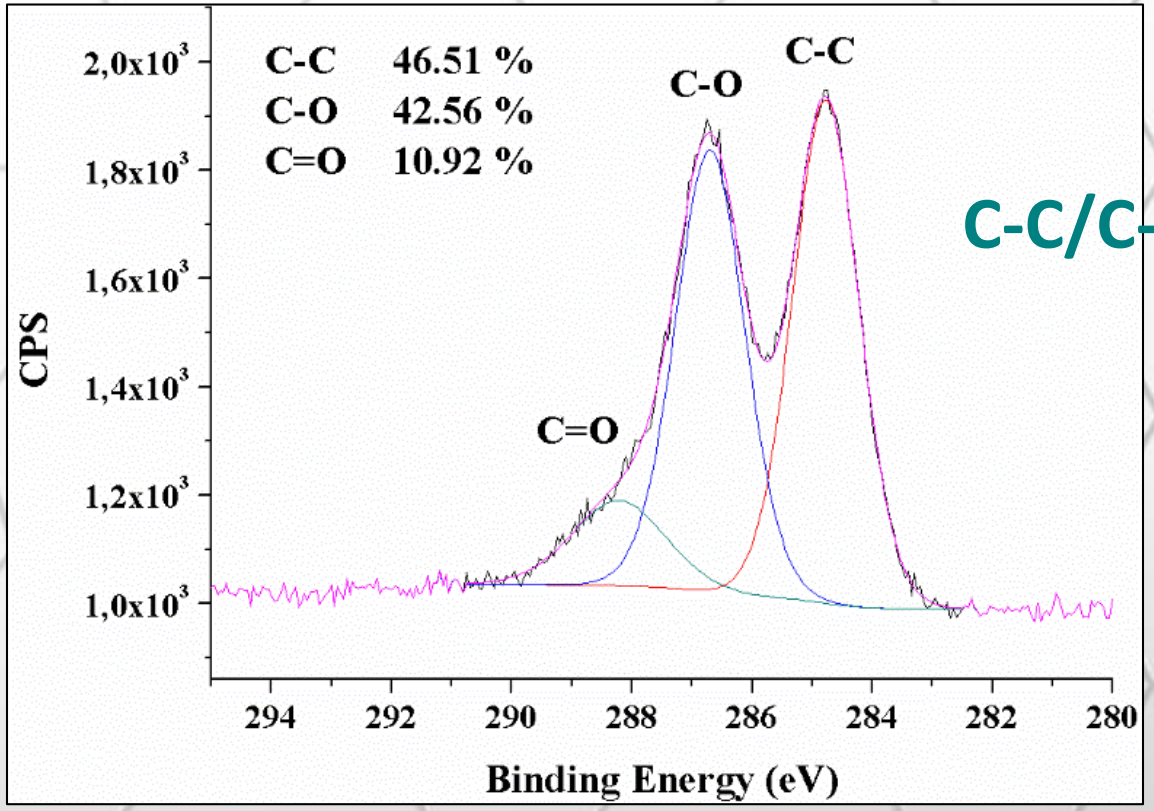




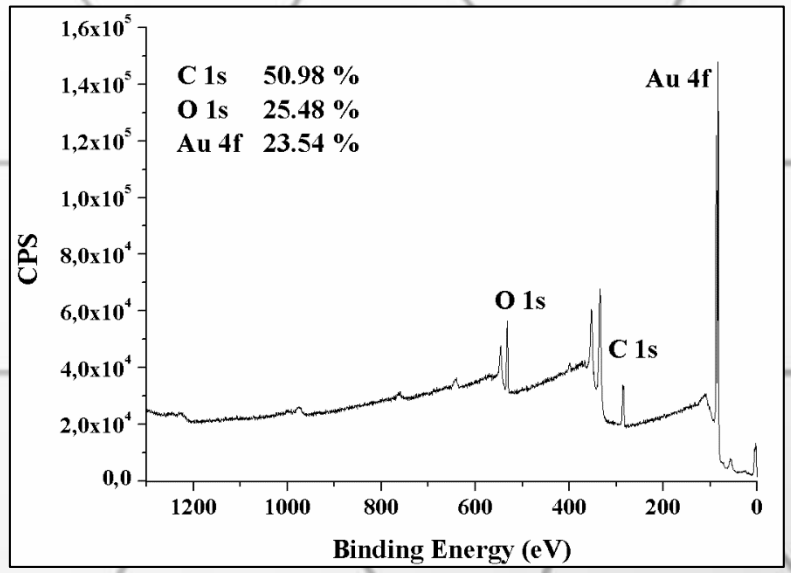
GO film	Contact Angle (°)	XPS (on Si)
As prepared	35 ± 3	(C/O ≈ 1)
70 °C reduced	55 ± 2	-
100 °C reduced	70 ± 2	(C/O ≈ 2)

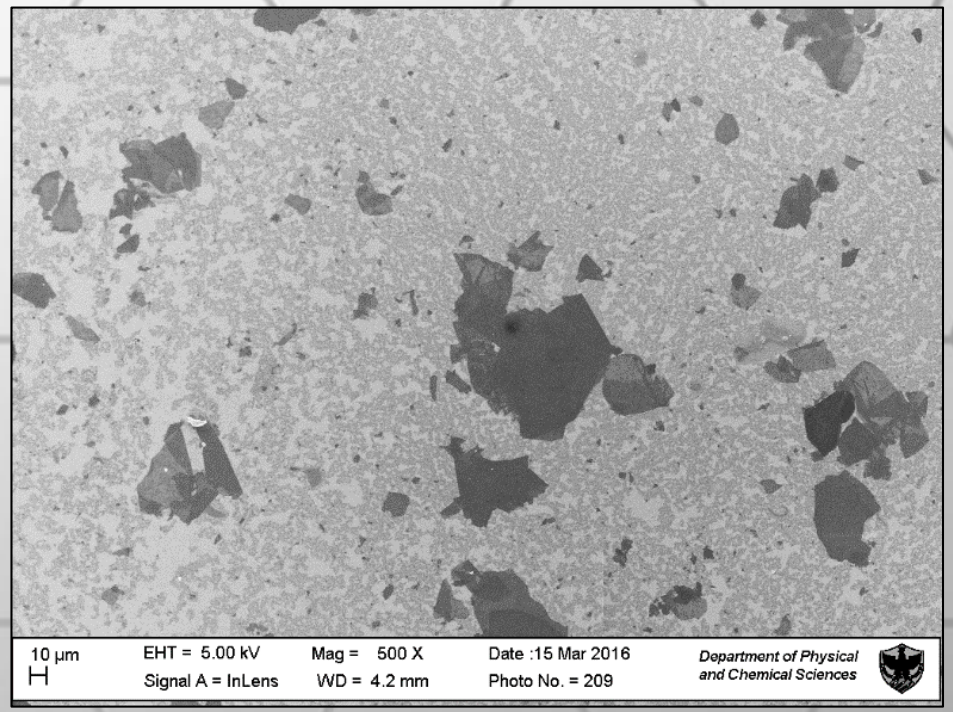
GO dispersion: XPS characterization

As prepared GO in water solution, deposited on Au



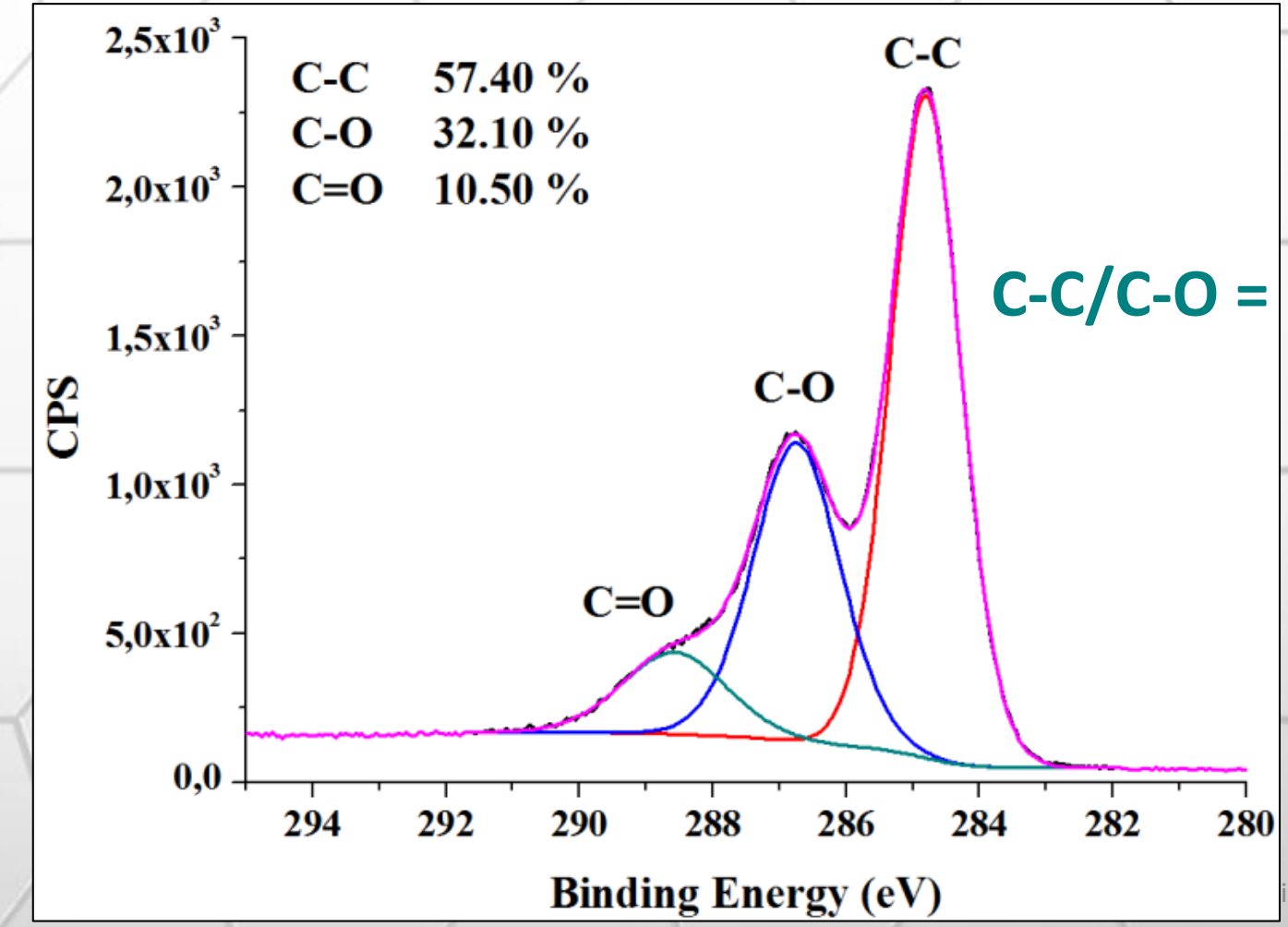
C-C/C-O = 1.09



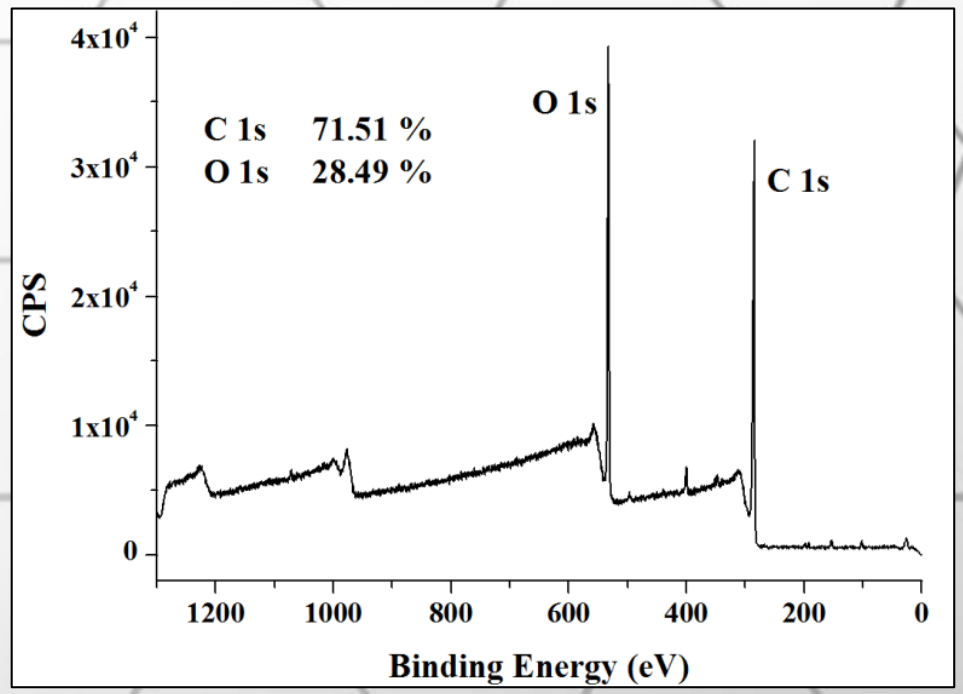


XPS C 1s spectra of pristine GO

GO film: XPS characterization

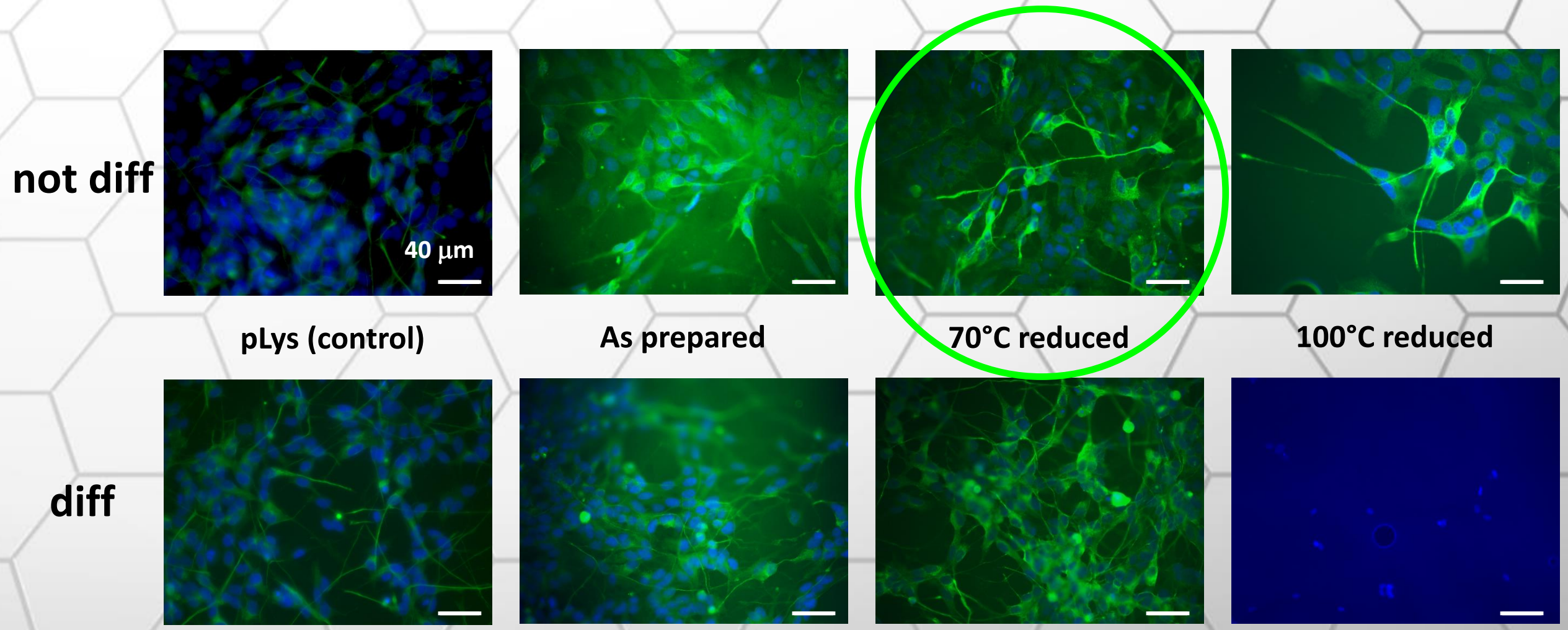


C-C/C-O = 1.79

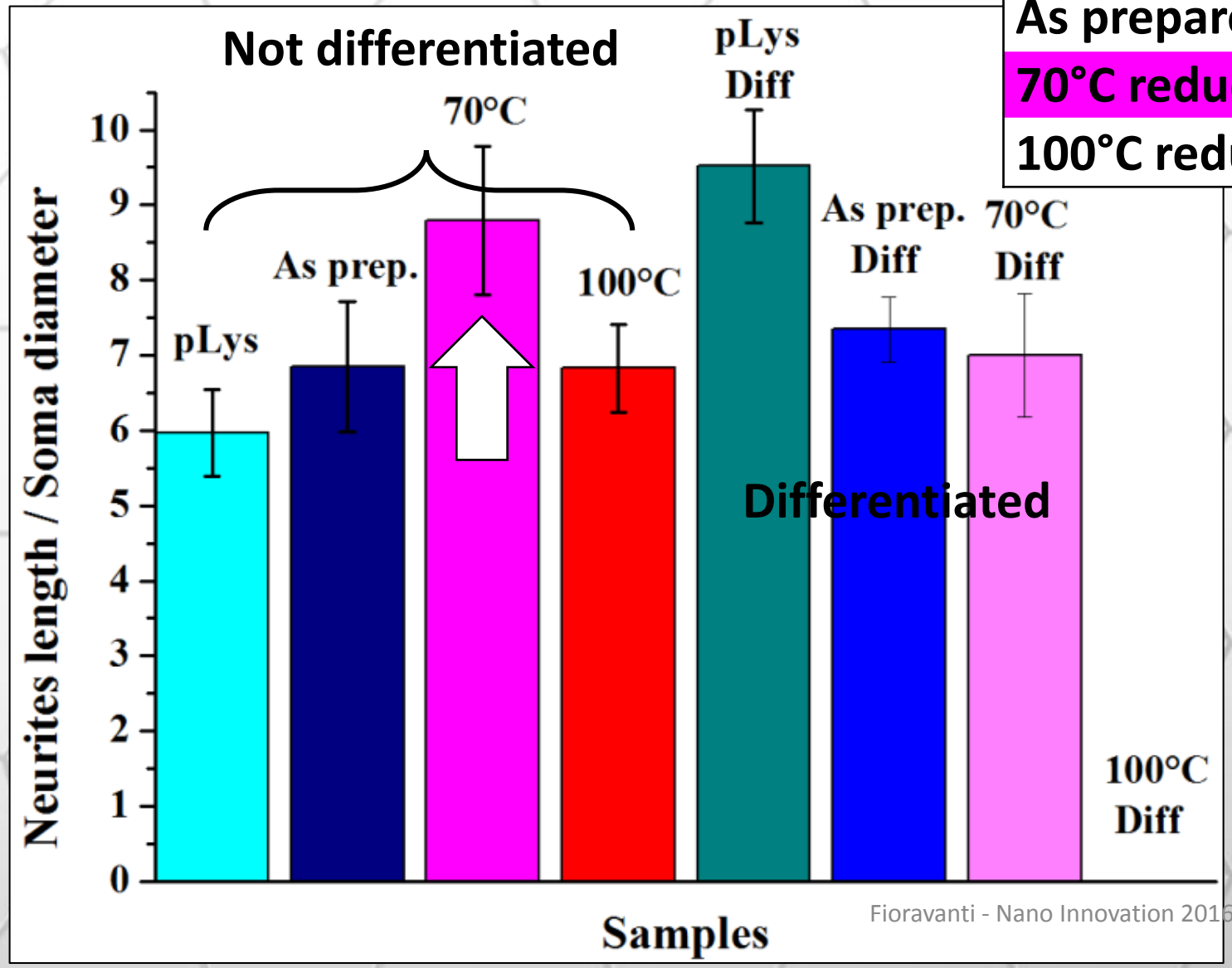


XPS C 1s spectra of GO film, reduced @100˚C

Effect of GO films on differentiation of SH-SY5Y



Neurites growth

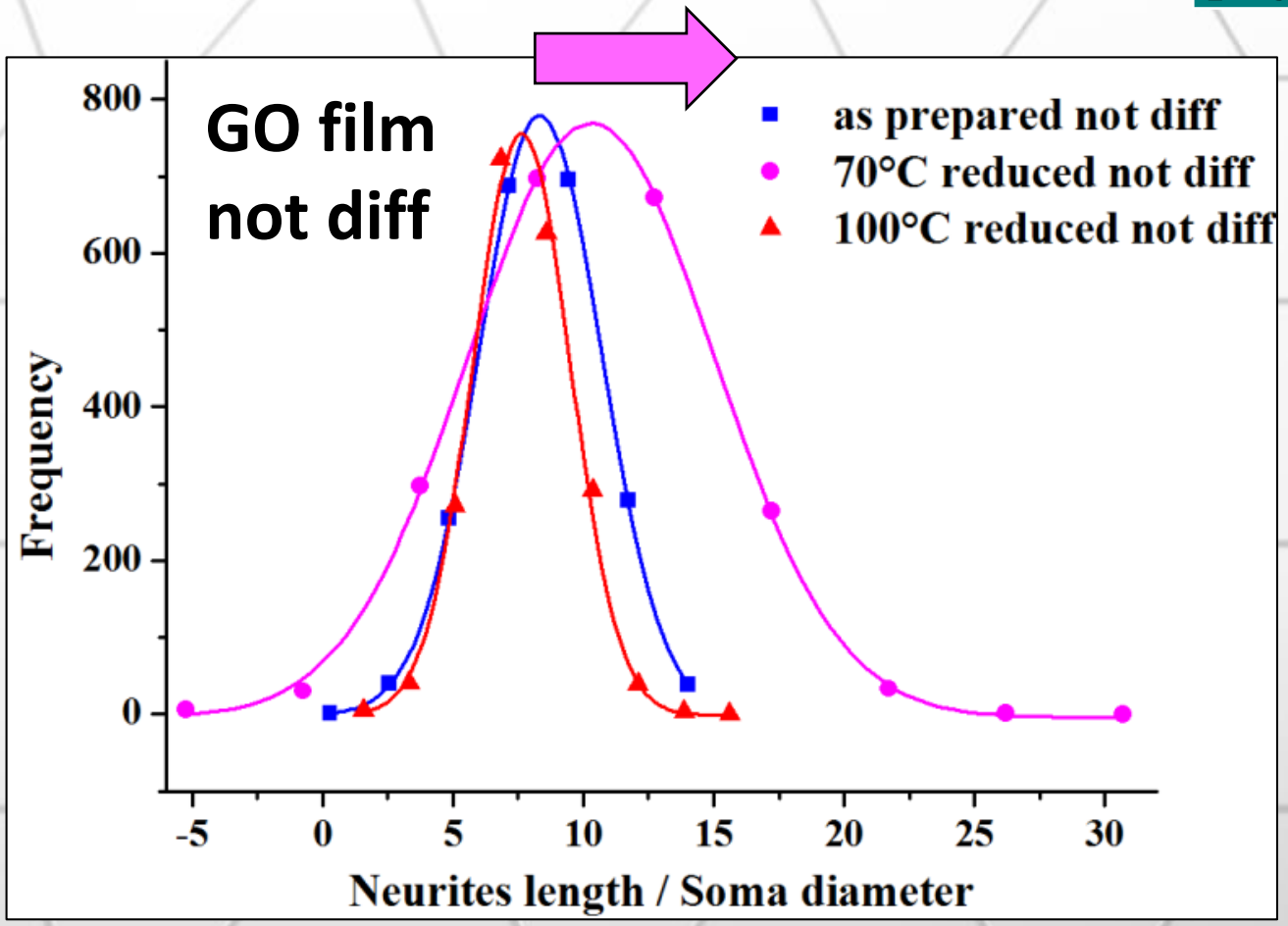


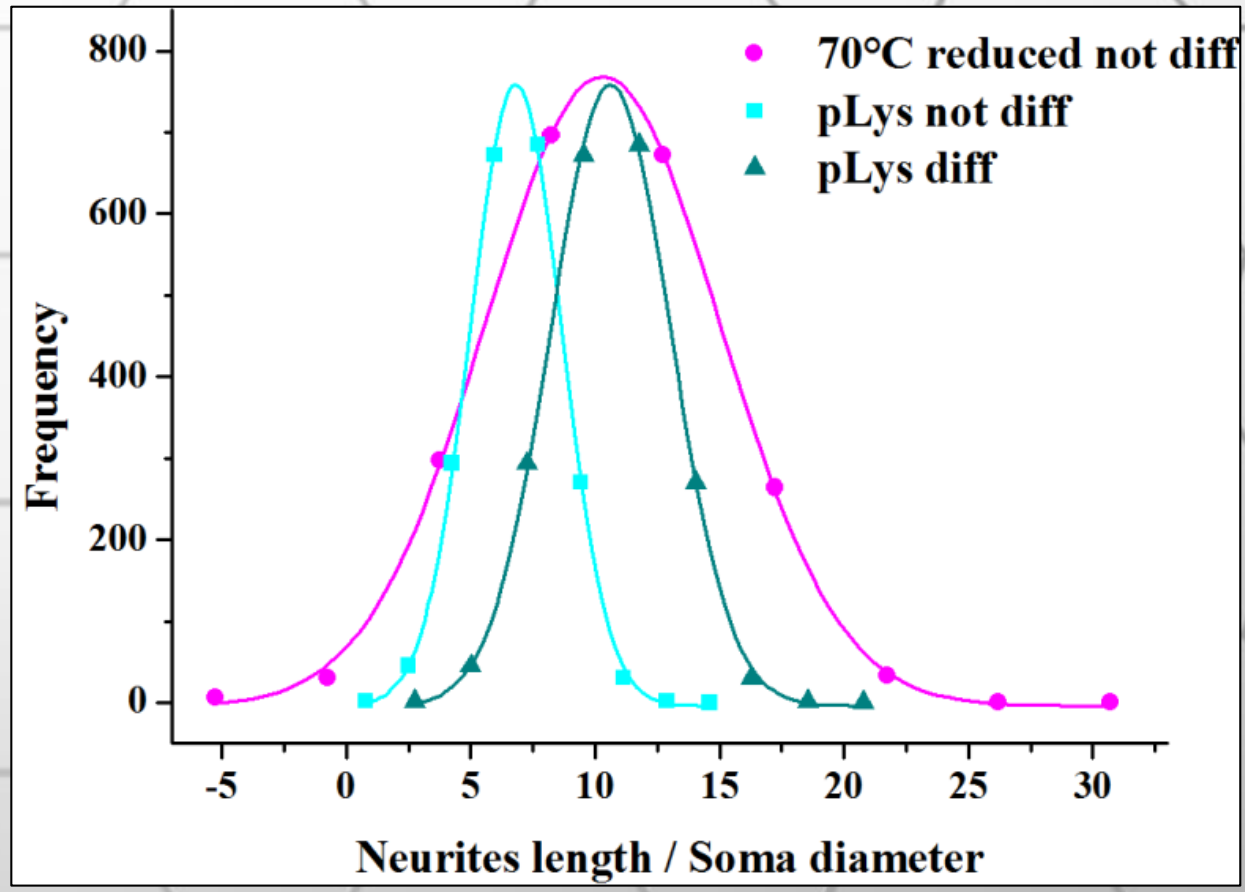
	Not Diff.	Diff.
pLys (control)	5.96 ± 0.58	9.52 ± 0.75
As prepared	6.67 ± 0.96	7.34 ± 0.43
70°C reduced	8.00 ± 0.99	7.00 ± 0.82
100°C reduced	6.83 ± 0.59	-

The **neurite length** was determined by comparing the neurite length with the mean diameter of cell soma and reported as neurite length/soma diameter.

Length distribution

pLys (control) not diff	5.96	±	0.58
70°C reduced not diff	8.79	±	0,99
pLys (control) diff	9.52	±	0.75

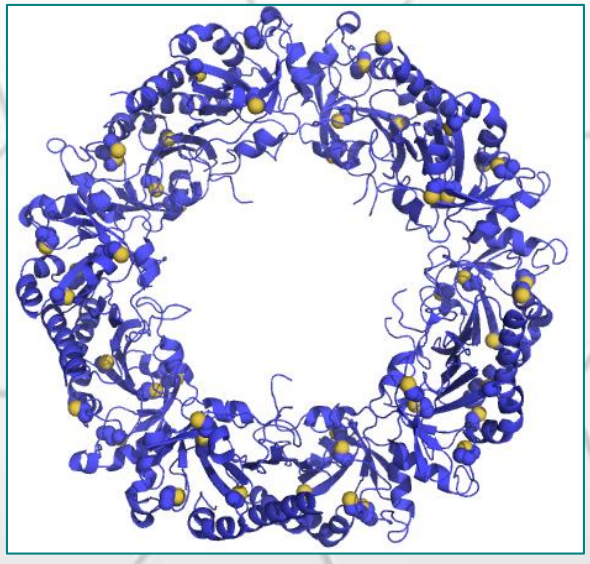




From film to hydrogel…

GO-protein Hydrogel

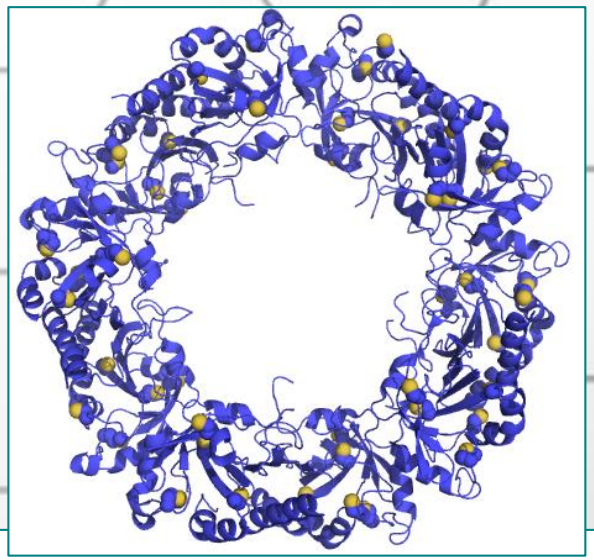
- Commercial GO sample
- Interaction with Peroxiredoxin (from *Schistosoma mansoni*)



Specifications from Data Sheet:

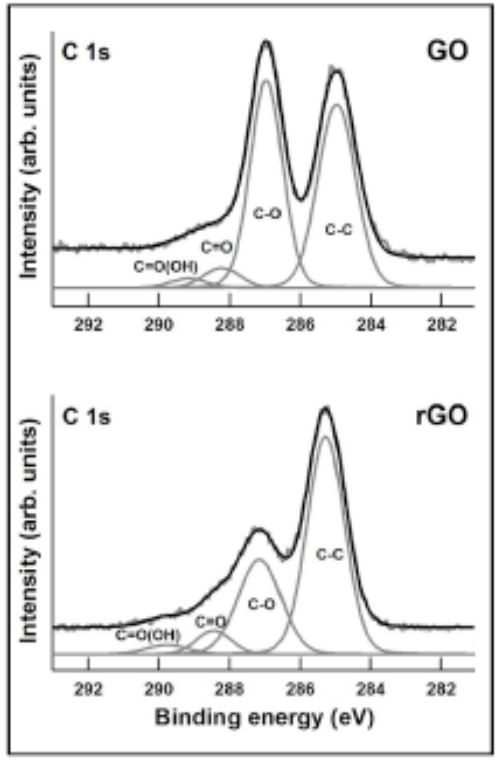
- Aqueous dispersion
- Concentration: 500 mg/L
- Composition: Carbon (79%), Oxygen (20%)
- Flake size: 0.3 - 0.7 microns
- Thickness: 1 atomic layer - at least 80%.
- Color: Brown

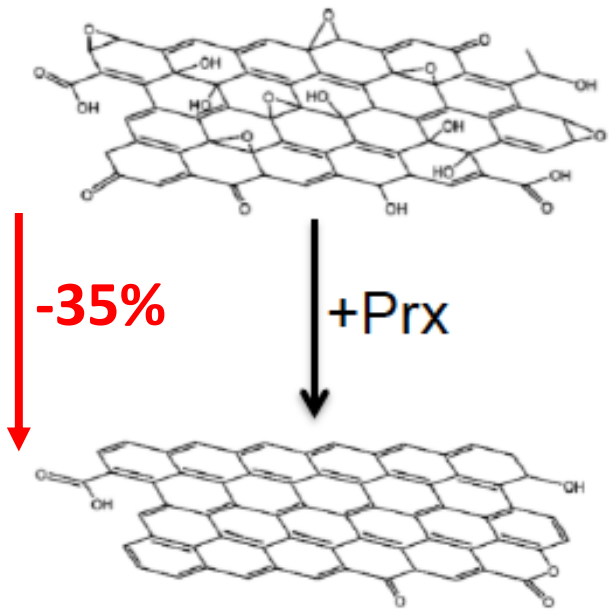
GO-Prx interaction by XPS data

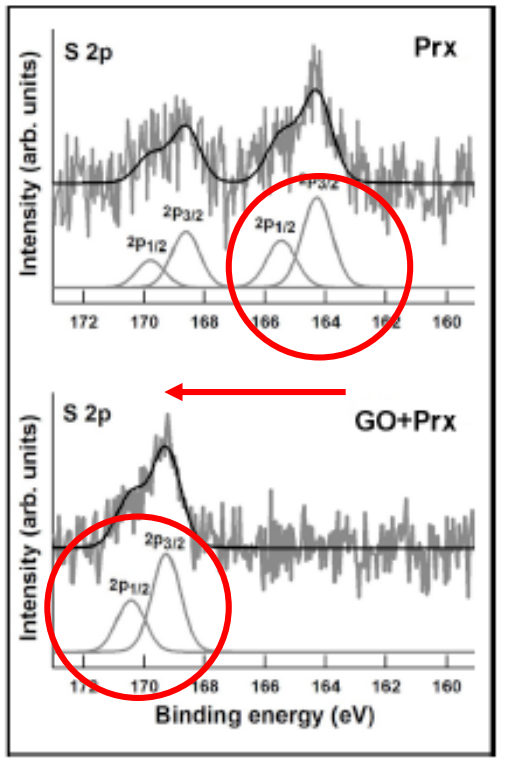


Peroxiredoxin is very rich of **cysteines** and **methionines**.

GO is partially reduced (- 35%) and **Prx is oxidized**.







Cys RSH
Met R-S-R

(sulfides)

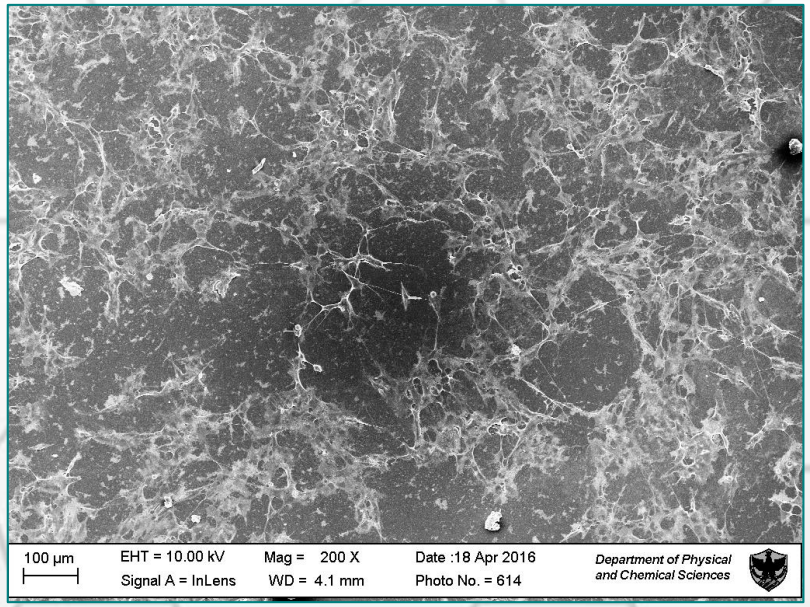
+GO

Cys RS(O)OH
Met R-S(O)$_2$-R

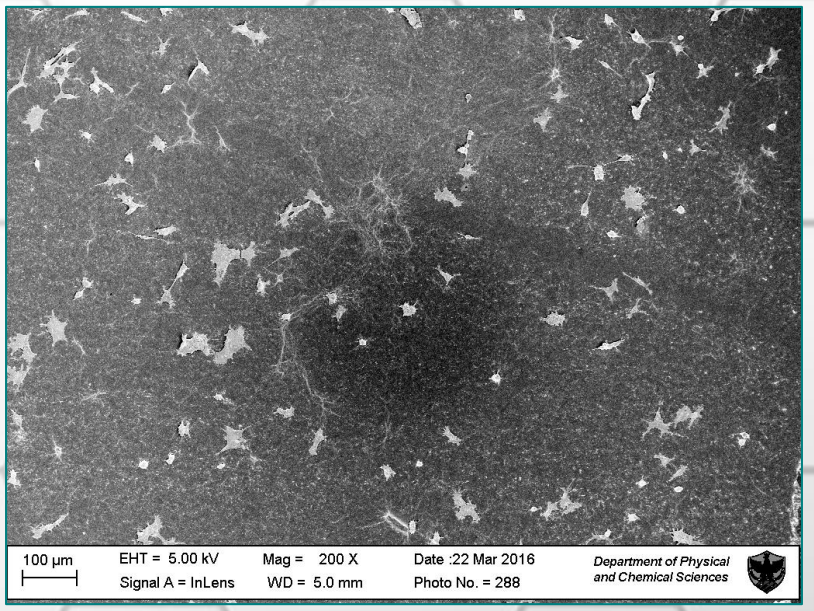
(sulfonic acids and sulfones)

Nanoscale, **2016**, 8, 6739–6753

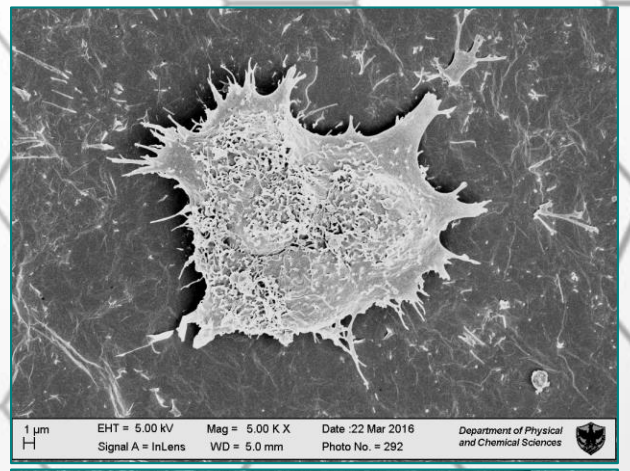
SHSY5Y on GO

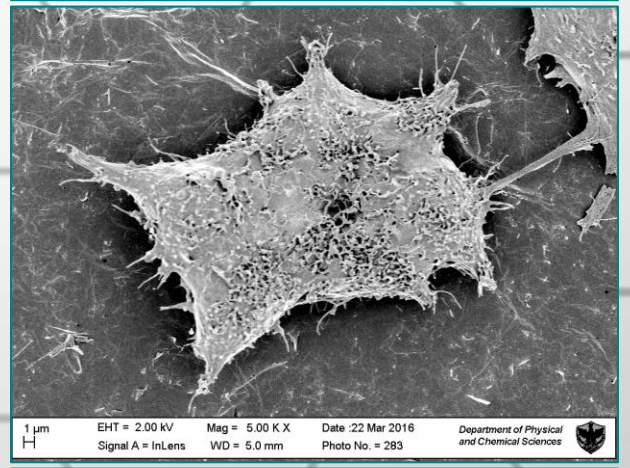


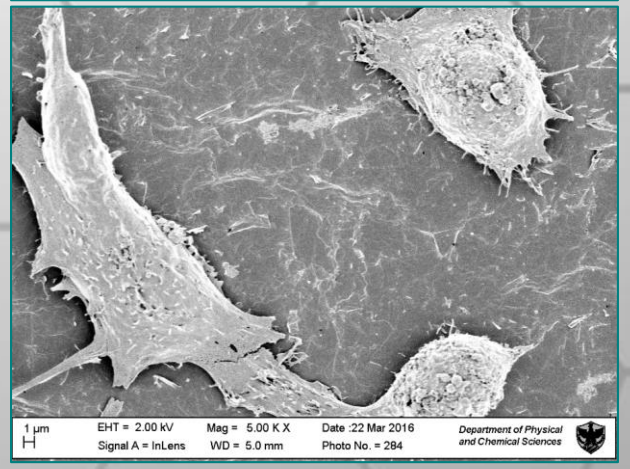
SHSY5Y (control)



GO + SHSY5Y

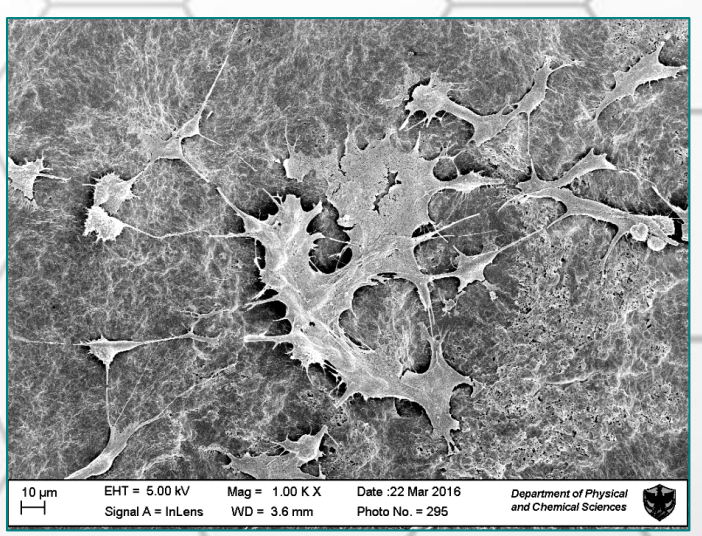


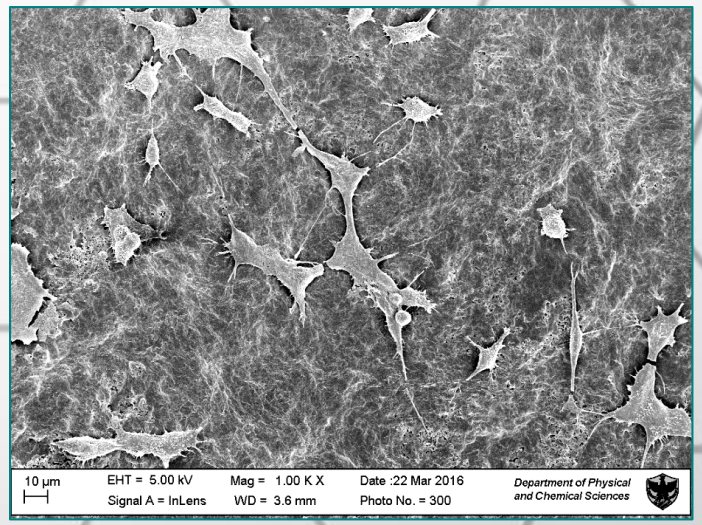


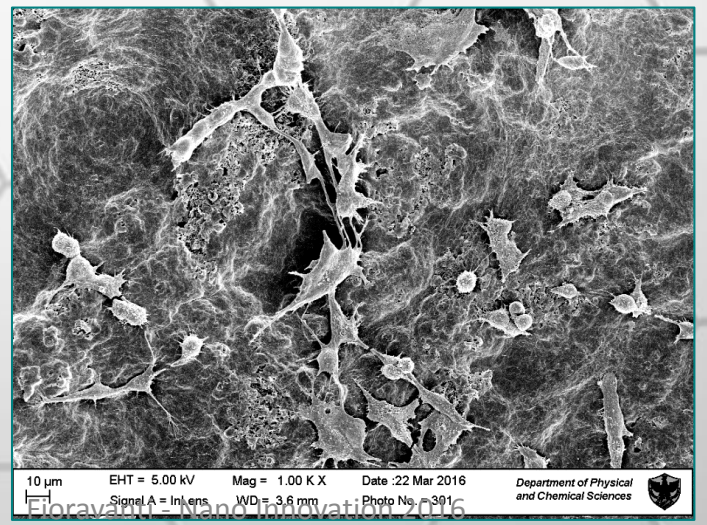


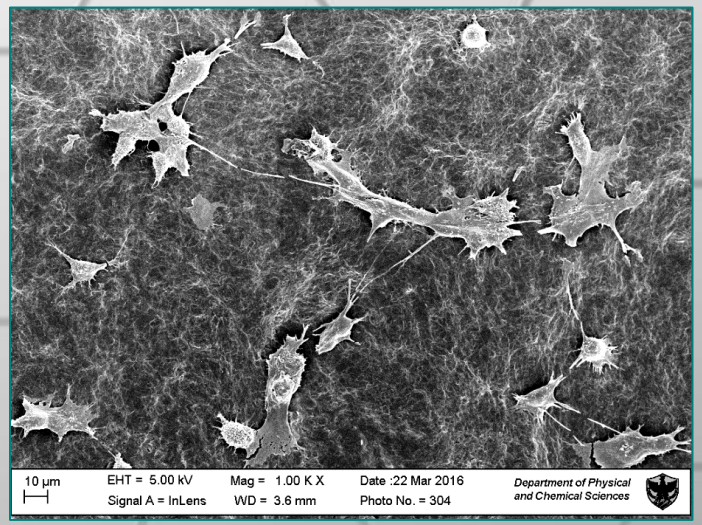
SHSY5Y + rGO-Peroxiredoxin (Prx) hydrogel

- Different shape (from 2D to 3D)
- Promoting cell differentiation



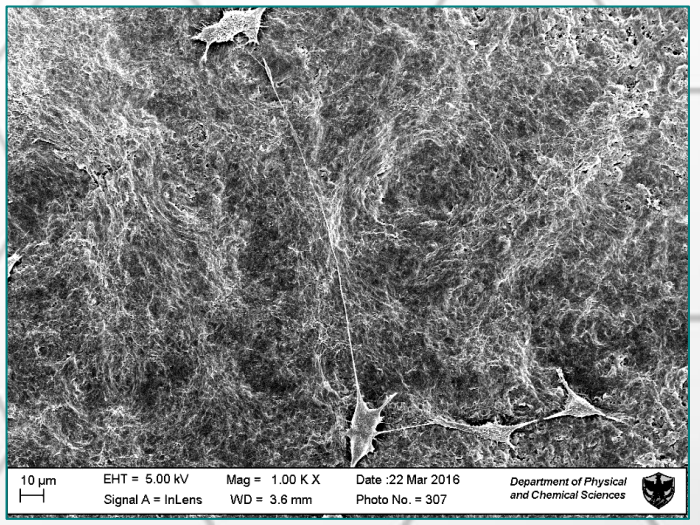


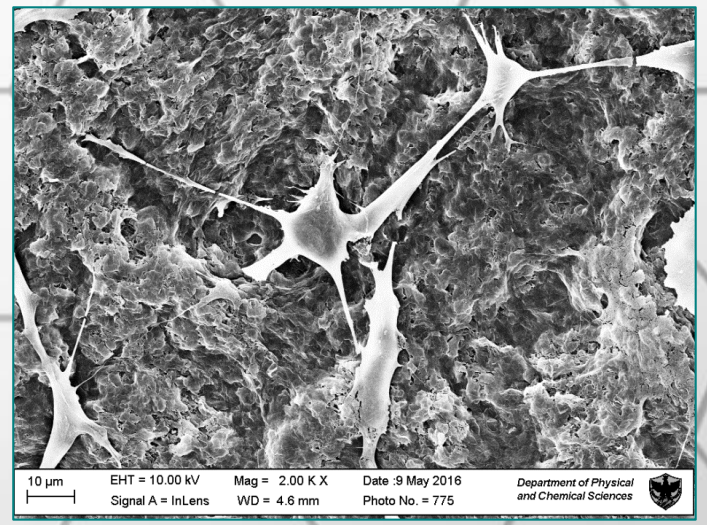


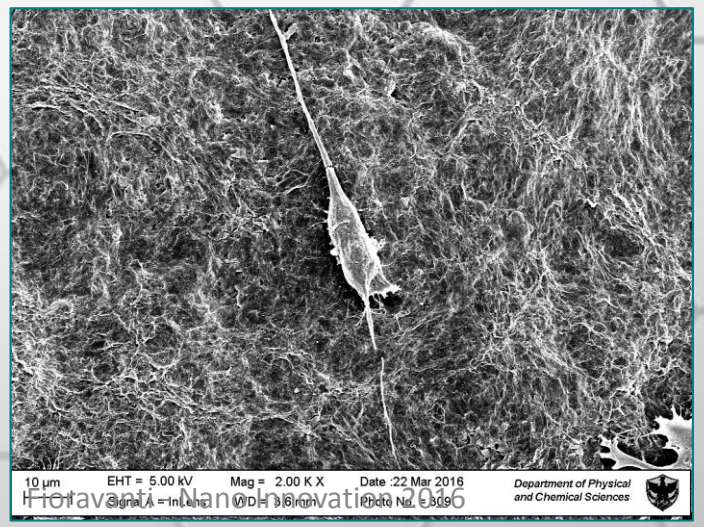


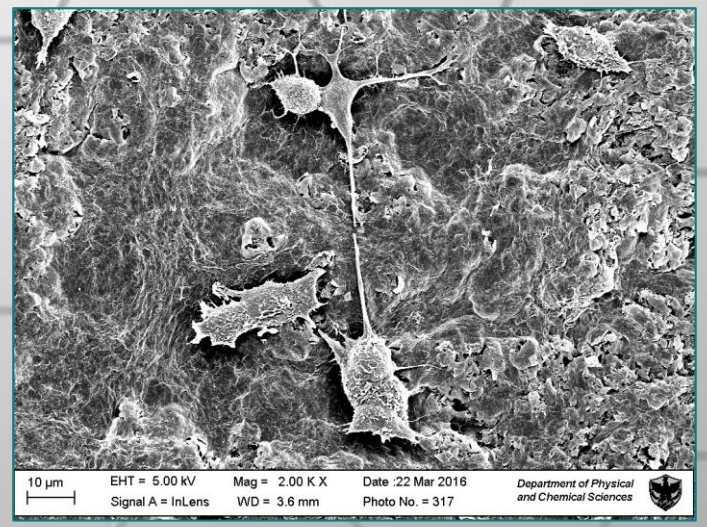
SHSY5Y + rGO-Peroxiredoxin-C48S

- Spunge shape (3D)
- Enhanced cell differentiation









Conclusions

- **Partially reduced GO promotes cell adhesion and differentiation** (for SHSY5Y)
- **No evident toxicity for GO film** (cells death only on 100 °C reduced)
- Good **elongation of neurites** (by comparison with control)
- Good platform for regenerative medicine

For the future:

- Quantification of differentiation (in terms of proteins level, marker expression etc… and hypothesis on the differentiation mechanism)
- Handier **3D scaffold** (for neuronal regeneration)

Collaborations

- The group of **Prof. A. Cimini and E. Benedetti** of the "Neurobiology Laboratory" - Department of Life, Health and Environmental Sciences (MESVA) - University of L'Aquila
- The group of **Prof. R. Ippoliti** of the "Molecular Biology Laboratory" - Department of Life, Health and Environmental Sciences (MESVA) - University of L'Aquila
- The group of **Prof. L. Ottaviano** of "XIL Laboratory for Interference Lithography" - Department of Physical and Chemical Sciences (DSFC) - University of L'Aquila

Thanks!!!

Department of Physical and Chemical Sciences

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