

# DETECCIÓN DE OXIDO DE GRAFENO EN SUSPENSIÓN ACUOSA (COMIRNATY™ (RD1))

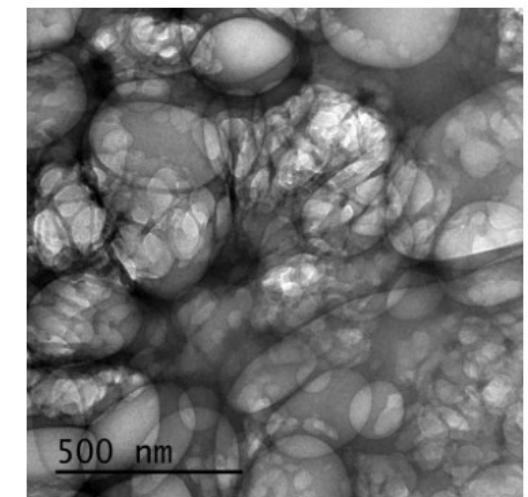
ESTUDIO OBSERVACIONAL EN MICROSCOPIA ÓPTICA Y ELECTRÓNICA

## Informe provisional (I)

28 de Junio de 2021



**NOTA:** questi ricercatori spagnoli hanno trovato l'ossido di grafene non solo nel qui nominato farmaco sperimentale anti-covid Comirnaty (erroneamente detto "vaccino"), ma ANCHE nei vaccini anti-influenzali somministrati durante il 2020



Prof. Dr. Pablo Campra Madrid

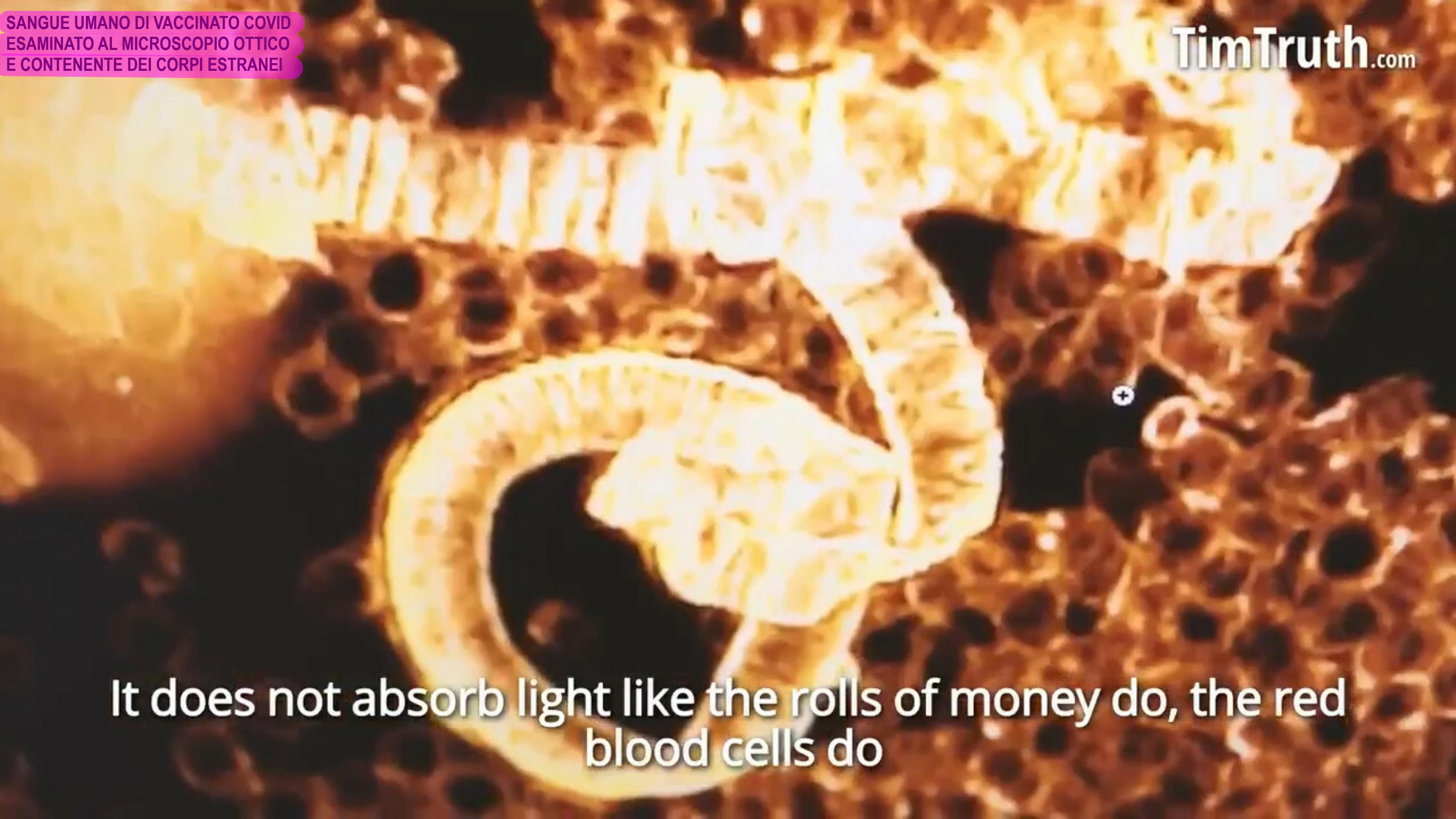
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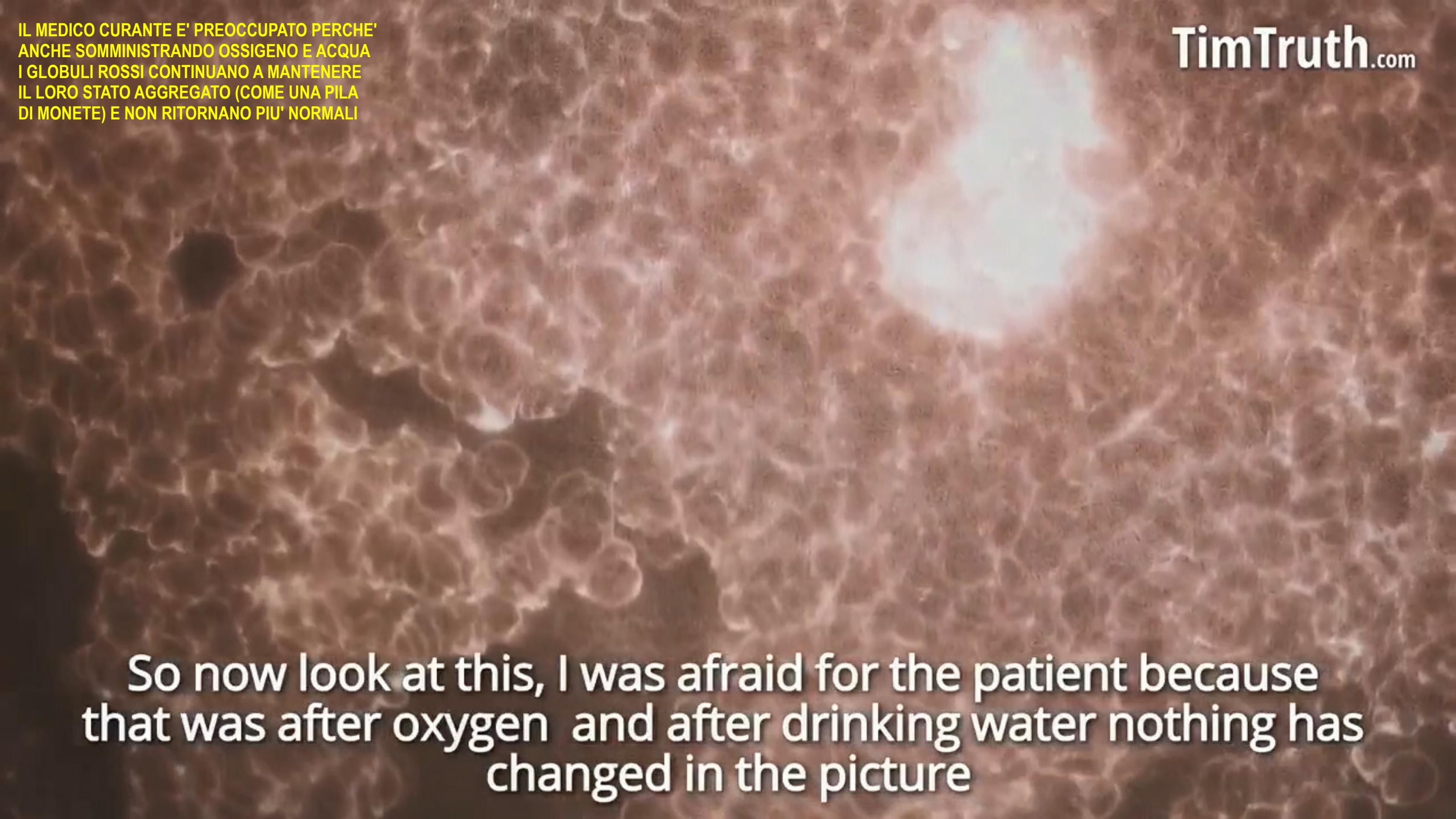
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A high-magnification optical microscope image of human blood. The image shows numerous red blood cells, which appear as small, dark, circular structures. Interspersed among them are larger, more complex cells, likely white blood cells or platelets, which have a more irregular, granular appearance. The overall color palette is dominated by shades of red, orange, and yellow, with some darker areas where shadows fall.

It does not absorb light like the rolls of money do, the red  
blood cells do

IL MEDICO CURANTE E' PREOCCUPATO PERCHE'  
ANCHE SOMMINISTRANDO OSSIGENO E ACQUA  
I GLOBULI ROSSI CONTINUANO A MANTENERE  
IL LORO STATO AGGREGATO (COME UNA PILA  
DI MONETE) E NON RITORNANO PIU' NORMALI

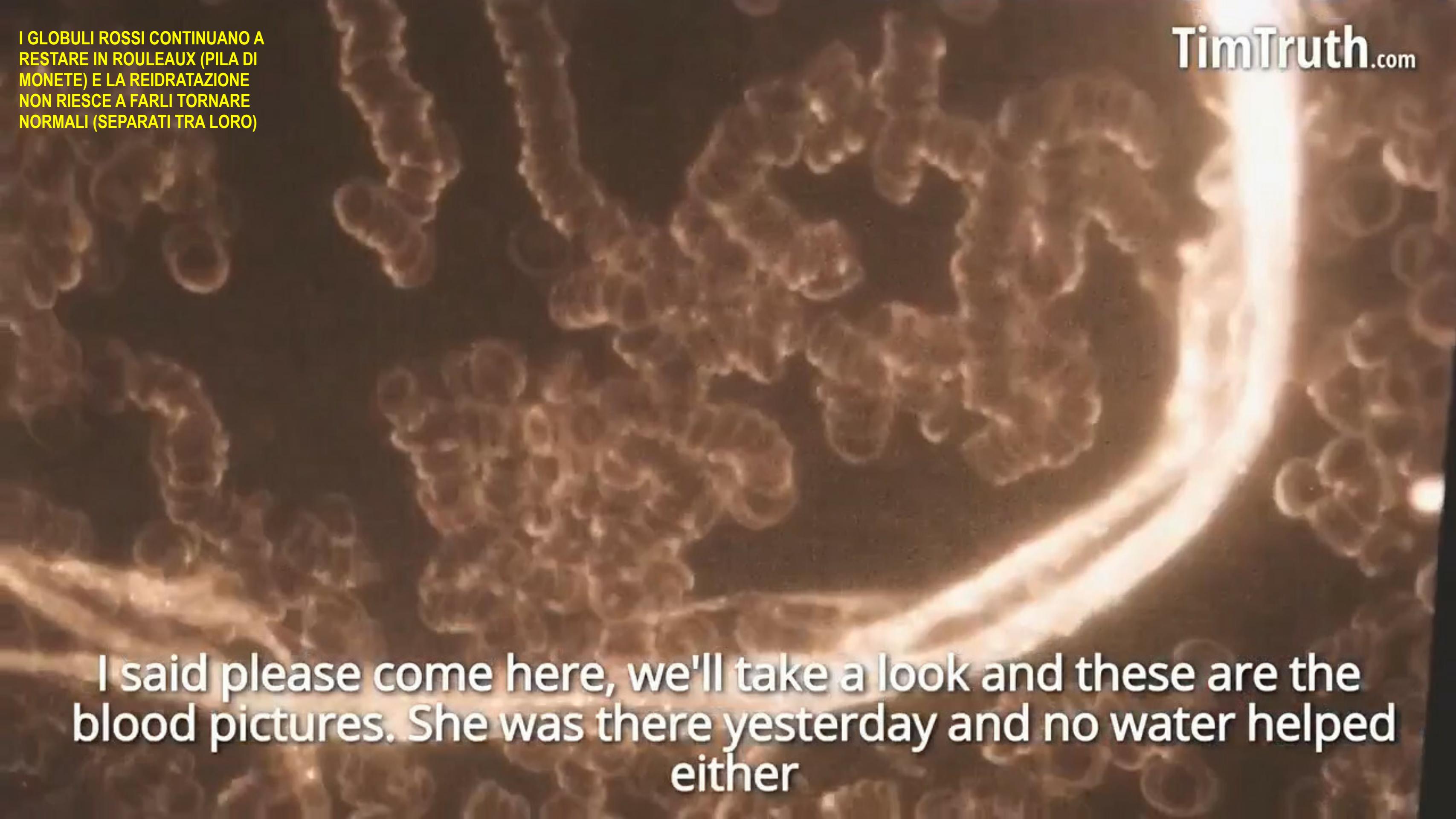
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A microscopic image showing numerous red blood cells. They appear as small, circular or oval shapes with a distinct biconcave center. The cells are densely packed and have a reddish-brown color. Some cells are more transparent than others, appearing darker. The overall texture is somewhat mottled and lacks the normal fluidity seen in healthy blood.

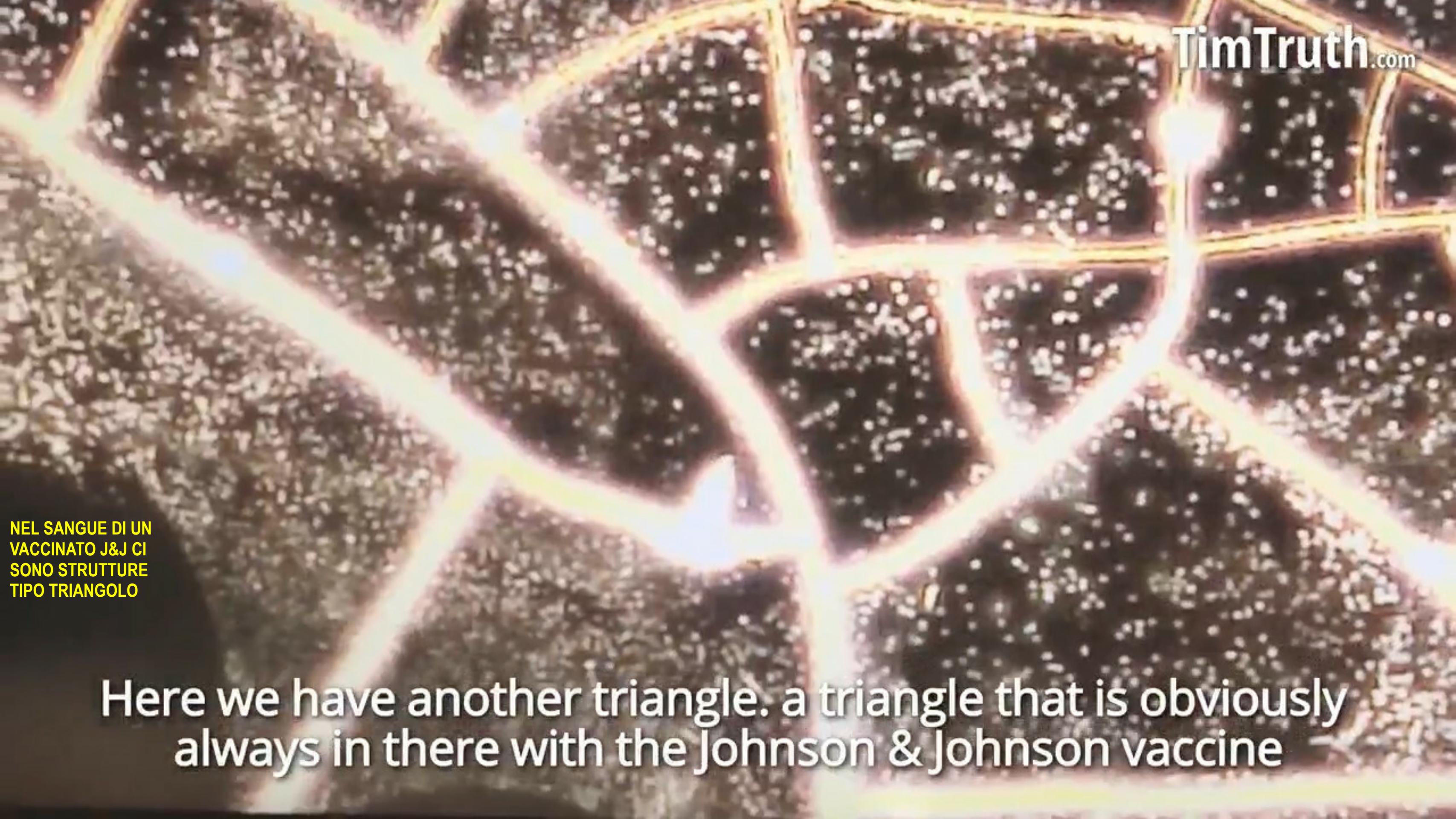
So now look at this, I was afraid for the patient because that was after oxygen and after drinking water nothing has changed in the picture

I GLOBULI ROSSI CONTINUANO A RESTARE IN ROULEAUX (PILA DI MONETE) E LA REIDRATAZIONE NON RIESCE A FARLI TORNARE NORMALI (SEPARATI TRA LORO)

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I said please come here, we'll take a look and these are the blood pictures. She was there yesterday and no water helped either



NEL SANGUE DI UN  
VACCINATO J&J CI  
SONO STRUTTURE  
TIPO TRIANGOLO

Here we have another triangle. a triangle that is obviously always in there with the Johnson & Johnson vaccine

> ACS Appl Mater Interfaces. 2014 Nov 26;6(22):19797-807. doi: 10.1021/am505084s.  
Epub 2014 Nov 12.

# In vitro hemocompatibility and toxic mechanism of graphene oxide on human peripheral blood T lymphocytes and serum albumin

Zhijia Ding <sup>1</sup>, Zhijun Zhang, Hongwei Ma, Yanyan Chen

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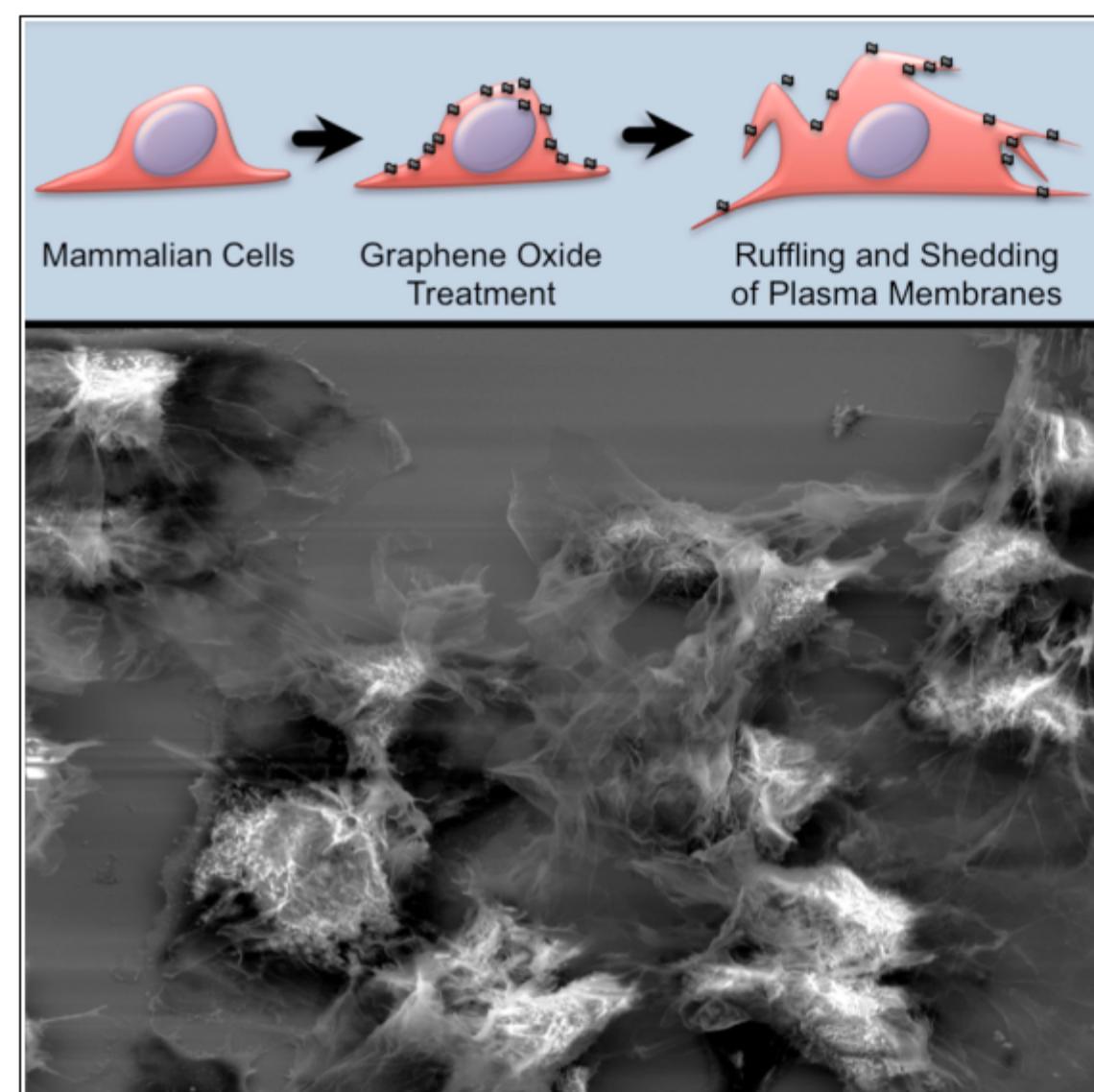
PMID: 25371999 DOI: [10.1021/am505084s](https://doi.org/10.1021/am505084s)

## Abstract

Graphene oxide (GO) has shown tremendous application potential as a biomedical material. However, its interactions with blood components are not yet well understood. In this work, we assess the toxicity of pristine GO (p-GO) and functionalized GO (GO-COOH and GO-PEI) to primary human peripheral blood T lymphocytes and human serum albumin (HSA), and also study the underlying toxic mechanism. Our results indicate that p-GO and GO-COOH have good biocompatibility to T lymphocytes at the concentration below 25  $\mu\text{g mL}^{-1}$ , but notable cytotoxicity above 50  $\mu\text{g mL}^{-1}$ . By contrast, GO-PEI exhibits significant toxicity even at 1.6  $\mu\text{g mL}^{-1}$ . Further investigations show that although p-GO does not enter into the cell or damage the membrane, its presence leads to the increase in reactive oxygen species (ROS), moderate DNA damage, and T lymphocyte apoptosis. Interestingly, little effect on T lymphocyte immune response suppression is observed in this process despite p-GO inflicting cell apoptosis. The toxic mechanism is that p-GO interacts directly with the protein receptors to inhibit their ligand-binding ability, leading to ROS-dependent passive apoptosis through the B-cell lymphoma-2 (Bcl-2) pathway. Compared with p-GO, GO-COOH exhibits a similar toxic effect on T lymphocytes except keeping a normal ROS level. A proposed toxic mechanism is that GO-COOH inhibits protein receptor-ligand binding, and passes the passive apoptosis signal to nucleus DNA through a ROS-independent mechanism. On the other hand, GO-PEI shows severe hematotoxicity to T lymphocytes by inducing membrane damage. For plasma protein HSA, the binding of GO-COOH results in minimal conformational change and HSA's binding capacity to bilirubin remains unaffected, while the binding of p-GO and GO-PEI exhibits strong toxicity on HSA. These findings on the interactions of two-dimensional nanomaterials and biological systems, along with the enquiry of the mechanisms, would provide essential support for further safety evaluation of the biomedical applications of GO.

## Article

# Graphene Oxide Nanosheets Stimulate Ruffling and Shedding of Mammalian Cell Plasma Membranes



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Yimo Han, David A. Muller,  
David A. Holowka, Barbara A.  
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## HIGHLIGHTS

Graphene oxide (GO) induces ruffling and shedding of mammalian cell plasma membranes

GO-induced plasma membrane responses induce loss of contact inhibition in RBL cells

GO-treated plasma membranes undergo nuanced structural and functional changes

Dichtel and colleagues show that graphene oxide (GO) induces significant ruffling and shedding of mammalian cell plasma membranes, in addition to a loss of contact inhibition by rat basophilic leukemia cells. These findings demonstrate profound non-lethal effects of GO and challenge assumptions, on the basis of its low toxicity, that GO has only benign effects on cells.

Original Article

# Impact of graphene oxide on the structure and function of important multiple blood components by a dose-dependent pattern

Ru Feng, Yueping Yu, Chaoxuan Shen, Yanpeng Jiao , Changren Zhou

First published: 25 September 2014 | <https://doi.org/10.1002/jbm.a.35341> | Citations: 24

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## Abstract

Graphene and its derivatives have become great concern in biomedical fields. Though many investigations about their toxicity have been reported, systematic investigation on the interaction with multiple blood components is lacking. In this work, we studied the effects of the graphene oxide (GO) on the structure and function of the blood components, especially, on morphology and hemolysis of red blood cells (RBCs), bovine serum albumin (BSA) and fibrinogen conformation, complement activation, and blood coagulation function. Scanning electron microscopy observation and hemolysis test results showed that the GO can affect RBC morphology and membrane integrity in a concentration-dependent way. Fluorescence and circular dichroism spectra showed that GO could alter the secondary structures and conformation of BSA and fibrinogen. In addition, the presence of GO could also trigger complement activation by detecting their key biomarker molecules in plasma. In the blood clotting process, the GO showed significant adverse effect on the activated partial thromboplastin time but not on prothrombin time of the platelet-poor plasma. Meanwhile, the GO also caused abnormal thromboelastography parameters of the whole blood coagulation. The results obtained in this study provides good insight into understanding the biomedical application of GO *in vivo*. © 2014 Wiley Periodicals, Inc. *J Biomed Mater Res Part A*: 103A: 2006–2014, 2015.

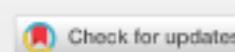
Original Article

# Low levels of graphene and graphene oxide inhibit cellular xenobiotic defense system mediated by efflux transporters

Su Liu, Wei Jiang, Bing Wu , Jing Yu, Haiyan Yu, Xu-Xiang Zhang, Cristina Torres-Duarte &amp; Gary N. Cherr

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Pages 597-606 | Received 18 Mar 2015, Accepted 04 Oct 2015, Published online: 10 Nov 2015

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## Abstract

Low levels of graphene and graphene oxide (GO) are considered to be environmentally safe. In this study, we analyzed the potential effects of graphene and GO at relatively low concentrations on cellular xenobiotic defense system mediated by efflux transporters. The results showed that graphene ( $<0.5\text{ }\mu\text{g/mL}$ ) and GO ( $<20\text{ }\mu\text{g/mL}$ ) did not decrease cell viability, generate reactive oxygen species, or disrupt mitochondrial function. However, graphene and GO at the nontoxic concentrations could increase calcein-AM (CAM, an indicator of membrane ATP-binding cassette (ABC) transporter) activity accumulation, indicating inhibition of ABC transporters' efflux capabilities. This inhibition was observed even at  $0.005\text{ }\mu\text{g/mL}$  graphene and  $0.05\text{ }\mu\text{g/mL}$  GO, which are 100 times and 400 times lower than their lowest toxic concentration from cytotoxicity experiments, respectively. The inhibition of ABC transporters significantly increased the toxicity of paraquat and arsenic, known substrates of ABC transporters. The inhibition of ABC transporters was found to be based on graphene and GO damaging the plasma membrane structure and fluidity, thus altering functions of transmembrane ABC transporters. This study demonstrates that low levels of graphene and GO are not environmentally safe since they can significantly make cell more susceptible to other xenobiotics, and this chemosensitizing activity should be considered in the risk assessment of graphene and GO.

> Biomaterials. 2016 Dec;109:12-22. doi: 10.1016/j.biomaterials.2016.09.005.  
Epub 2016 Sep 13.

# Ultrastrong trapping of VEGF by graphene oxide: Anti-angiogenesis application

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Irving Po-Jung Lai <sup>1</sup>, Ju-Yi Mao <sup>1</sup>, Pang-Hung Hsu <sup>1</sup>, Han-Jia Lin <sup>1</sup>, Wen-Shyong Tzou <sup>1</sup>,  
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Affiliations + expand

PMID: 27639528 DOI: [10.1016/j.biomaterials.2016.09.005](https://doi.org/10.1016/j.biomaterials.2016.09.005)

## Abstract

Angiogenesis is the process of formation of new blood vessels, which is essential to human biology, and also plays a crucial role in several pathologies such as tumor growth and metastasis, exudative age-related macular degeneration, and ischemia. Vascular endothelial growth factor (VEGF), in particular, VEGF-A<sub>165</sub> is the most important pro-angiogenic factor for angiogenesis. Thus, blocking the interaction between VEGFs and their receptors is considered an effective anti-angiogenic strategy. We demonstrate for that first time that bovine serum albumin-capped graphene oxide (BSA-GO) exhibits high stability in physiological saline solution and possesses ultrastrong binding affinity towards VEGF-A<sub>165</sub> [dissociation constant ( $K_d$ )  $\sim 3 \times 10^{-12}$  M], which is at least five orders of magnitude stronger than that of high-abundant plasma proteins such as human serum albumin, fibrinogen, transferrin, and immunoglobulin G. Due to the surprising binding specificity of BSA-GO for VEGF-A<sub>165</sub> in complex plasma fluid, we have also studied the anti-angiogenic effects in vitro and in vivo. Results show that BSA-GO not only effectively inhibits the proliferation, migration and tube formation of human umbilical vein endothelial cells, but also strongly disturbs the physiological process of angiogenesis in chick chorioallantoic membrane and blocks VEGF-A<sub>165</sub>-induced blood vessel formation in rabbit corneal neovascularization. Our findings indicate that GO nanomaterials can potentially act as therapeutic anti-angiogenic agents via ultrastrong VEGF adsorption and its activity suppression.

**Keywords:** Anti-angiogenesis; Blood vessel; Corneal neovascularization; Graphene oxide; Serum albumin; Vascular endothelial growth factor.

> Sci Rep. 2017 Jan 12;7:40572. doi: 10.1038/srep40572.

# Differential cytotoxic effects of graphene and graphene oxide on skin keratinocytes

Marco Pelin <sup>1 2</sup>, Laura Fusco <sup>2</sup>, Verónica León <sup>3</sup>, Cristina Martín <sup>3</sup>, Alejandro Criado <sup>2 4</sup>, Silvio Sosa <sup>1</sup>, Ester Vázquez <sup>3</sup>, Aurelia Tubaro <sup>1</sup>, Maurizio Prato <sup>2 4 5</sup>

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PMID: 28079192 PMCID: PMC5227695 DOI: 10.1038/srep40572

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## Abstract

Impressive properties make graphene-based materials (GBMs) promising tools for nanoelectronics and biomedicine. However, safety concerns need to be cleared before mass production of GBMs starts. As skin, together with lungs, displays the highest exposure to GBMs, it is of fundamental importance to understand what happens when GBMs get in contact with skin cells. The present study was carried out on HaCaT keratinocytes, an in vitro model of skin toxicity, on which the effects of four GBMs were evaluated: a few layer graphene, prepared by ball-milling treatment (FLG), and three samples of graphene oxide (GOs, a research-grade GO1, and two commercial GOs, GO2 and GO3). Even though no significant effects were observed after 24 h, after 72 h the less oxidized compound (FLG) was the less cytotoxic, inducing mitochondrial and plasma-membrane damages with EC<sub>50</sub>s of 62.8 µg/mL (WST-8 assay) and 45.5 µg/mL (propidium iodide uptake), respectively. By contrast, the largest and most oxidized compound, GO3, was the most cytotoxic, inducing mitochondrial and plasma-membrane damages with EC<sub>50</sub>s of 5.4 and 2.9 µg/mL, respectively. These results suggest that only high concentrations and long exposure times to FLG and GOs could impair mitochondrial activity associated with plasma membrane damage, suggesting low cytotoxic effects at the skin level.

# Graphene oxide significantly inhibits cell growth at sublethal concentrations by causing extracellular iron deficiency

Qilin Yu, Bing Zhang, Jianrong Li, Tingting Du, Xiao Yi, Mingchun Li  Wei Chen  & Pedro J. J. Alvarez ... show less

Pages 1102-1114 | Received 20 May 2017, Accepted 14 Oct 2017, Published online: 09 Nov 2017

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## Abstract

Graphene oxide (GO)-based materials are increasingly being used in medical materials and consumer products. However, their sublethal effects on biological systems are poorly understood. Here, we report that GO (at 10 to 160 mg/L) induced significant inhibitory effects on the growth of different unicellular organisms, including eukaryotes (i.e. *Saccharomyces cerevisiae*, *Candida albicans*, and *Komagataella pastoris*) and prokaryotes (*Pseudomonas fluorescens*). Growth inhibition could not be explained by commonly reported cytotoxicity mechanisms such as plasma membrane damage or oxidative stress. Based on transcriptomic analysis and measurement of extra- and intracellular iron concentrations, we show that the inhibitory effect of GO was mainly attributable to iron deficiency caused by binding to the O-functional groups of GO, which sequestered iron and disrupted iron-related physiological and metabolic processes. This inhibitory mechanism was corroborated with supplementary experiments, where adding bathophenanthroline disulfonate—an iron chelating agent—to the culture medium exerted similar inhibition, whereas removing surface O-functional groups of GO decreased iron sequestration and significantly alleviated the inhibitory effect. These findings highlight a potential indirect detrimental effect of nanomaterials (i.e. scavenging of critical nutrients), and encourage research on potential biomedical applications of GO-based materials to sequester iron and enhance treatment of iron-dependent diseases such as cancer and some pathogenic infections.

Related

> ACS Nano. 2018 Feb 27;12(2):1373-1389. doi: 10.1021/acsnano.7b07734.

Epub 2018 Jan 31.

# Live Imaging of Label-Free Graphene Oxide Reveals Critical Factors Causing Oxidative-Stress-Mediated Cellular Responses

Sandra Vranić <sup>1 2</sup>, Artur Filipe Rodrigues <sup>1 2</sup>, Maurizio Buggio <sup>1 2</sup>, Leon Newman <sup>1 2</sup>, Michael R H White <sup>3</sup>, David G Spiller <sup>4</sup>, Cyrill Bussy <sup>1 2</sup>, Kostas Kostarelos <sup>1 2</sup>

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PMID: 29286639 DOI: [10.1021/acsnano.7b07734](https://doi.org/10.1021/acsnano.7b07734)

## Abstract

The interest in graphene and its translation into commercial products has been expanding at a high pace. Based on previously described pulmonary safety concerns for carbon nanomaterials, there is a great need to define parameters guiding interactions between graphene-based materials and the pulmonary system. The aim of the present study was to determine the importance of two critical parameters: lateral dimensions of the material and coating with proteins in relation to each other and their pulmonary impact. Endotoxin-free materials with distinct lateral dimensions, s-GO (50-200 nm) and I-GO (5-15 μm), were produced and thoroughly characterized. Exploiting intrinsic fluorescence of graphene oxide (GO) and using confocal live-cell imaging, the behavior of the cells in response to the material was visualized in real time. Although BEAS-2B cells internalized GO efficiently, I-GO was linked to higher plasma membrane interactions correlated with elevated reactive oxygen species (ROS) levels, pro-inflammatory response, and greater cytotoxicity, in agreement with the oxidative stress paradigm. For both GO types, the presence of serum alleviated lipid peroxidation of plasma membrane and decreased intracellular ROS levels. However, protein coating was not enough to entirely mitigate toxicity and inflammatory response induced by I-GO. In vitro results were validated in vivo, as I-GO was more prone to induce pulmonary granulomatous response in mice compared to s-GO. In conclusion, the lateral dimension of GO played a more important role than serum protein coating in determining biological responses to the material. It was also demonstrated that time-lapse imaging of live cells interacting with label-free GO sheets can be used as a tool to assess GO-induced cytotoxicity.

**Keywords:** 2D materials; confocal live-cell imaging; graphene; inflammation; lungs; nanotoxicology.

> J Hazard Mater. 2021 Jul 15;414:125472. doi: 10.1016/j.jhazmat.2021.125472.  
Epub 2021 Feb 20.

# 2D graphene oxide particles induce unwanted loss in pluripotency and trigger early differentiation in human pluripotent stem cells

Jiwoong Heo <sup>1</sup>, Jaewon Choi <sup>2</sup>, Jin Young Kim <sup>3</sup>, Hyejoong Jeong <sup>1</sup>, Daheui Choi <sup>1</sup>,  
Uiyoung Han <sup>1</sup>, Ju Hyun Park <sup>4</sup>, Hee Ho Park <sup>5</sup>, Jinkee Hong <sup>6</sup>

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## Abstract

The potential health hazards of particulates, such as micro/nano-sized plastics and carbon materials have recently received extensive attention. However, their toxicological properties in association with stem cell differentiation is still relatively unexplored. In this study, we elucidated the cytotoxic effects of 2D graphene oxide (GO), in relation to differentiation of human induced pluripotent stem cells (hiPSCs). Supplementation of GO to hiPSCs demonstrated uptake of GO through the plasma membrane and intracellular accumulation was observed. Increasing the concentration of GO led to reduced viability and increased likelihood of hiPSC colony detachment. Moreover, treatment of GO resulted in significant loss in pluripotency markers, OCT-4 and NANOG. In particular, when hiPSCs were cultured with GO in cardiomyocyte induction medium, upregulation of cardiomyocyte marker, NKX2.5, along with observation of early triggering of differentiation were observed. Taken together, our results highlight the risk in the uptake and accumulation of GO on the stem cell development by unwanted loss in pluripotency and accelerated initiation of differentiation.

**Keywords:** 2D graphene oxide; Cytotoxicity; Early triggering of differentiation; Pluripotency; Unwanted differentiation; hiPSC.

# Crucial Role of Lateral Size for Graphene Oxide in Activating Macrophages and Stimulating Pro-inflammatory Responses in Cells and Animals

Juan Ma<sup>†</sup>, Rui Liu<sup>†</sup>, Xiang Wang<sup>‡</sup>, Qian Liu<sup>†</sup>, Yunan Chen<sup>†</sup>, Russell P. Valle<sup>§</sup>, Yi Y. Zuo<sup>§</sup>, Tian Xia<sup>\*‡</sup>, and Sijin Liu<sup>\*†</sup>

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Publication Date: September 21, 2015 ▾

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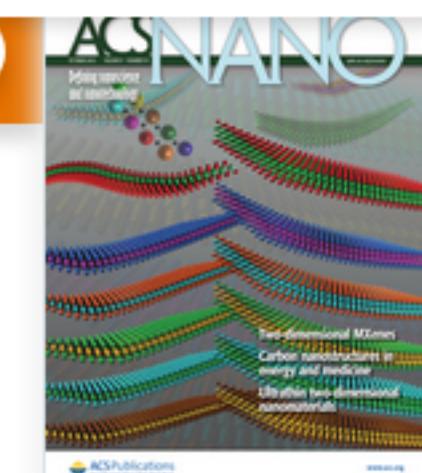
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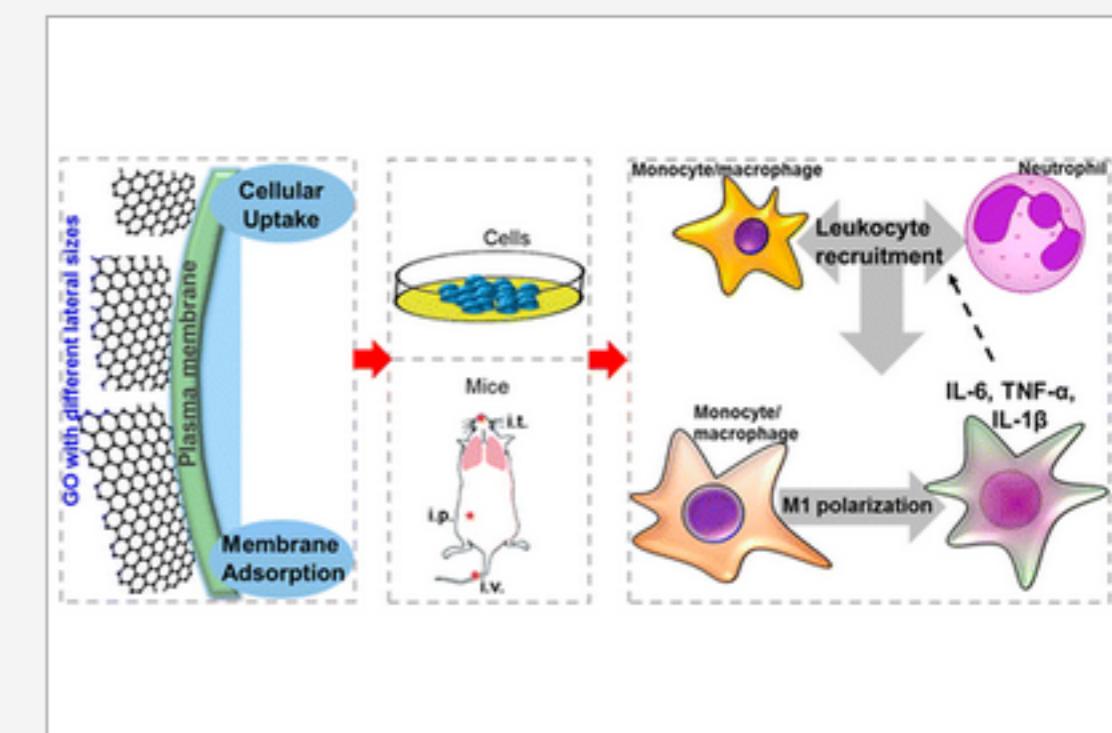
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Supporting Info (1) »

**SUBJECTS:** Immunology, Peptides and proteins, ▾

## Abstract

Graphene oxide (GO) is increasingly used in biomedical applications because it possesses not only the unique properties of graphene including large surface area and flexibility but also hydrophilicity and dispersibility in aqueous solutions. However, there are conflicting results on its biocompatibility and biosafety partially due to large variations in physicochemical properties of GO, and the role of these properties including lateral size in the biological or toxicological effects of GO is still unclear. In this study, we focused on the role of lateral size by preparing a panel of GO samples with differential lateral sizes using the same starting material. We found that, in comparison to its smaller counterpart, larger GO showed a stronger adsorption onto the plasma membrane with less phagocytosis, which elicited more robust interaction with toll-like receptors and more potent activation of NF-κB pathways. By contrast, smaller GO sheets were more likely taken up by cells. As a result, larger GO promoted greater M1 polarization, associated with enhanced production of inflammatory cytokines and recruitment of immune cells. The *in vitro* results correlated well with local and systemic inflammatory responses after GO administration into the abdominal cavity, lung, or bloodstream through the tail vein. Together, our study delineated the size-dependent M1 induction of macrophages and pro-inflammatory responses of GO *in vitro* and *in vivo*. Our data also unearthed the detailed mechanism underlying these effects: a size-dependent interaction between GO and the plasma membrane.



# Graphene Oxide Promotes Cancer Metastasis through Associating with Plasma Membrane To Promote TGF- $\beta$ Signaling-Dependent Epithelial–Mesenchymal Transition

Jianqiang Zhu, Bin Li, Ming Xu, Rui Liu, Tian Xia, Zhihong Zhang\*, Yong Xu, and Sijin Liu\*

Cite this: ACS Nano 2020, 14, 1, 818–827

Publication Date: December 26, 2019

<https://doi.org/10.1021/acsnano.9b07891>

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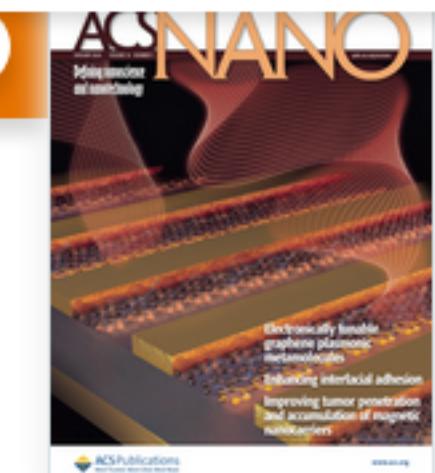
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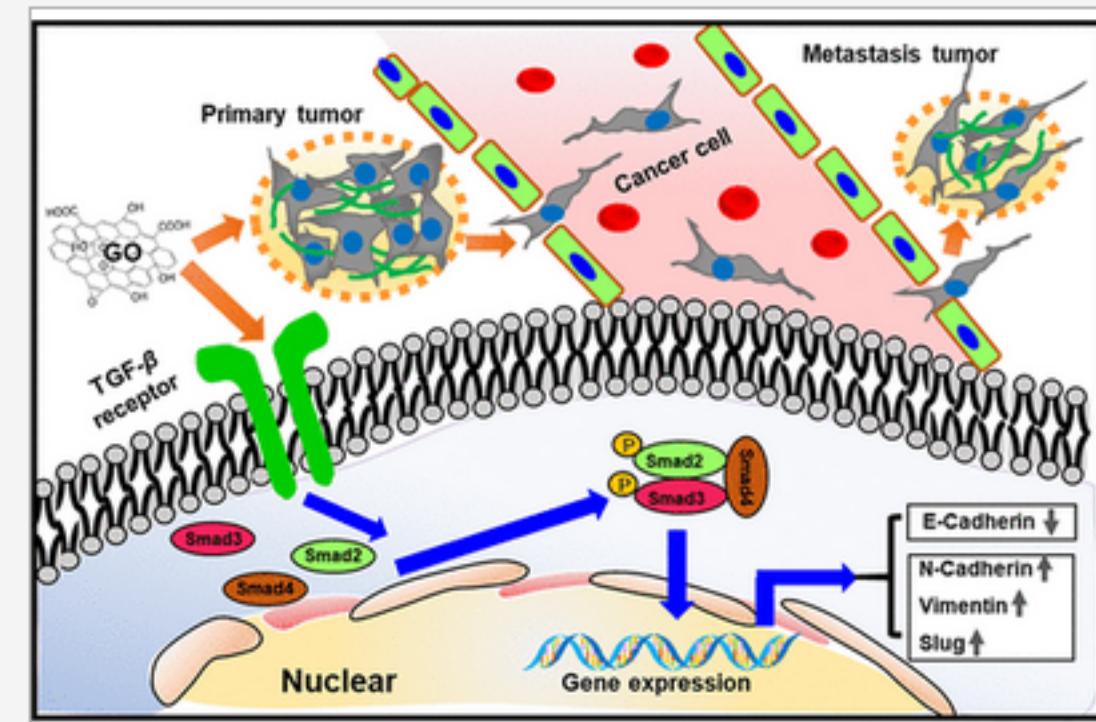
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SI Supporting Info (1) »

**SUBJECTS:** Cell signaling, Cancer, Plasma membrane, Two dimensional materials, Cells

## Abstract

Nanomedicines are being developed to treat diverse diseases; however, inadvertent or unintended health effects have to be considered, especially for those targeting cancers. For cancers, occurrence of metastasis hints an advanced phase of cancer progression, and nanomedicines *per se* should be evaluated for their effects on existing metastatic tumors and triggering metastases. Graphene-based 2D nanomaterials, such as graphene oxide (GO), due to its unique characteristics, have been extensively studied for biomedical applications including cancer therapy. However, the potential effect of GO on metastasis has not been determined yet. Herein, we found that low-dose GO could induce significant morphological and structural changes of the cellular membrane within cancer cells, suggesting an epithelial-mesenchymal transition (EMT), with enhanced invasion/migration and the alterations of representative EMT indicators in GO-treated cells. These changes resulted in enhanced lung metastasis of cancer cells in various metastasis models. The mechanistic investigations unveiled that GO increased the protein levels of the TGF- $\beta$  receptor, leading to a constitutively activated TGF- $\beta$ -Smad2/3 signaling pathway that drives the EMT. Collectively, our findings enhance the understanding of the unintended side and detrimental effects of GO nanosheets in increasing the progression of metastatic tumors. Thus, the likelihood of pro-EMT effects upon low-dose GO exposure should be considered when developing GO nanomedicines.



ARTICLE



<https://doi.org/10.1038/s41467-020-20546-w>

OPEN

# Graphene active sensor arrays for long-term and wireless mapping of wide frequency band epicortical brain activity

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Graphene active sensors have demonstrated promising capabilities for the detection of electrophysiological signals in the brain. Their functional properties, together with their flexibility as well as their expected stability and biocompatibility have raised them as a promising building block for large-scale sensing neural interfaces. However, in order to provide reliable tools for neuroscience and biomedical engineering applications, the maturity of this technology must be thoroughly studied. Here, we evaluate the performance of 64-channel graphene sensor arrays in terms of homogeneity, sensitivity and stability using a wireless, quasi-commercial headstage and demonstrate the biocompatibility of epicortical graphene chronic implants. Furthermore, to illustrate the potential of the technology to detect cortical signals from infra-slow to high-gamma frequency bands, we perform proof-of-concept long-term wireless recording in a freely behaving rodent. Our work demonstrates the maturity of the graphene-based technology, which represents a promising candidate for chronic, wide frequency band neural sensing interfaces.

<https://www.businesswire.com/news/home/20210330005388/en/INBRAIN-Neuroelectronics-Secures-17-Million-in-Series-A-Funding-for-First-AI-Powered-Graphene-Brain-Interface>

# INBRAIN Neuroelectronics Secures \$17 Million in Series A Funding for First AI-Powered Graphene-Brain Interface

*Funding enables company to advance first-in-human studies for its flagship product, a less-invasive neuromodulation device for treating neurological conditions using artificial intelligence and graphene electrodes*

March 30, 2021 07:00 AM Eastern Daylight Time

BARCELONA, Spain--(BUSINESS WIRE)--INBRAIN Neuroelectronics S.L., a company in the intersection between Medtech, Deeptech and Digital Health dedicated to developing the world's first intelligent graphene-brain interface, today announced \$16.8 million in Series A funding for its disruptive system for treating epilepsy and Parkinson's disease.

"Led by an extraordinary team of

The investment, co-led by Asabys Partners and Alta Life Sciences, and joined by V squared Ventures and TruVenture GmbH, includes the



INBRAIN NEUROELECTRONICS  
S.L.

## Release Summary

INBRAIN Neuroelectronics has closed \$17 million in Series A financing to support first-in-

# Polymer-Plastics Technology and Engineering



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## **Millimeter wave absorbing property of flexible graphene/acrylonitrile-butadiene rubber composite in 5G frequency band**

Yukun Chen, Xingfeng Fu, Lie Liu, Ying Zhang, Liming Cao, Daosheng Yuan & Pingan Liu

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# DETECCIÓN DE OXIDO DE GRAFENO EN SUSPENSIÓN ACUOSA (*COMIRNATY™* (RD1))

ESTUDIO OBSERVACIONAL EN MICROSCOPIA ÓPTICA Y ELECTRÓNICA

## Informe provisional (I) 28 de Junio de 2021



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ESCUELA SUPERIOR DE INGENIERIA

UNIVERSIDAD DE ALMERÍA, ESPAÑA

## **AVISO IMPORTANTE**

**Seguidamente se presenta un estudio microscópico, observacional y meramente descriptivo de la muestra problema.**

**La identificación definitiva del material dominante en la muestra precisa de ulteriores fraccionamientos y análisis espectroscópicos específicos que permitan caracterizar la estructura del material.**

# Antecedentes

- D. Ricardo Delgado Martín solicita PRESTACIÓN DE SERVICIOS de Investigación a la UAL denominada:

***“DETECCIÓN DE GRAFENO EN MUESTRA DE SUSPENSIÓN ACUOSA”***

- El 10/06/2021 se recibe por mensajería 1 vial, etiquetado con el texto siguiente:

- ***“COMIRNATY™ .Sterile concentrate. COVID-19 mRNA. 6 doses after dilution.***
- ***Discard date/time:PAA165994.LOT/EXP: EY3014 08/2021”***

- Procedencia y trazabilidad: se desconoce
- Estado de conservación: refrigerado
- Mantenimiento durante el estudio: refrigerado
- Codificación de la muestra problema a analizar: RD1

# Observaciones preliminares de la muestra problema RD1

Descripción:

- Vial sellado, con goma y tapa de aluminio intactas, de 2ml de capacidad, conteniendo una suspensión acuosa turbia de 0,45 ml.
- Se realiza extracción y cuantificación de RNA
- Se observa presencia de microbiología nanométrica no caracterizada, visible a 600X en microscopio óptico

## Procesamiento de la muestra

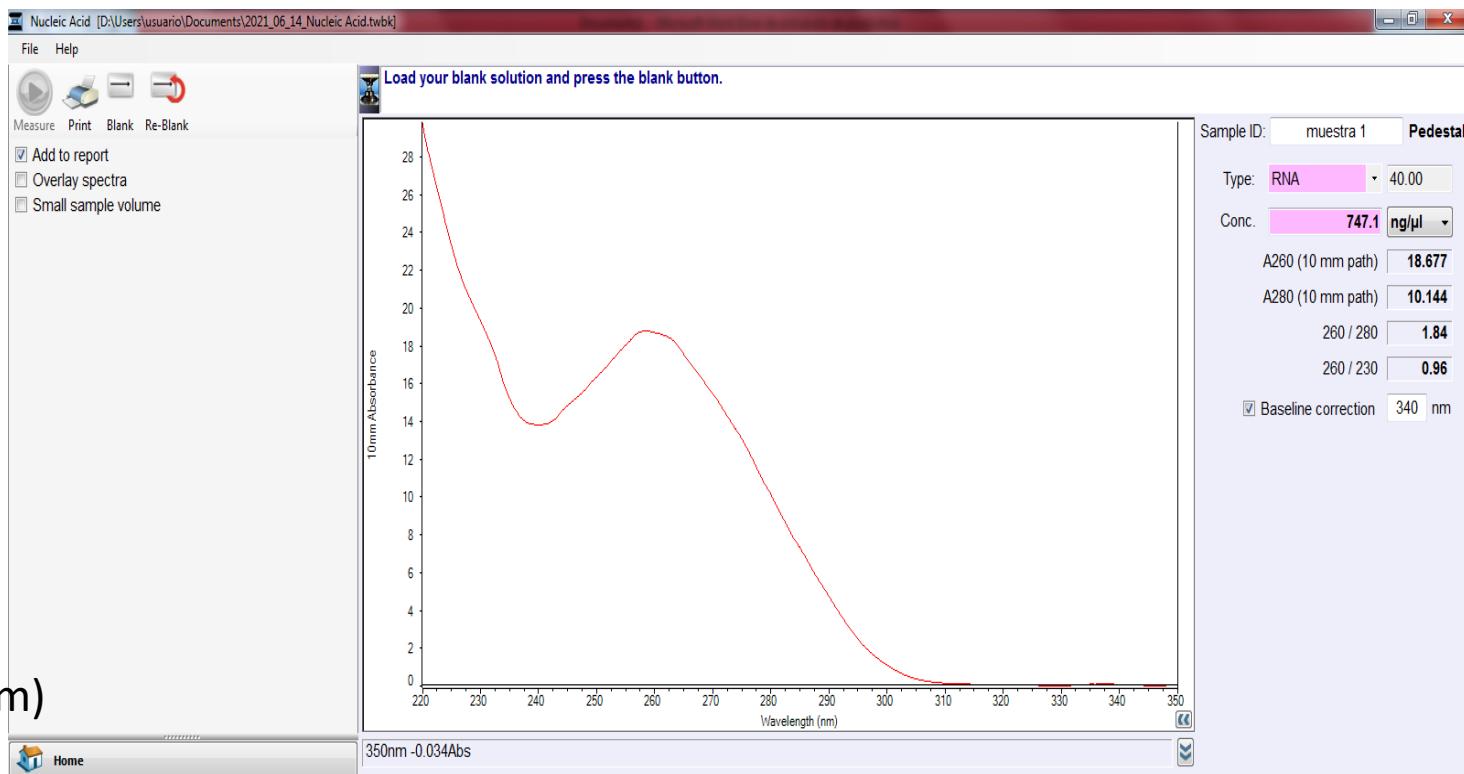
1. Dilución en suero fisiológico estéril al 0,9% (0,45 ml + 1,2 ml)
2. Fraccionamiento por polaridad: 1,2 ml hexano+120 ul de muestra RD1
3. Extracción de fase hidrofílica
4. Extracción y cuantificación de RNA en la muestra
5. Microscopía electrónica y ópticas de fase acuosa

# Análisis preliminar: extracción y cuantificación de Rna en la muestra

1. Extracción RNA: Kit <https://www.fishersci.es/shop/products/ambion-purelink-rna-mini-kit-7/10307963>
2. Cuantificación de absorbancia UV total en espectrofotómetro  
NanoDrop™ <https://www.thermofisher.com/order/catalog/product/ND-2000#/ND-2000>
3. Cuantificación específica de Rna por fluorescencia  
QUBIT2.0: <https://www.thermofisher.com/es/es/home/references/newsletters-and-journals/bioprobes-journal-of-cell-biology-applications/bioprobes-issues-2011/bioprobes-64-april-2011/the-qubit-2-0-fluorometer-april-2011.html>

# Espectro de absorción UV de la fase acuosa de la muestra RD1 (Equipo Nano-drop)

Máximo de absorción de la MUESTRA RD1 (260-270 nm)



- RNA. Presenta máximos habituales a 260 nm. Concentración total estimada por fluorometria QUBIT2.0 : **6 ng/ul**
- El espectro revela presencia de elevada cantidad de sustancias o sustancia diferentes al Rna con máximo de absorción en la misma región, con un total estimado en **747 ng/ul** (estimación no calibrada)
- El oxido de grafeno reducido (RGO) presenta máximos de absorción a 270 nm, **compatibles** con el espectro obtenido (*Thema et al, 2013. Journal of Chemistry ID 150536*)
- **El máximo de absorción obtenido NO PERMITE DESCARTAR la presencia de grafeno en la muestra. La cantidad mínima de RNA detectado por QUBIT2.0 sólo explica un porcentaje residual de la absorción total UV de la muestra.**

**OBJETIVO:** Identificación microscópica de derivados de grafeno

**METODOLOGIA:**

1. Toma de imágenes en microscopia óptica y electrónica
2. Comparativa con imágenes de literatura y patrón de oxido de grafeno reducido

# MICROSCOPIA ELECTRONICA DE TRANSMISIÓN (TEM)

Microscopio electrónico JEM-2100Plus

Tensión: 200 kV

Resolución 0,14 nm

Aumento hasta x1.200.000

# MICROSCOPIA ELECTRONICA DE TRANSMISIÓN (TEM)

La microscopía electrónica (TEM) se utiliza habitualmente para obtener imágenes de nanomateriales de grafeno. Se ha convertido en un instrumento bastante estándar y fácil de usar que es capaz de obtener imágenes de láminas de grafeno en capas individuales.

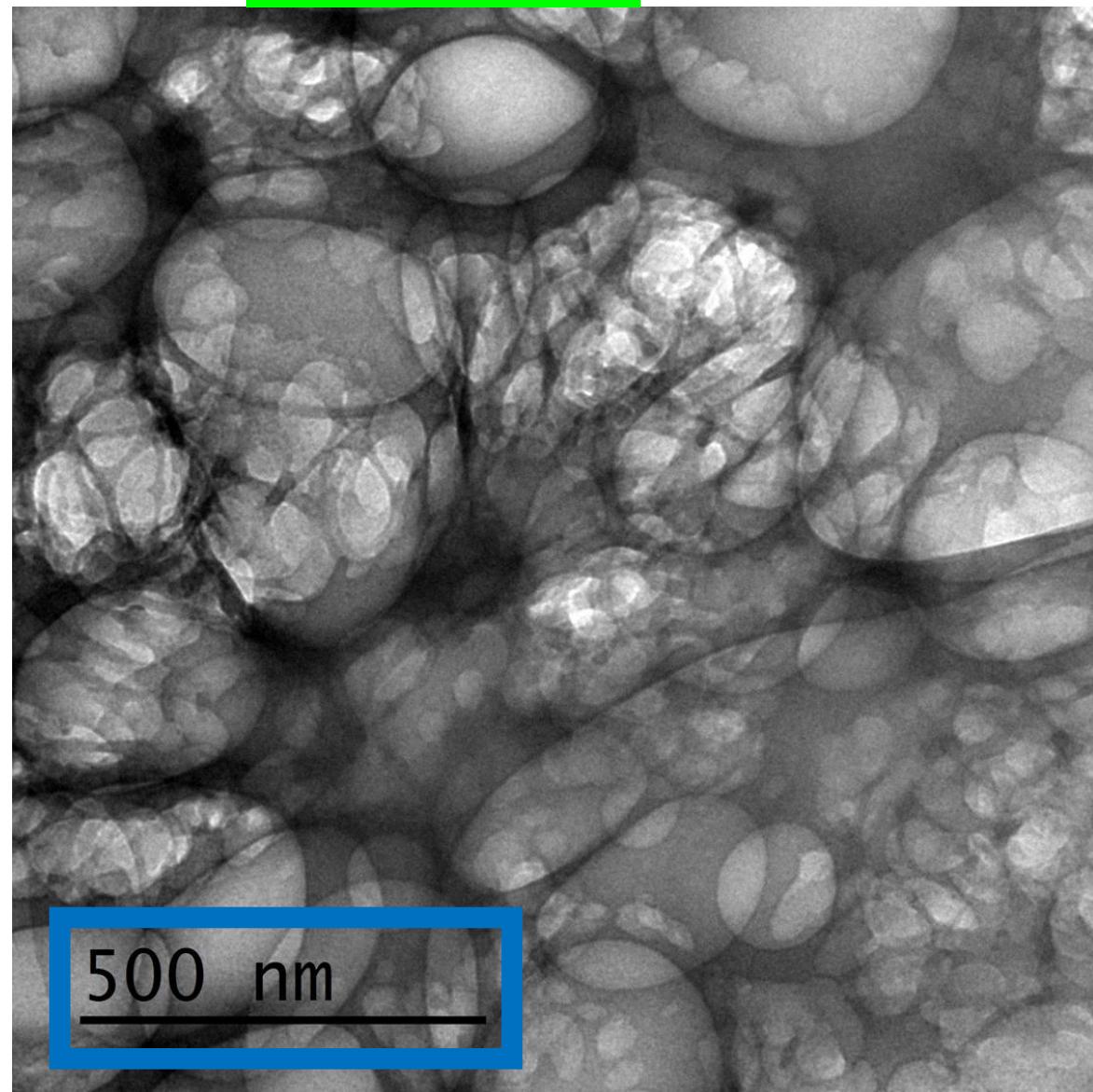
## DESCRIPCIÓN DE LA IMAGEN anterior

(de: Choucair et al, 2009. Gram-scale production of graphene based on solvothermal synthesis and sonication. *Nature Nanotechnology* 4(1):30-3

- Figura 2: “*Imágenes TEM de las láminas de grafeno aglomeradas. La misma región de muestra se ve con diferentes aumentos y muestra claramente el grado de formación de la lámina y la tendencia de las láminas a fusionarse en regiones superpuestas. Es evidente una estructura inherente en forma de lámina que muestra una intrincada matriz de pliegues de largo alcance. Como las imágenes se toman en modo de transmisión, la opacidad relativa de las láminas individuales es el resultado de regiones interfaciales con superposición entre láminas individuales. Las láminas se extienden en dimensiones laterales sobre escalas de longitud micrométrica, que van desde 100 nm hasta más de 1.000 nm.*

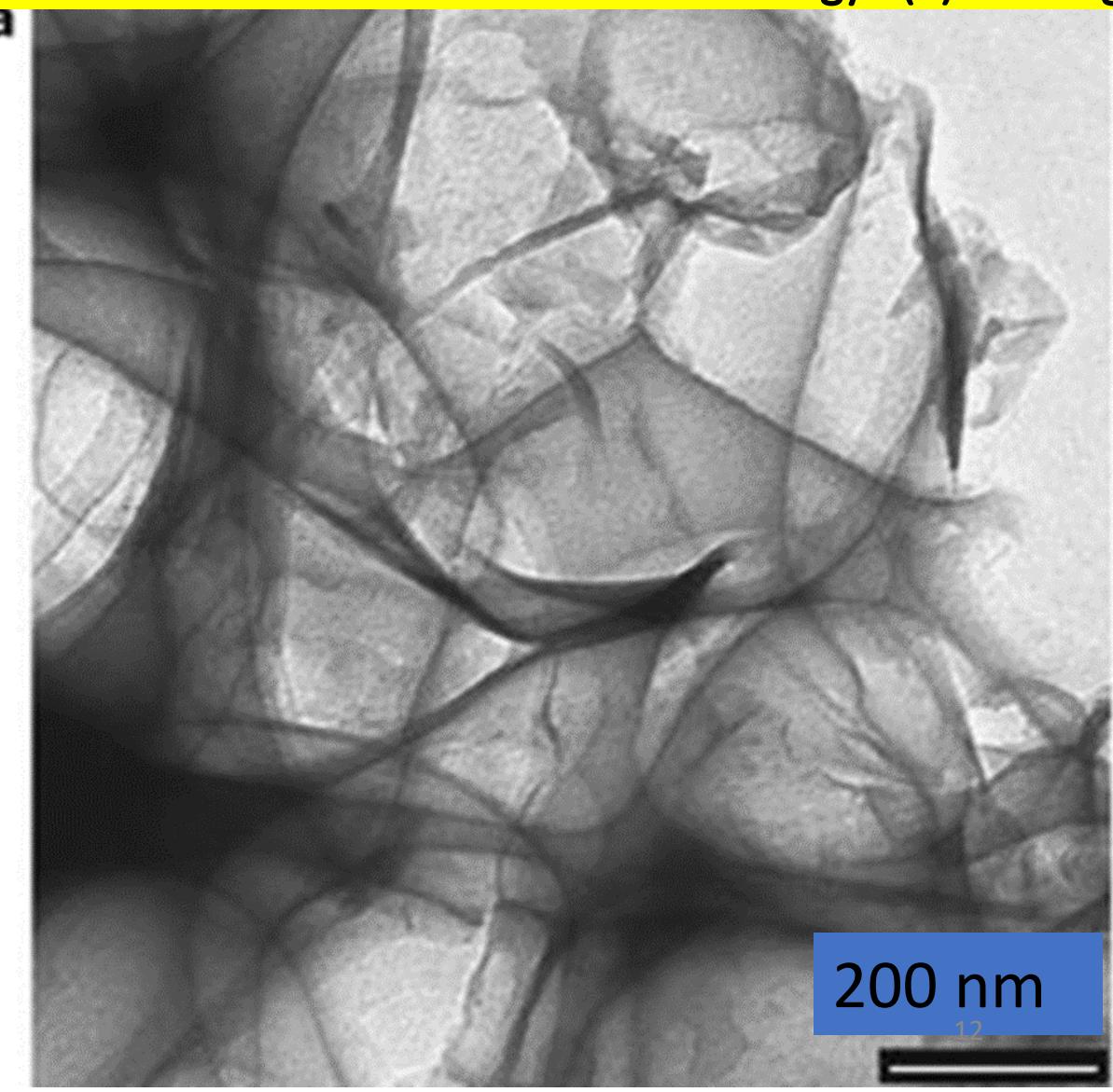
# RESULTADOS: Comparativa de muestra problema (RD1) con una imagen TEM de literatura

MUESTRA RD1



500 nm

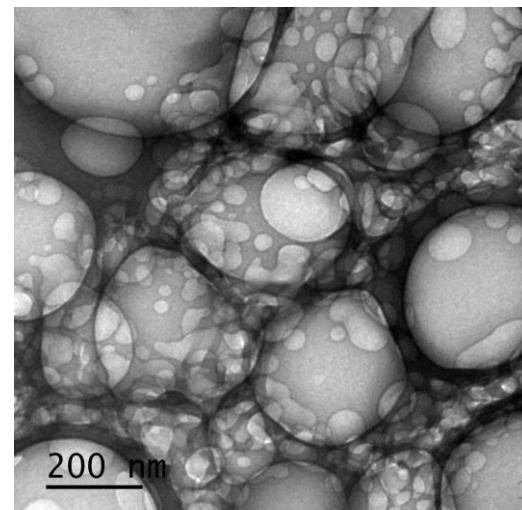
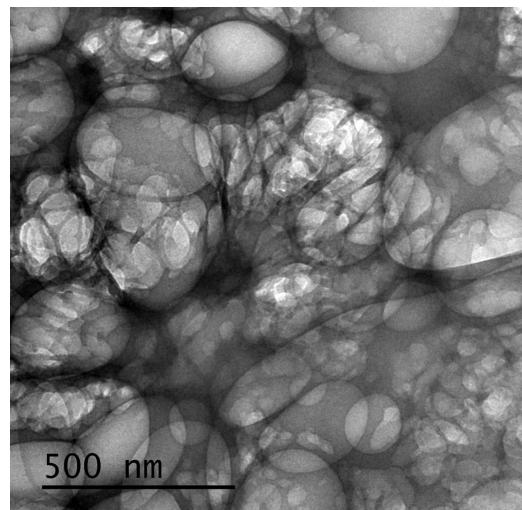
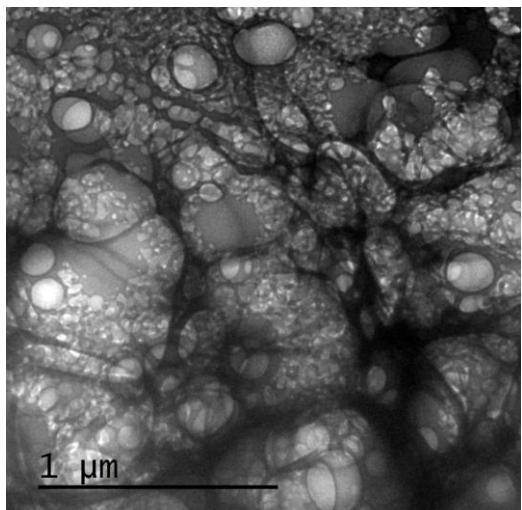
Choucair et al 2009. Nature Nanotechnology 4(1):30-3 Fig 2



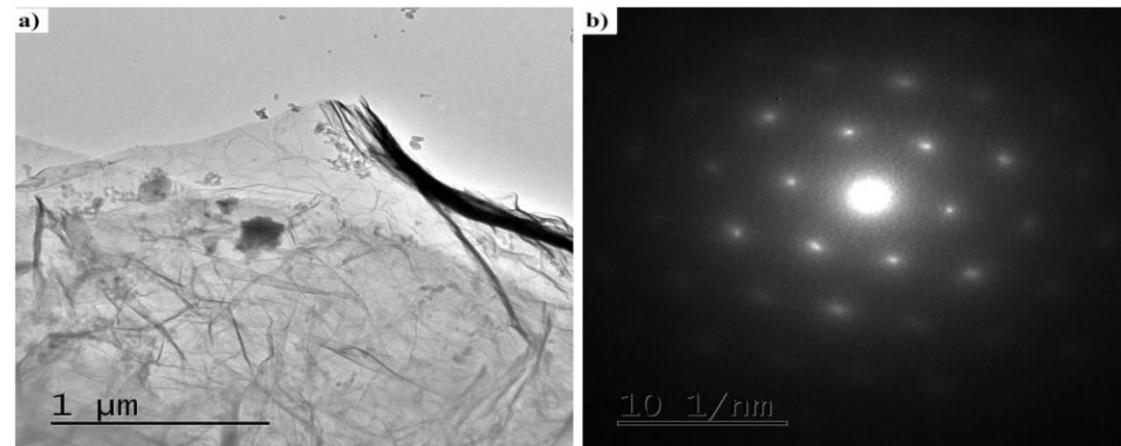
200 nm

## RESULTADOS: DESCRIPCIÓN DE LAS IMAGENES TEM DE LA MUESTRA PROBLEMA RD1

Las imágenes TEM de la muestra RD1 **en general PRESENTAN UNA ELEVADA SEMEJANZA** con imágenes de **oxido de grafeno** de literatura obtenidas por la misma técnica TEM, con aumentos parecidos. Se puede observar una intrincada **matriz o malla de láminas flexibles translúcidas plegadas sobre sí mismas**, con mezcla de aglomeraciones multicapa más oscuras y de monocapas no plegadas de color mas claro. Aparecen zonas lineares más oscuras debidas a la superposición local de láminas y a la disposición local de láminas individuales en paralelo al haz de electrones. Tras la malla aparece una elevada densidad de **formas claras redondeadas y elípticas sin identificar**, posiblemente correspondientes a orificios generados por forzamiento mecánico de la malla durante el tratamiento. Mostramos aquí 3 imágenes con aumento progresivo:



- **NOTA importante:** Para una IDENTIFICACIÓN definitiva del GRAFENO por TEM, es necesario complementar la observación con la caracterización estructural mediante obtención por EDS de un PATRÓN DE DIFRACCIÓN DE ELECTRONES característico (como la figura b mostrada abajo). El patrón correspondiente al grafito o el grafeno presenta una simetría hexagonal, y generalmente cuenta con varios hexágonos concéntricos. **No ha sido posible por el momento obtener este patrón por la escasez de muestra disponible para su procesamiento, y la disposición caótica y la densidad de los pliegues.**



# Microscopio Óptico

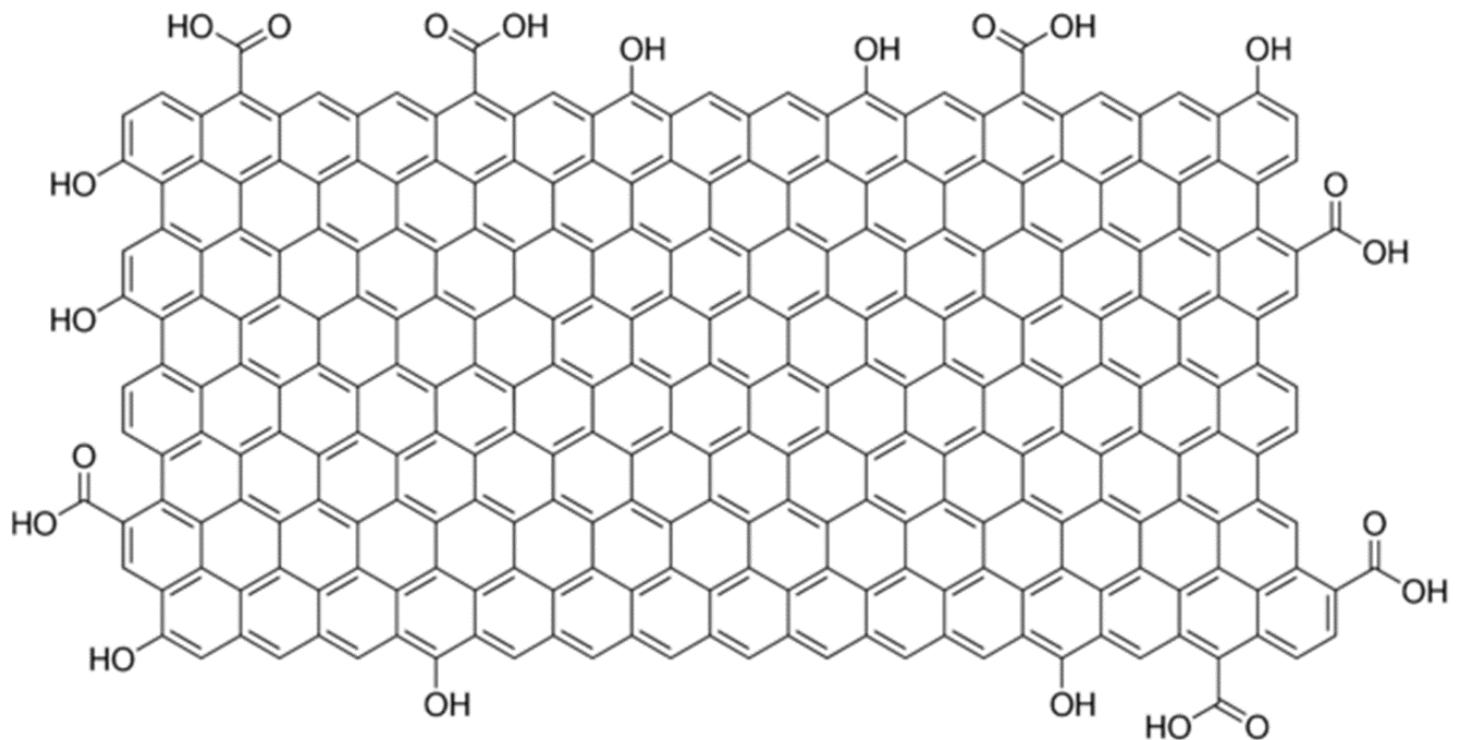
## Microscopio Biológico CX43

Objetivos PLAN Fluor de 10x, 20x (DIC) y 40x (DIC)

Ocular: 10x

- Condensador ajustado en posición intermedia con efecto 3D (entre campo Claro (BF) y campo oscuro (DF))

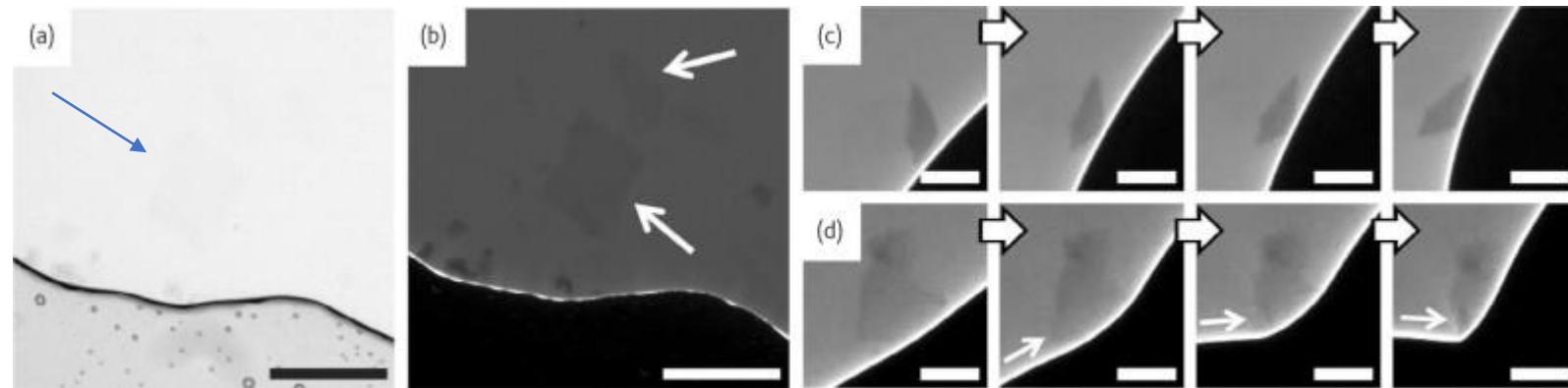
# PATRON DE OXIDO DE GRAFENO REDUCIDO



# IDENTIFICACIÓN DE OXIDO DE GRAFENO Y SUS CARACTERISTICAS ESTRUCTURALES POR MICROSCOPIA OPTICA

Los materiales de grafeno esencialmente constan de una sola capa atómica. Esto hace que la observación del microscopio óptico basado en absorbancia sea difícil, aunque es posible adquirir imágenes ópticas de láminas de grafeno suspendidas bajo luz transmitida de campo claro ( Fig. a ). El grafeno oxidado (GO) tiene un color mucho más pálido que le reducido (rGO).

Sin embargo, bajo **iluminación reflectante**, la obtención de **imágenes ópticas de alto contraste de grafeno** e incluso láminas de GO se ha reportado en literatura. Modificando el ángulo de incidencia de la iluminación, mediante ajuste apropiado del condensador (campo claro y campo oscuro), esta ha sido la técnica empleada para aumentar el contraste en muestra RD1 del presente informe y obtener imágenes de la rugosidad en la superficie de las láminas con efecto 3D.



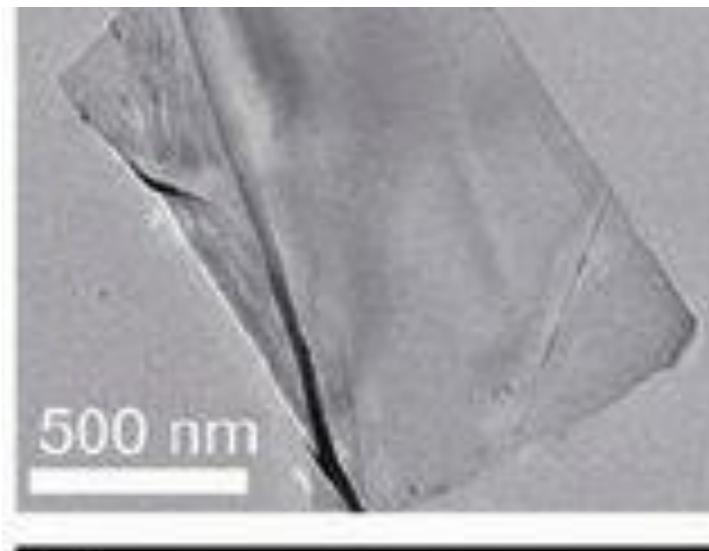
a) Campo claro. b-d) Microscopía de extinción de fluorescencia (FQM)

Kim et al, 2010. Seeing graphene-based sheets, Materials Today, Volume 13, 2010, Pages 28-38,

## Imagen de literatura TEM de bajo aumento

*“La figura muestra una imagen TEM de grafeno bicapa con bordes que tienden a enrollarse y doblarse ligeramente”*

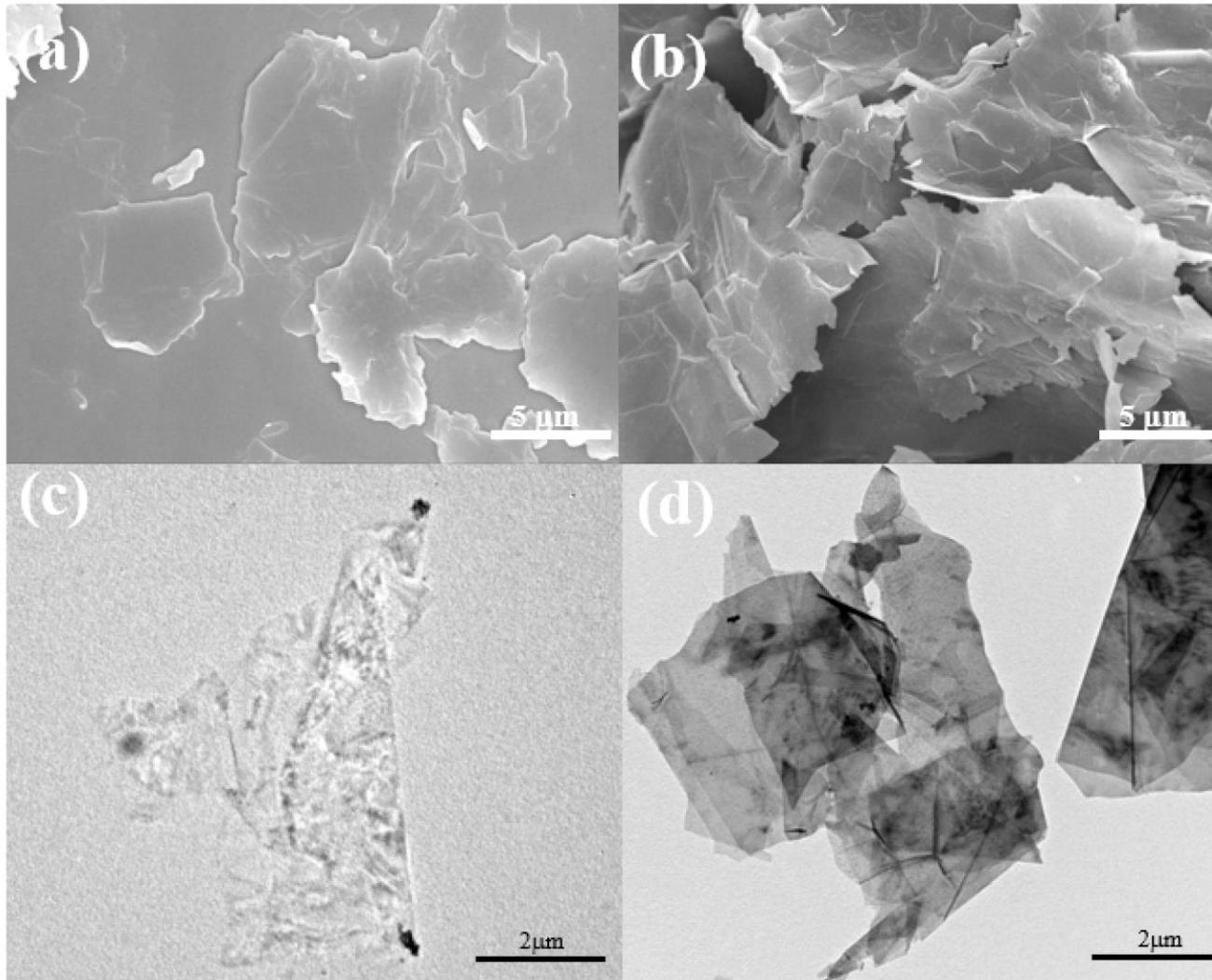
*Qian, W., Hao, R., Hou, Y. et al. Solvothermal-assisted exfoliation process to produce graphene with high yield and high quality. Nano Res. 2, 706–712 (2009).*



## IMAGENES DE LITERATURA. MICROSCOPIA ELECTRONICA A BAJO AUMENTO MICROSCOPIA ELECTRONICA DE BARRIDO (SEM) (a) y (b) y TRANSMISION (TEM) (c) y (d)

**Effects of Graphene Nanosheets with Different Lateral Sizes as Conductive Additives on the Electrochemical Performance of LiNi<sub>0.5</sub>Co<sub>0.2</sub>Mn<sub>0.3</sub>O<sub>2</sub> Cathode Materials for Li Ion Batteries.** Figure 2. SEM images of different graphene sheet sizes: (a) GN-13 and (b) GN-28, and transmission electron microscopy (TEM) images of different graphene sheet sizes: (c) GN-13 and (d) GN-28.

Husu et al. Polymers 2020, 12(5), 1162



# Robust Magnetized Graphene Oxide Platform for In Situ Peptide Synthesis and FRET-Based Protease Detection

Kim et al, Sensors 2020, 20(18), 5275

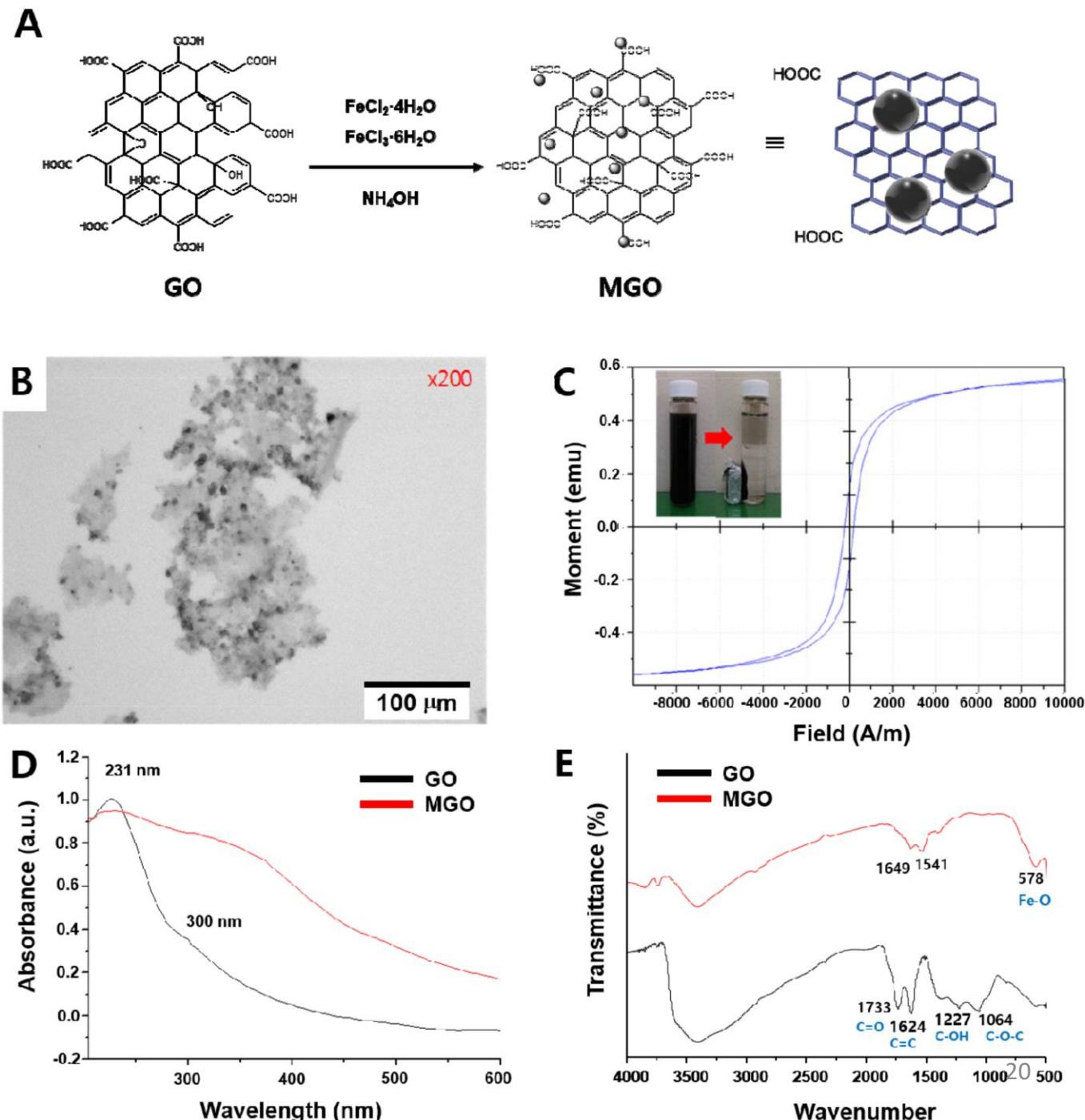
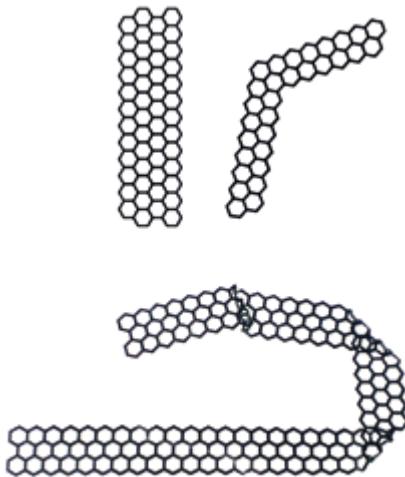


Figure 1. Preparation and characterization of graphene oxide (GO) and magnetic graphene oxide (MGO). (A) Schematic of MGO synthesis procedure. (B) **Optical microscopy image of MGO**. (C) Magnetic hysteresis loop of MGO. (D) UV/Vis absorption spectra of GO and MGO. (E) FT-IR spectra of GO and MGO.

# Comparativa de muestra RD1 al microscopio óptico con imágenes del patrón de OXIDO DE GRAFENO REDUCIDO (rGO)

Las imágenes ópticas de las láminas presentes en la muestra RD1 revelan gran similitud con las láminas exfoliadas a partir de sonicación del patrón rGO. Ambas muestras presentan láminas translúcidas interiormente rugosas, con perfiles irregulares, plegadas sobre sí mismas y con tendencia a enrollarse en los bordes. Las formas y dimensiones de las láminas son muy variables, presentando en ambas muestras láminas en cintas o bandas plegadas sobre sí mismas (*ribbons*).

**En el ANEXO adjunto se muestran imágenes alternas de MUESTRA PATRON DE rGO y MUESTRA PROBLEMA RD1**



# CONCLUSIONES Y RECOMENDACIONES

1. El estudio microscópico de la muestra aporta **sólidas evidencias de presencia probable de derivados de grafeno, si bien la microscopía no proporciona una prueba concluyente**. La identificación definitiva de grafeno, grafeno oxidado (GO) o grafeno oxidado reducido (rGO) en la muestra RD1 precisa de la **CARACTERIZACIÓN ESTRUCTURAL** mediante el análisis de patrones espectrales específicos comparables a los publicados en literatura y a los obtenidos a partir de muestra patrón, obtenidos con técnicas espectroscópicas como XPS, EDS, RMN, FTIR o Raman, entre otras.
2. Los análisis de este informe corresponden a **UNA SOLA MUESTRA, limitada en volumen total disponible para procesar**. Es por tanto necesario realizar un muestreo significativo de viales similares para extraer conclusiones generalizables a muestras comparables, registrando origen, trazabilidad y control de calidad durante la conservación y transporte previas a los análisis.

# Exención de responsabilidad



- Los resultados y conclusiones de este informe no implican posición institucional alguna de la Universidad de Almería
- Ni el Investigador Principal ni La Universidad de Almería asumen responsabilidad alguna de los contenidos y opiniones de terceros sobre el presente informe a partir de su posible difusión en redes sociales o medios de comunicación, ni de las conclusiones que puedan extraerse del mismo que no hayan sido explicitadas en el texto.

VER ANEXO FOTOGRAFIAS DE LA MUESTRA



# Resultado

La firma digital es correcta. El CSV es válido.

## Documentos



MICROSCOPIA DE VIAL CORMINATY. DR. CAMPRA.pdf  
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## Firmantes



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