Investigating multiple endpoints for the interaction assessment of a graphene oxide-silver nanocomposite with macrophage

Luis A. V. Luna, Ana C. M. Moraes, Douglas S. Silva, Nahiara E. Zorgi, Silvio R. Consonni, Selma Giorgio, Oswaldo L. Alves

Department of Cellular and Structural Biology – Institute of Biology
Laboratory of Solid State Chemistry (LQES) – Institute of Chemistry
State University of Campinas, Brazil

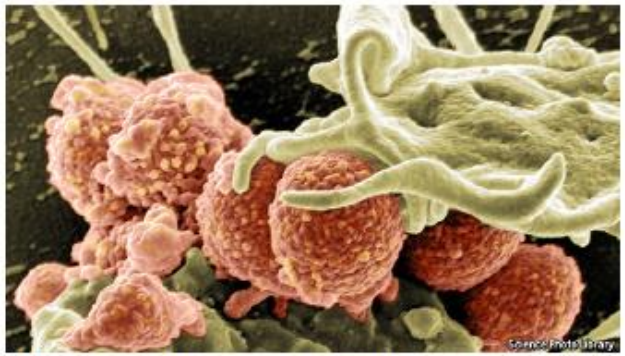
November 2016

Introduction

The Economist

Antibiotic resistance

The grim prospect



The evolution of pathogens is making many medical problems worse. Time to take drug resistance seriously

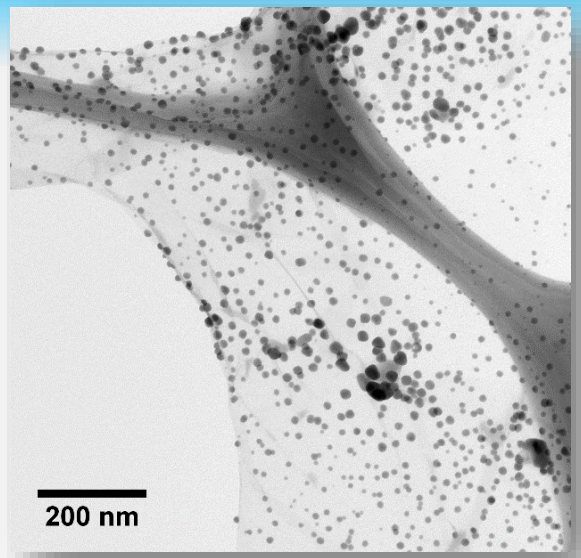
May 21st 2016 | From the print edition

Emergent alternatives from the nanotechnology

Silver-based nanomaterials

Introduction

Graphene oxide functionalized with silver nanoparticles (GOAg)



GOAg nanocomposite:

- *High surface area permits support the silver nanoparticles*
- *Improve the contact between silver nanoparticle and bacteria*
- *Increased antibacterial activity*

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Anti-adhesion and antibacterial activity of silver nanoparticles supported on graphene oxide sheets

Andreia Fonseca de Faria[a,*], Diego Stéfani Teodoro Martinez[a], Stela Maris Meister Meira[b], Ana Carolina Mazarin de Moraes[a], Adriano Brandelli[b], Antonio Gomes Souza Filho[c], Oswaldo Luiz Alves[a,*]

International Journal of Nanomedicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Graphene oxide-silver nanocomposite as a promising biocidal agent against methicillin-resistant *Staphylococcus aureus*

Ana Carolina Mazarin de Moraes[1]
Bruna Araujo Lima[2]
Andreia Fonseca de Faria[1]
Marcelo Brocchi[2]
Oswaldo Luiz Alves[1]

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
2 November 2015
Number of times this article has been viewed

Introduction

GOAg nanocomposite is very effective, but is it safe ?

How does this nanocomposite interact with mammalian cells ?

What are the nanotoxicological outcomes ?

Introduction

Why use macrophages as a model to assess the safety of nanomaterials ?

- Found in all tissues
- Part of innate immunity
- Professional phagocytes
- Destruction of microbes
- Production of ROS and NO

- Antigens presentation
- Cleaning process
- Tissue repair
- Cell recruitment
- Inflammation process

S. Giorgio, *Inflamm. Res.* **2013**, *62*, 835-843.

Y. Okabe, R. Medzhitov, *Nat Immunol* **2016**, *17*, 9-17..

Objectives

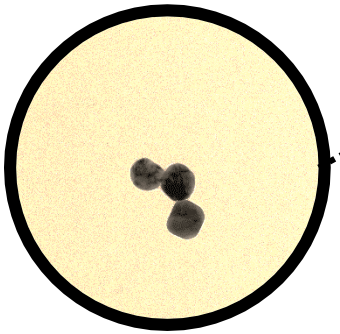
- Assessing the biocompatibility of the nanocomposite graphene oxide functionalized with silver nanoparticles (GOAg) and the pristine counterparts (GO and AgNP) using macrophages.
- Unveiling possible toxic mechanisms.
- Understanding how the nanocomposite interacts with the macrophage cells.

Methods

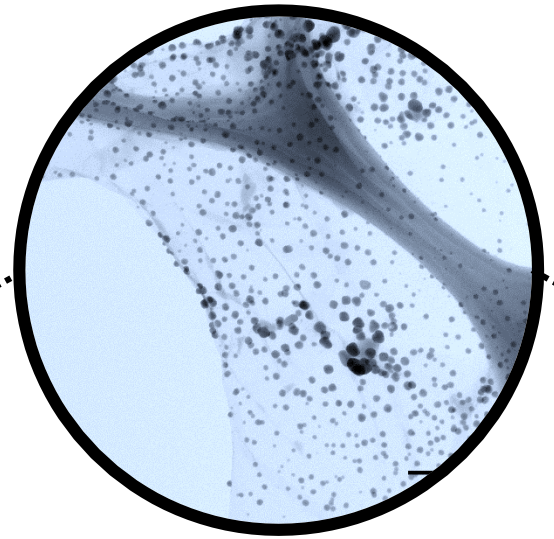
1. Synthesis

Nanocomposite (GOAg)

Citrate stabilized Silver nanoparticles (AgNP)

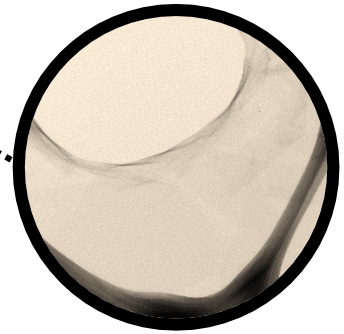


Turkevitch *et al.*, Discuss Faraday Soc. 1951



Turkevitch (*in situ*)

Pristine Graphene oxide (GO)



Hummers *et al.*, J Am Chem Soc. 1958

2. Physicochemical characterization

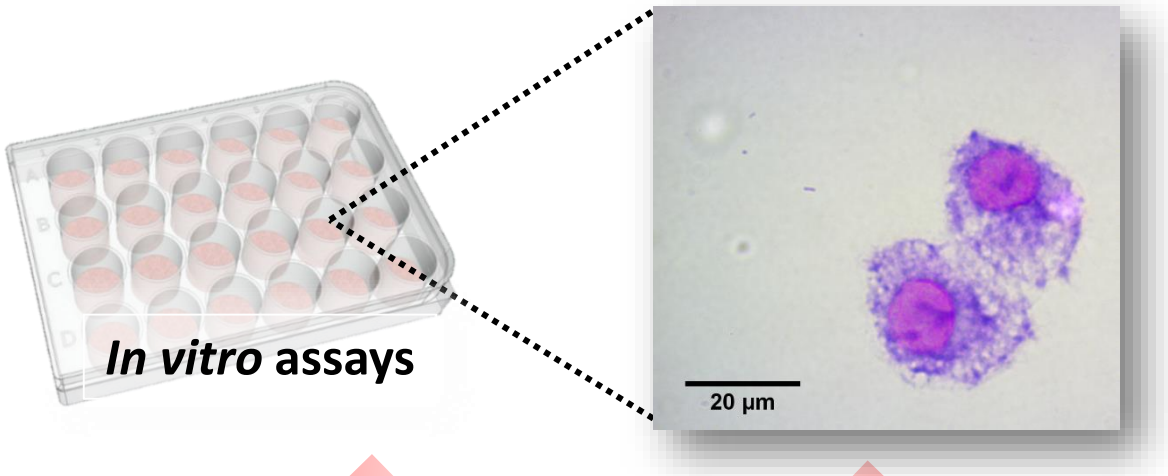
- Plasmon resonance band: UV-vis
- Morphology: TEM and AFM
- Cristallinity: DRX
- Silver concentration: ICP OES
- Surface charge: Zeta potential
- Hydrodynamic size: DLS

TEM images: LQES (IQ-Unicamp)

Methods

3. Biocompatibility with the macrophages

Cell model: mouse macrophages J774



Cytotoxicity

- Cell exclusion assay: Trypan blue
- Oxidative Stress: superoxide probe

Nanomaterials-cell interaction

- Cell membrane interaction: SEM
- Internalization: TEM and ICP-MS

Immunomodulation

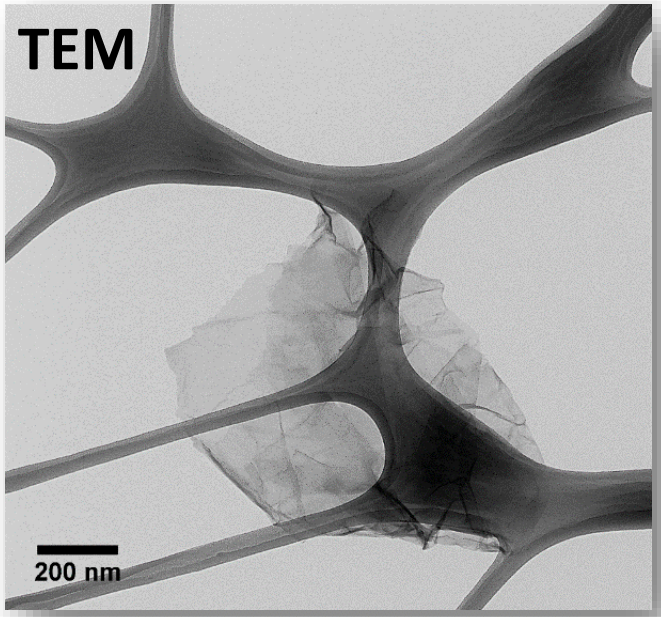
- Pro-inflammatory cytokines in culture supernatants: CBA Flow cytometry

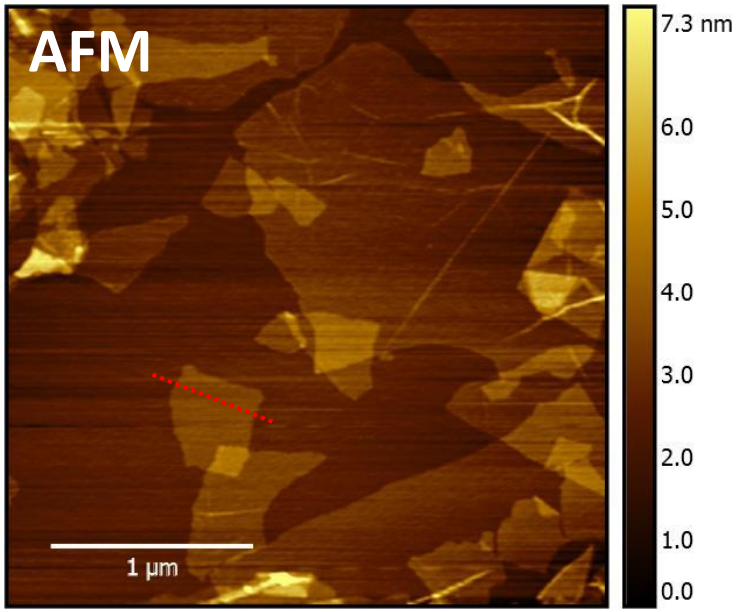
Macrophage stained by Giemsa (Lableish)

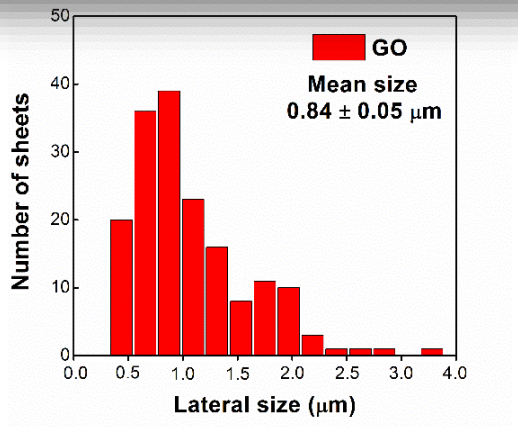
Results

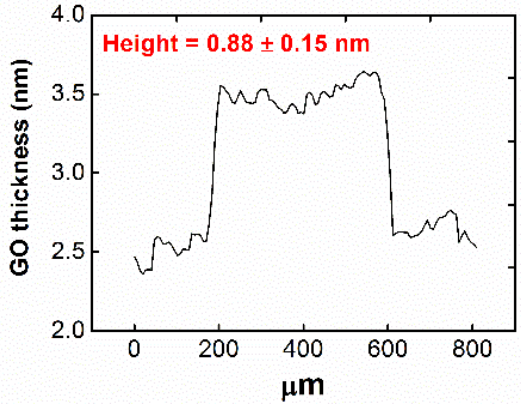
Synthesis and Physicochemical Characterization

1. Graphene Oxide





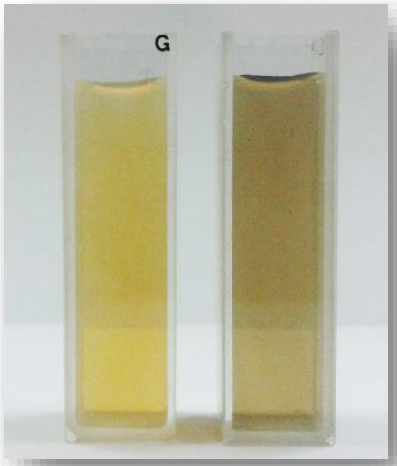




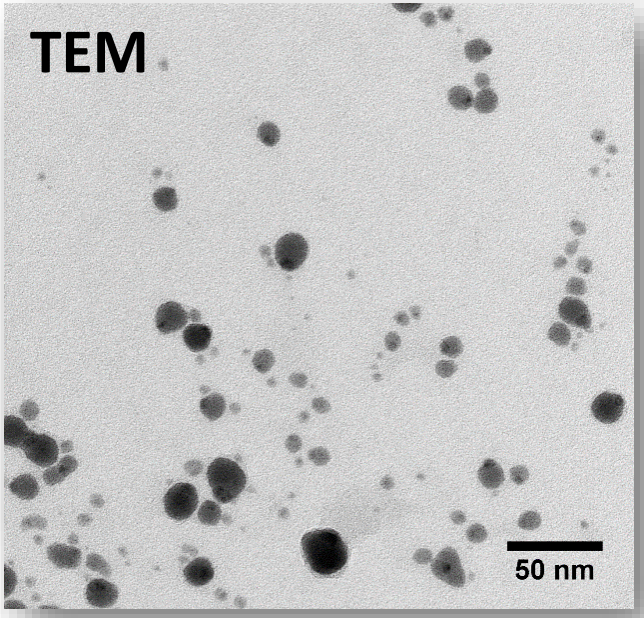
Results

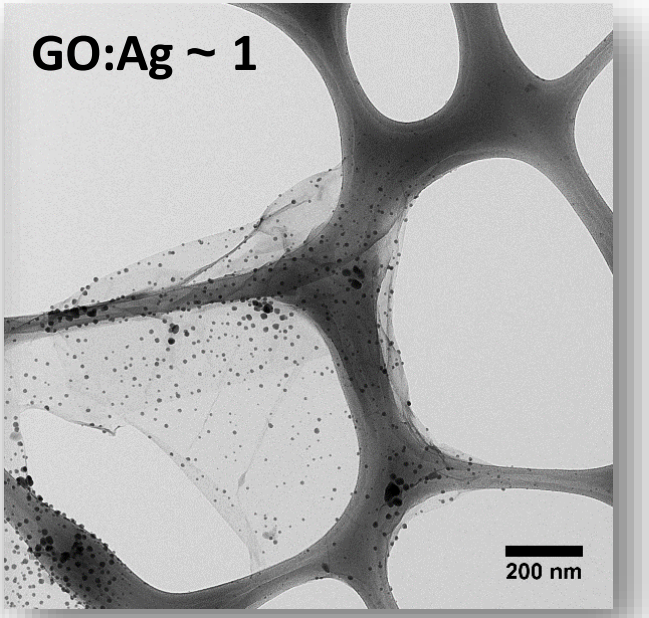
Synthesis and Physicochemical Characterization

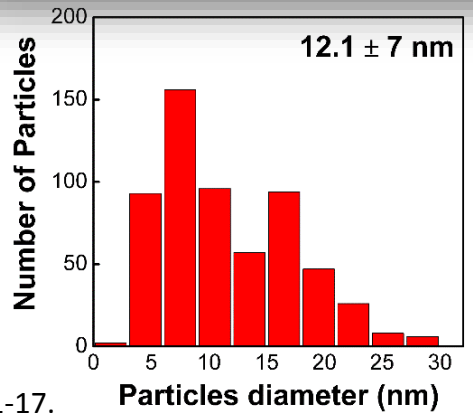
2. Pristine silver nanoparticles (AgNP) and nanocomposite (GOAg)

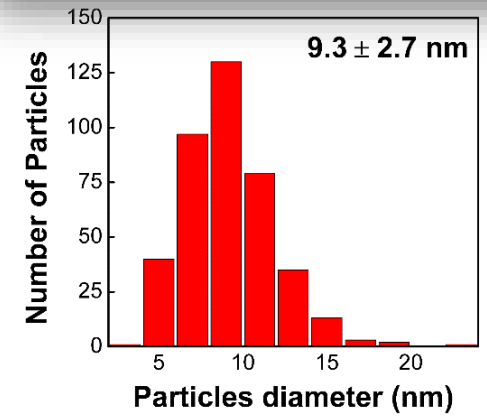


AgNP GOAg





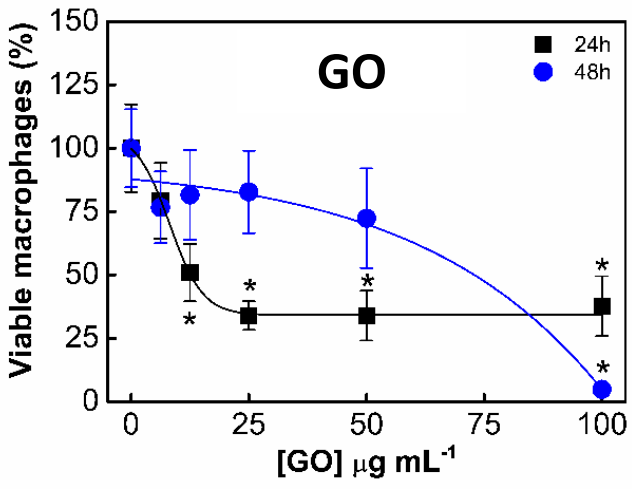


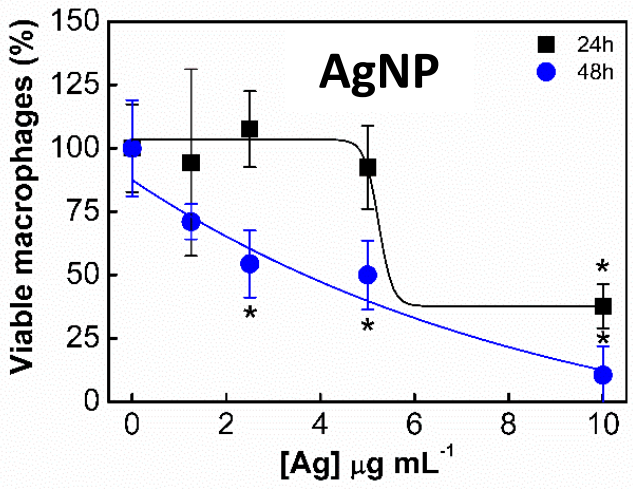


Luna, et al., *J Nanobiotechnol* **2016**, *14*, 1-17.

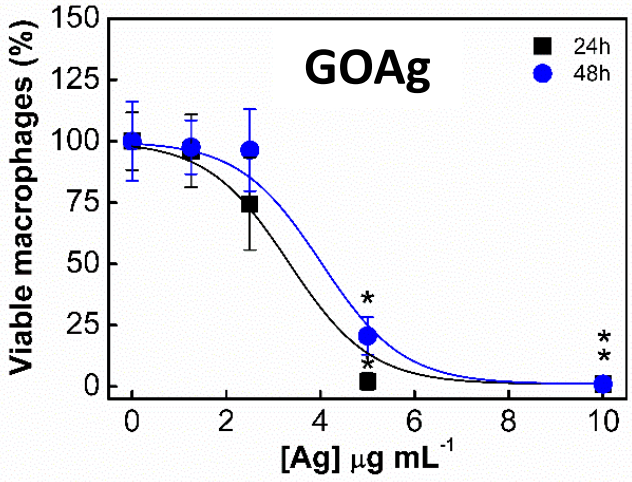
Results – Cytotoxicity*

Exposure to the pristine nanomaterials



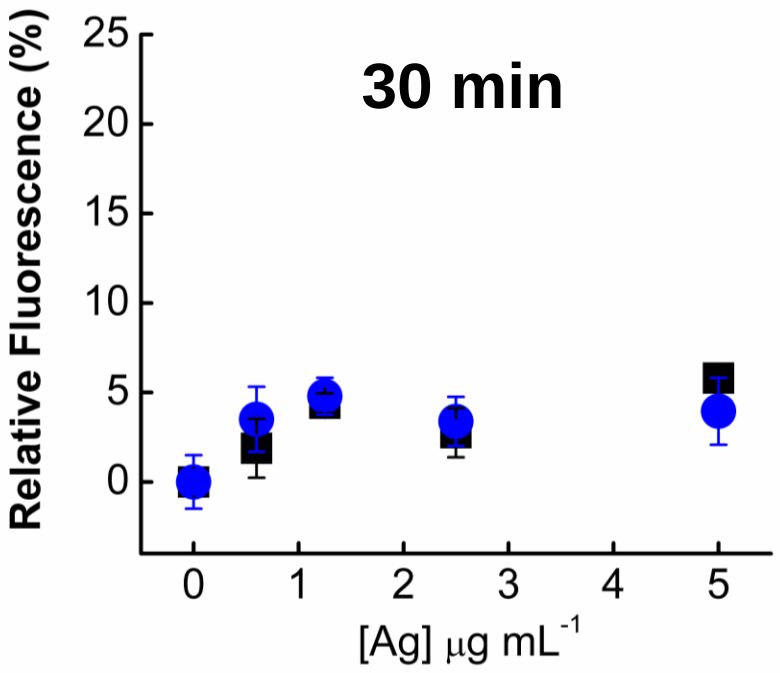


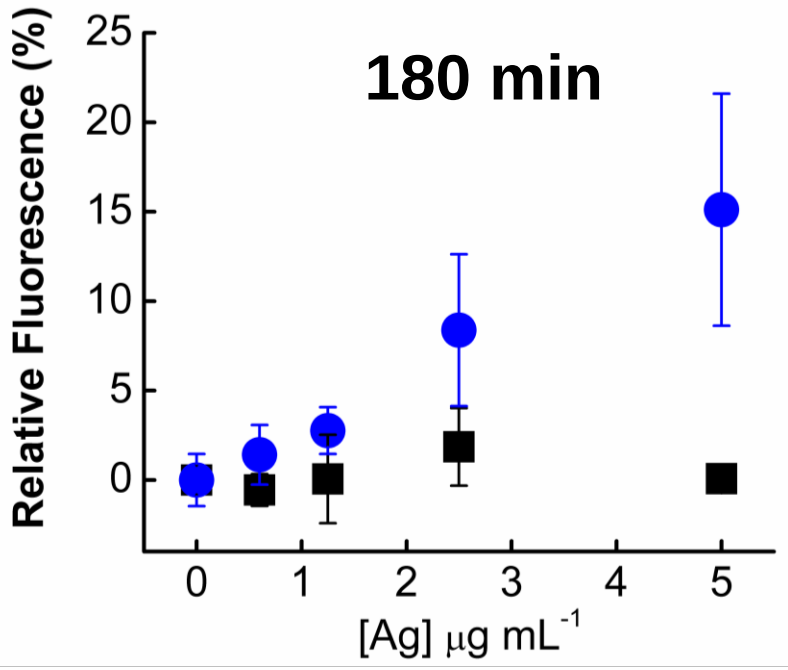
Exposure to the nanocomposite



*Trypan Blue exclusion assay (ANOVA: $P < 0.05$)

Results – Oxidative Stress (ROS)

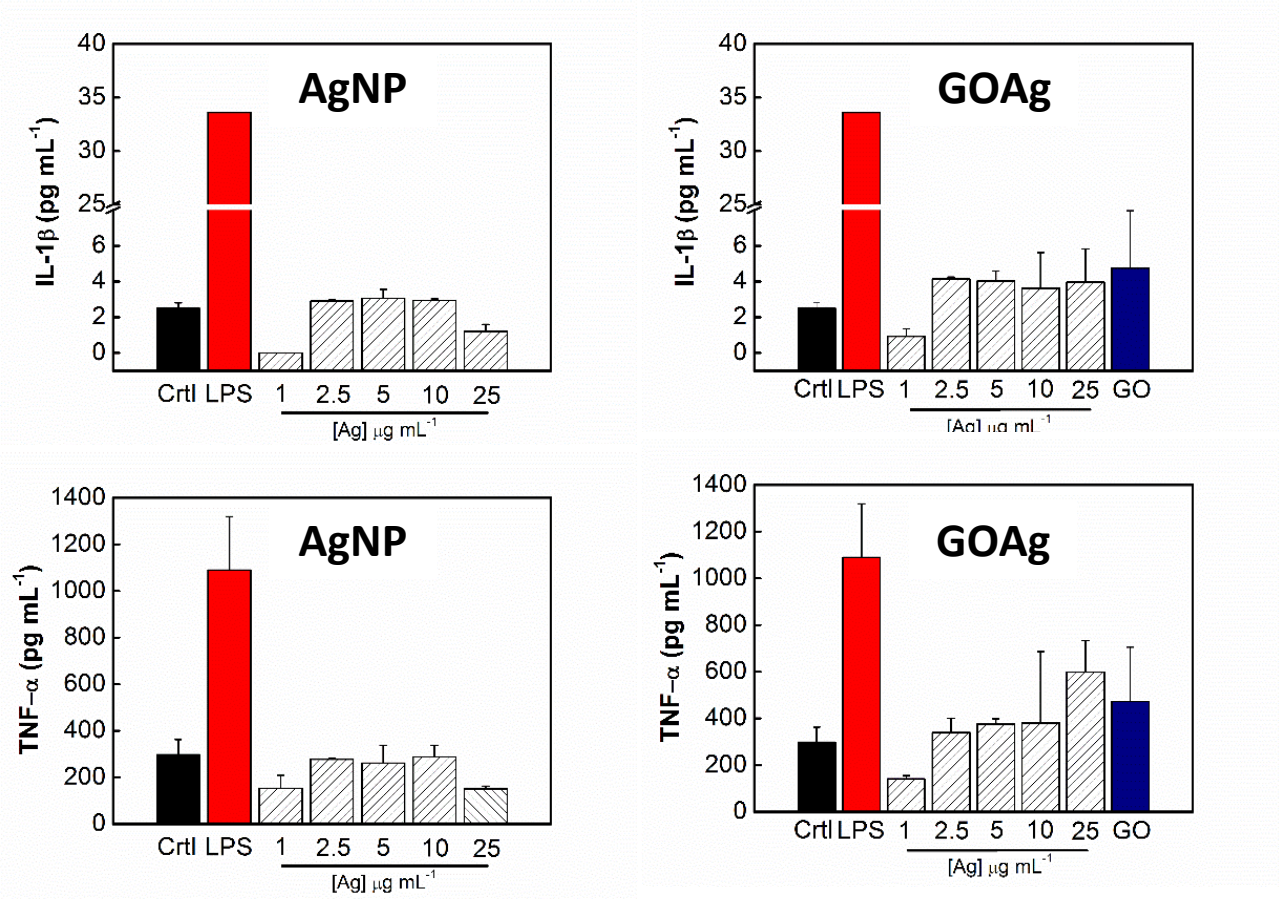




AgNP GOAg

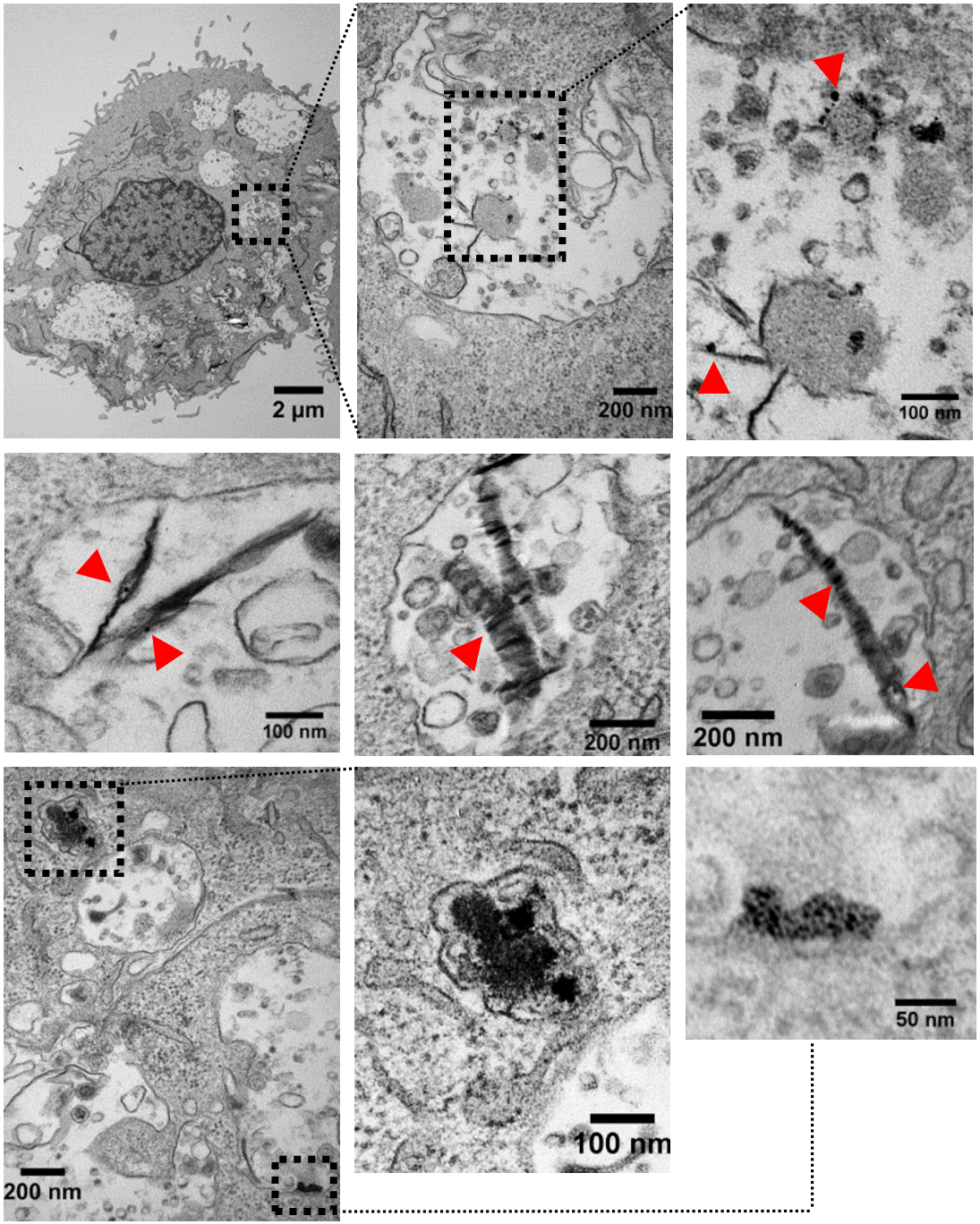
Results – Immunomodulation

Does the nanocomposite cause inflammation ?



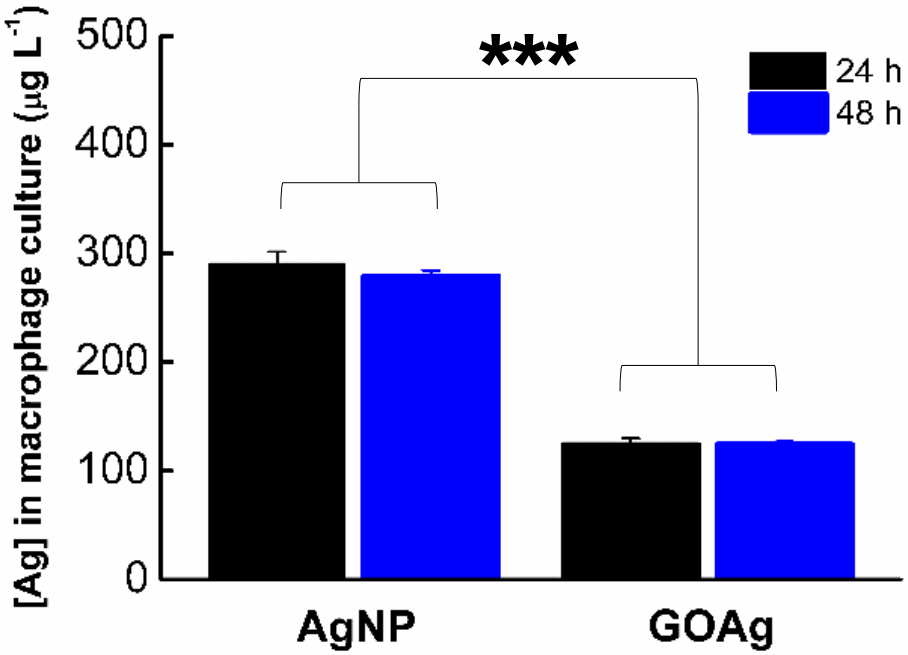
*FACS Canto II, BD Biosciences CBA Beads. LPS: *E. coli* bacteria lipopolysaccharides (Sigma)

Nanocomposite Internalization



Results – Internalization

Is the nanocomposite a platform for delivery of silver nanoparticles ?



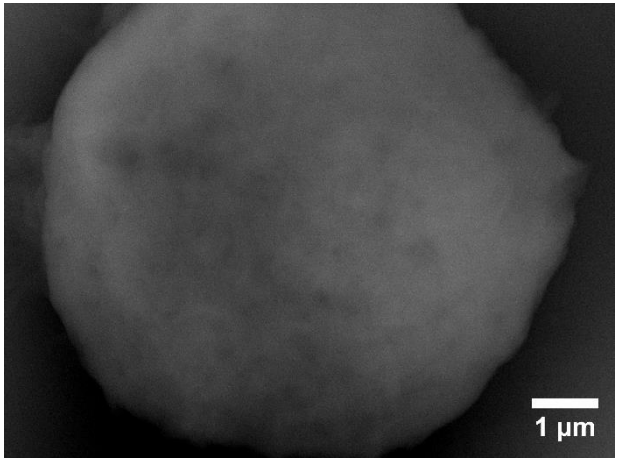
*Cells exposed to non-lethal dosages of nanomaterials. ICP-MS ($P < 0.001$)

Results – Nano Cell interaction

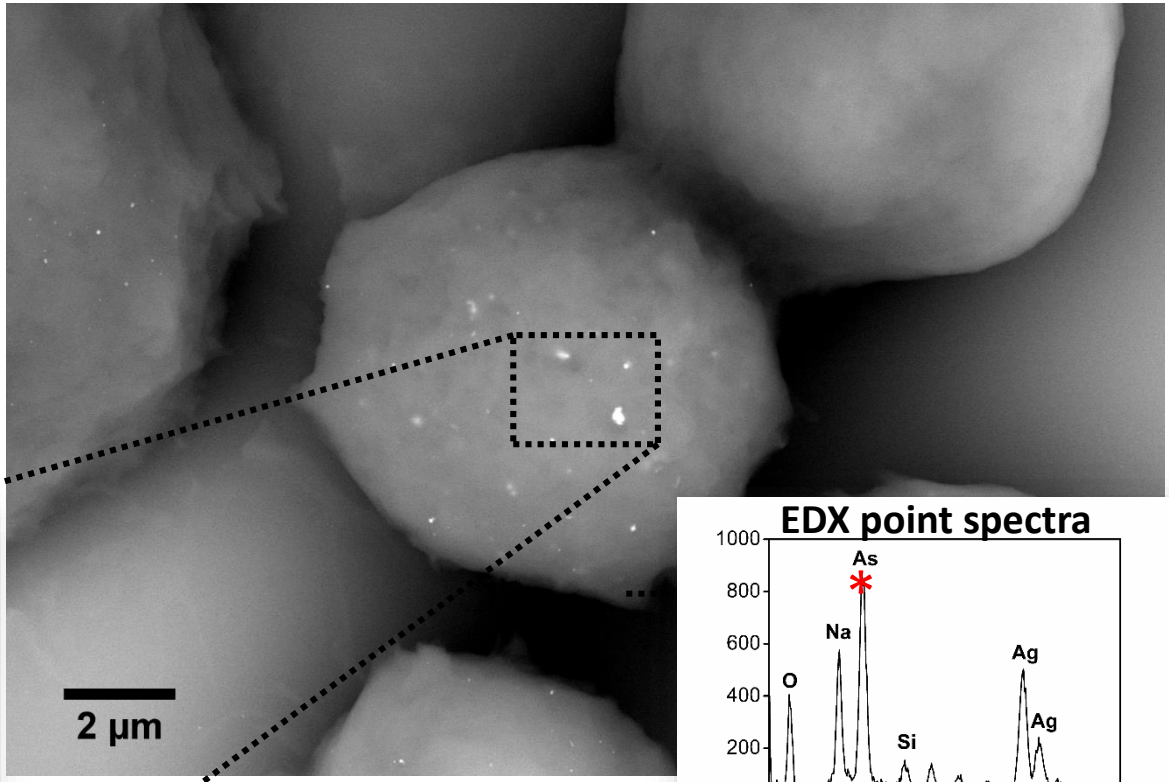
A microscopy study

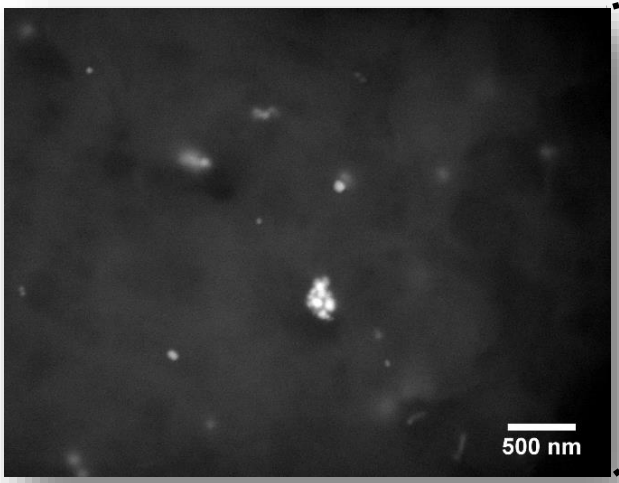
How do these nanomaterials interact with cells ?

Non-exposed macrophages



Exposed to pristine AgNP





Substrate: Silicon wafer

*Fixative agent: Cacodylate buffer [$Na(CH_3)_2\ AsO_2\ .\ 3H_2O$]

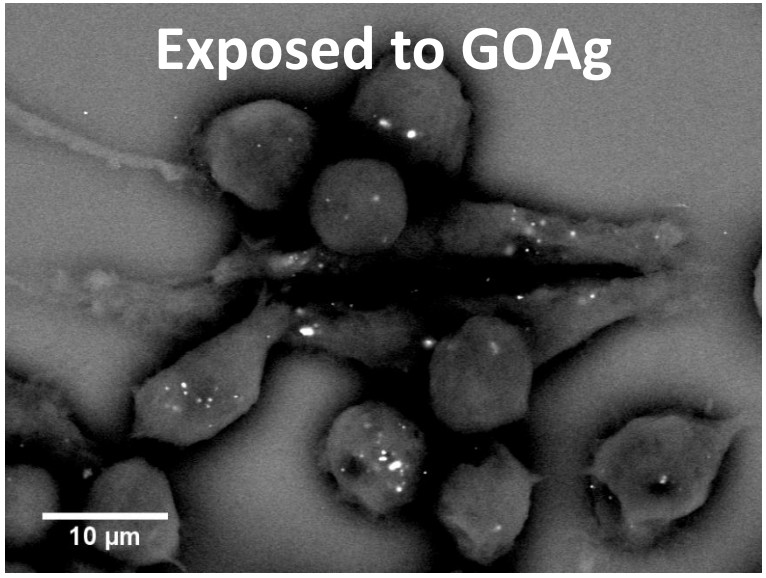
FEG-SEM Backscatter Detector/ non-metalized samples

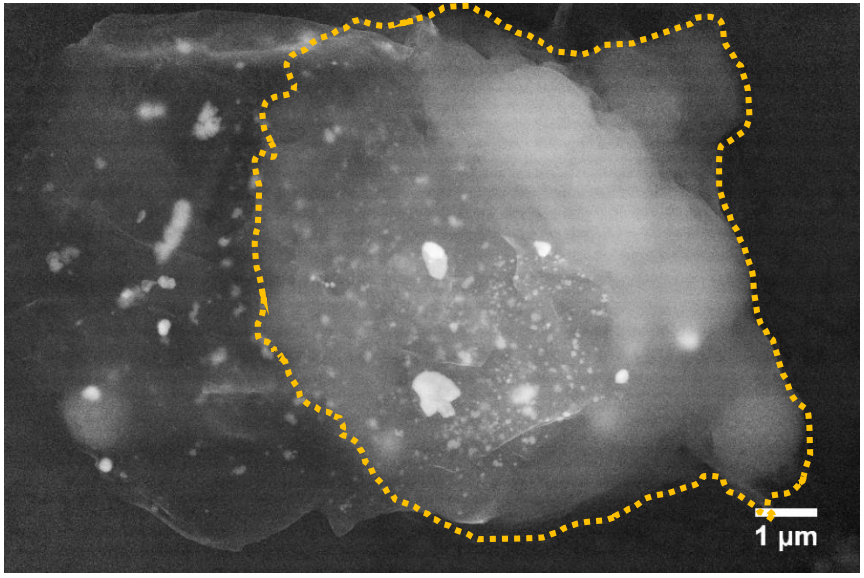
Results – Nano Cell interaction

A microscopy study

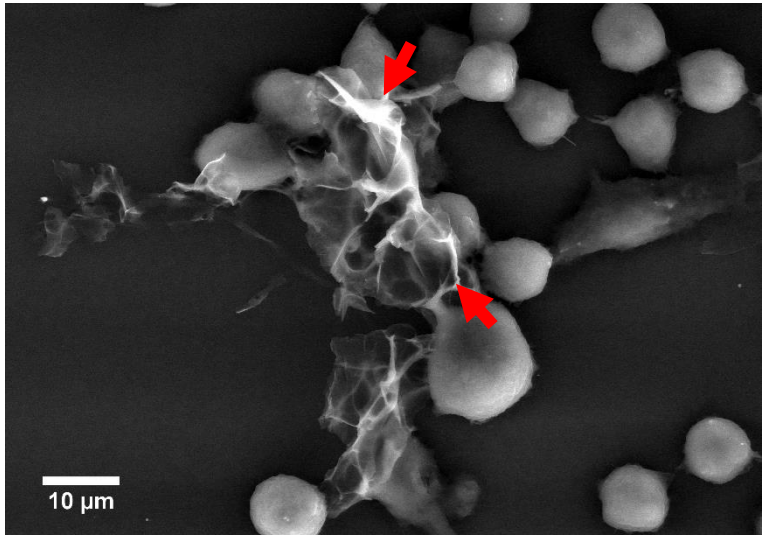
Graphene oxide changes the way silver nanoparticles interact with the cell

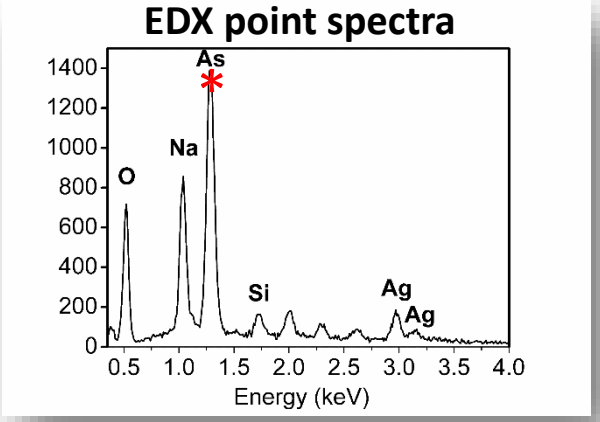
Backscatter detector





Large Field detector





Substrate: Silicon wafer

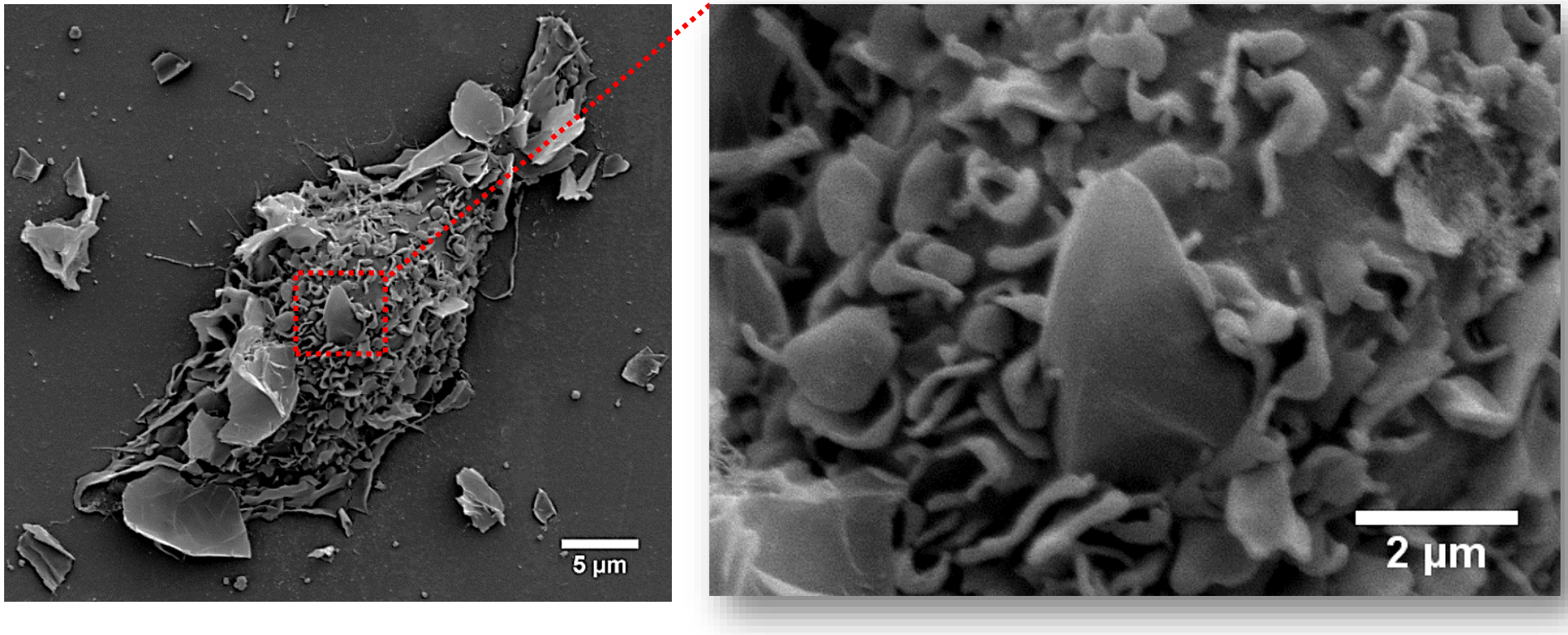
*Fixative agent: Cacodylate buffer [$Na(CH_3)_2\ AsO_2\ .\ 3H_2O$]

FEG-SEM/ non-metalized samples

Results – Nano Cell interaction

A microscopy study

Macrophages exposed to the nanocomposite



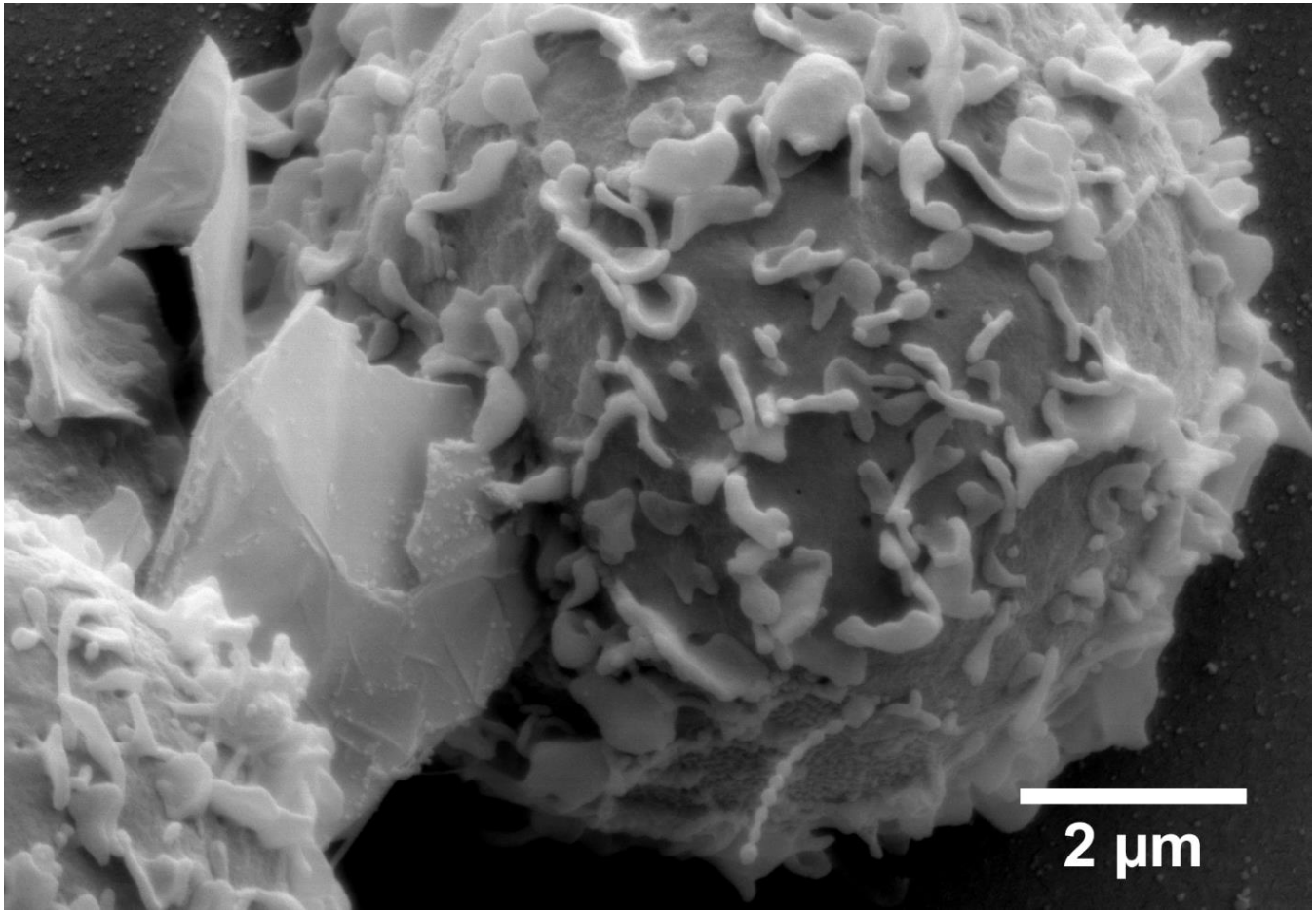
Substrate: glass coverslips
FEG-SEM/ Gold metallization

Results – Nano Cell interaction

A microscopy study

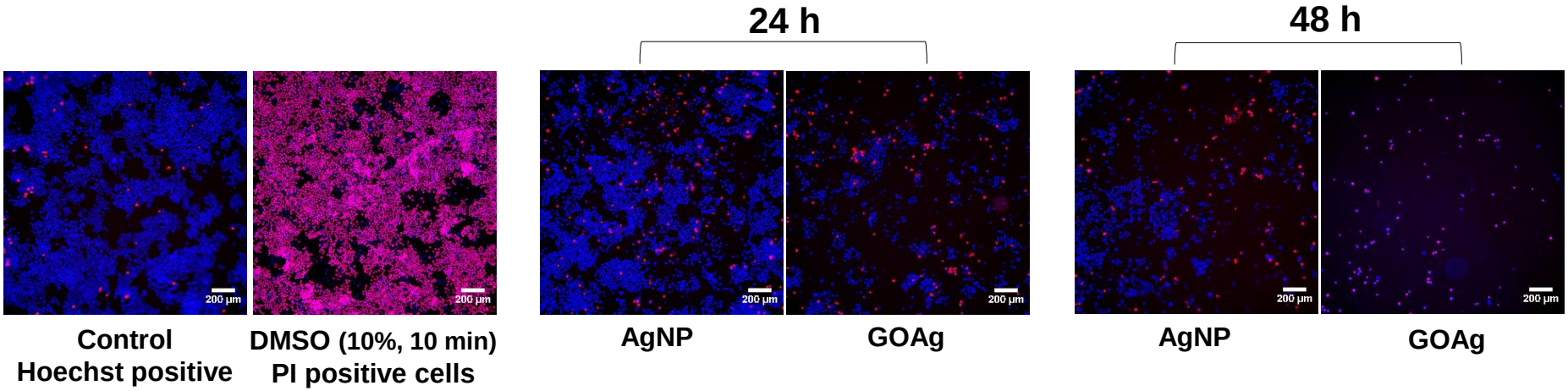
The nanocomposite with large lateral size and its aggregates may difficult macrophage endocytosis

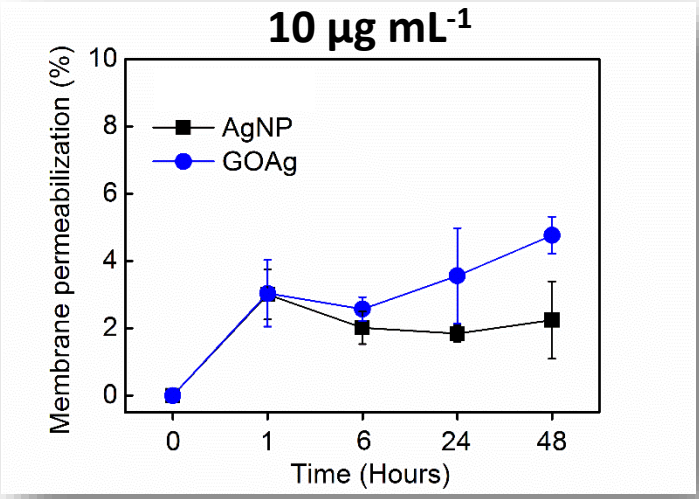
Macrophages exposed to the nanocomposite

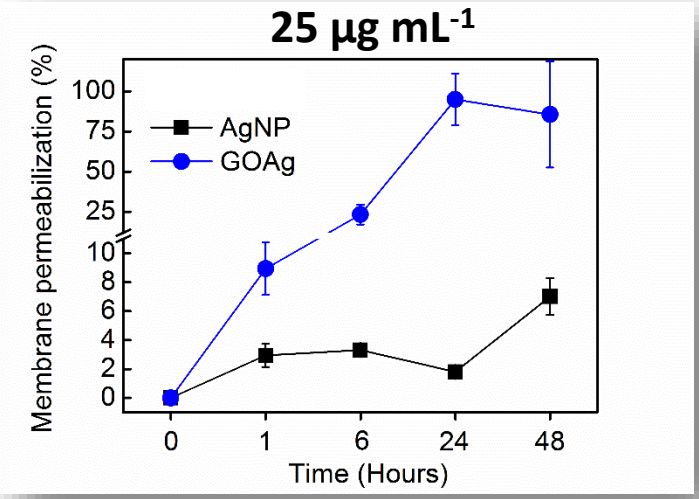


Preliminary Results

Kinetics of cell membrane permeation using High-Content*







* Photos of the remaining cells, still adherent to the microplate (25 µg mL^{-1}). Micro Confocal High-Content Imaging©.

Conclusions

1. Exposure to GOAg resulted in a synergistic toxicity to the macrophages.

2. GOAg presented higher toxicity and early oxidative stress, but did not present inflammatory potential.

3. GO sheets maximize the contact between AgNP and cell membrane.

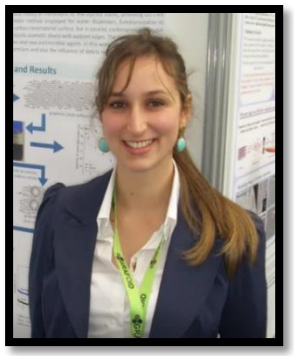
4. GOAg was difficult to be internalized by the macrophages, but it increased cell permeability.

5. Probably due to a frustrated internalization.

Next steps…

Acknowledgements

Collaborators:


Dr. Ana Moraes


Dr. Nahiara Zorgi


Dr. Douglas Soares


Dr. Catarinie Diniz


Dr. Sílvio Consonni

Advisors:


Prof. Selma Giorgio


Prof. Oswaldo Luiz Alves

Financial support and research facilities:

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

Nanoestruturas
NANOBIOSS
Biossistemas

iTAL
INSTITUTO DE TECNOLOGIA DE ALIMENTOS

Life Sciences Core Facility
LaCTAD-Unicamp

Merci !