Polymeric particulated carriers in drug delivery:

Obtention, study and characterization

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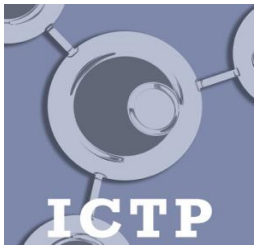

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Outline

- Drug Delivery Systems (DDS)
  - ✓Polymeric micro and nanoparticles
- Biopolymers used in drug delivery science
  - ✓PLGA
  - ✓PLA
- DDS we are working on
  - ✓BSA loaded PLGA nanoparticles
  - ✓Polilisyne loaded PLA/PEG-b-PLA microparticles
  - ✓Tamoxifen loaded PLGA nanoparticles

DDS

Drug delivery systems

can be defined as devices capable of perform mechanisms to introduce therapeutic agents into the body

- Act as a vehicle for a wide variety of therapeutic agents.
- Protect drug molecules from degradation in the human organism before reaching the target site.
- Modulate release rate in the target site to achieve the adequate pharmacological response.
- Be customized in order to effectively reach the specific target in the human body.
- Achieving intra or extracellular drug delivery depending of the therapeutic goal.
- Be biocompatible and be able to biodegrade in order to be safe for human administration

Polymeric Micro and Nanoparticles

Polymeric micro and nanoparticles are micron and submicron size entities made from a wide variety of polymers. Because of their potential ability to improve current disease therapies these micro and nanodevices are being extensively used as drug carriers and controlled release systems in the field of medicine and pharmacy.

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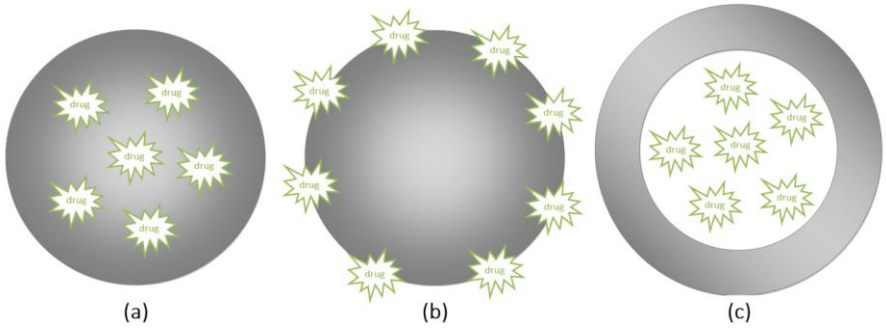


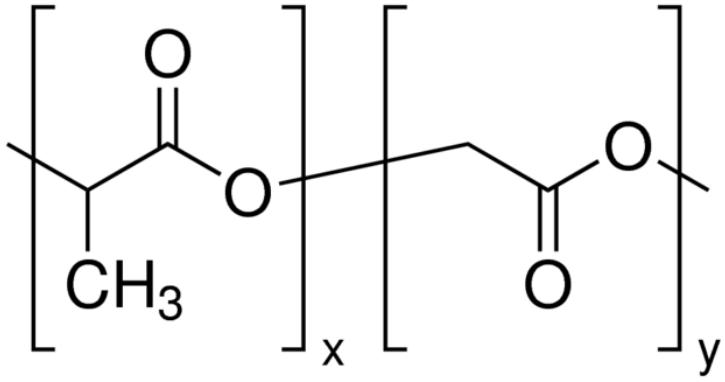
Micro/nanoparticle carrying drug molecules within it matrix. (b) Micro/nanoparticle carrying drug molecules in its surface. (c) Micro/nanocapsule carrying drug molecules in its internal cavity.

Active pharmaceutical ingredients can be encapsulated, covalently attached, or adsorbed onto such carriers

PLGA

Poly(lactic-co-glycolic acid)

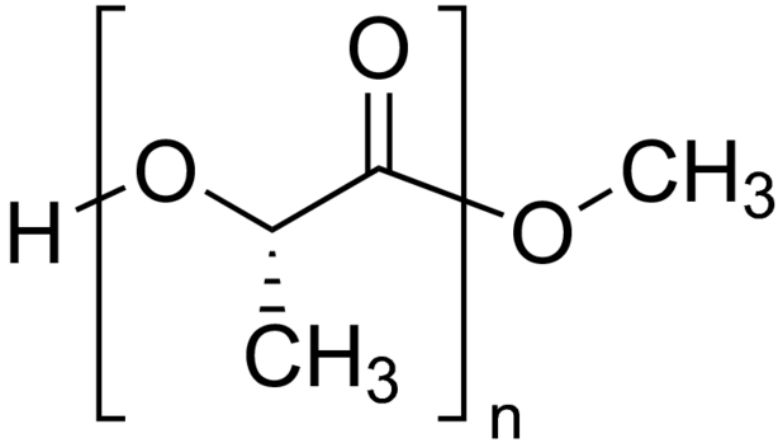
is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility.



Polymers	Thermal & Mechanical Properties			Degradation Properties		Processing and Applications	
	Melting Temperature (°C)	Glass Transition Temperature (°C)	Tensile Modulus (GPa)	Time (Months)	Products	Solvent	Applications
Poly- latic-co-glycolic PLGA (50/50)	Amorphous	50–55	1.4–2.8	3–6	D,L-lactic acid and glycolic acid	Choloroform Dichlorometane Etylacetate Acetone Tetrahydrofuran hexafluoroisopropanol	Suture, drug delivery

PLLA

Polylactic acid is an aliphatic ester of lactic acid derived from renewable resources such as corn starch or sugarcane. It is a biodegradable polymer which has gained commercial interest due to its easy manufacturing. Because of the presence of two chiral centers, lactic acid is a chiral molecule, and therefore it has two stereoisomers: L- and D-lactic acid.

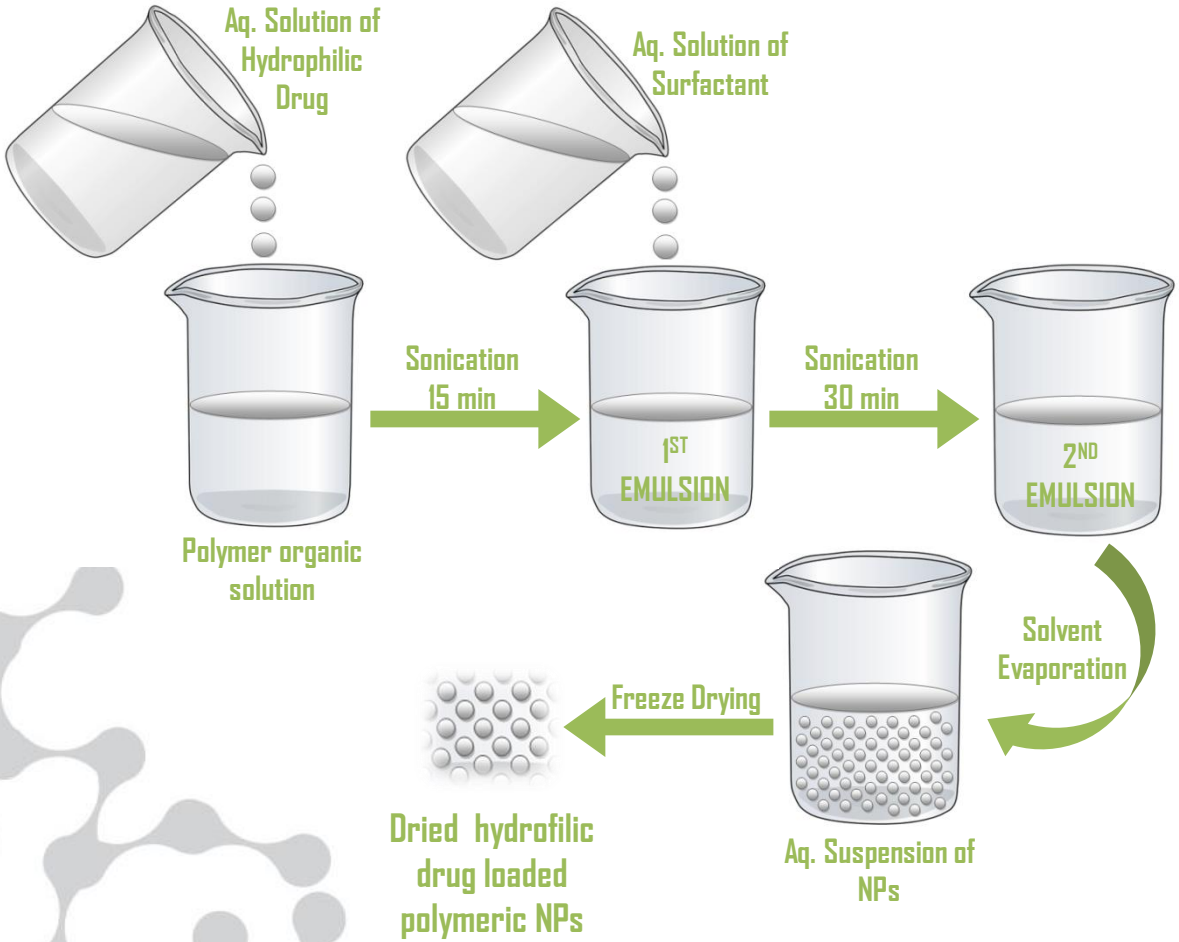


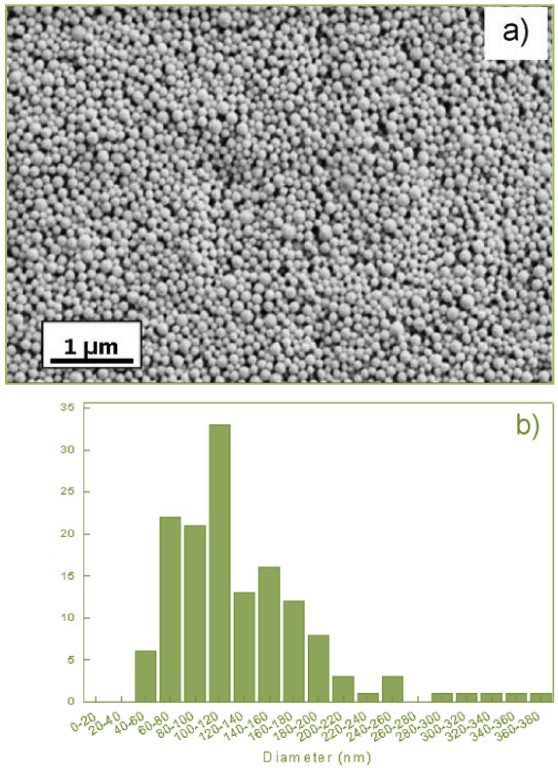
Polymers	Thermal & Mechanical Properties			Degradation Properties		Processing and Applications	
	Melting Temperature (°C)	Glass Transition Temperature (°C)	Tensile Modulus (GPa)	Time (Months)	Products	Solvent	Applications
Polylactic acid PLA	173–178	60–65	1.5–2.7	12–18	L-lactic acid	Choloroform Dioxane Dichlorometane Etylacetate Acetone Tetrahydrofuran hexafluoroisopropanol	Fracture fixation, interference screws, suture anchors, meniscus repair

BSA loaded PLGA NPs

Double Emulsión Technique

Polymer	BSA w/w %	Size (nm)	PdI	% EE
PLGA	10	293±5	0.034±0.01	65





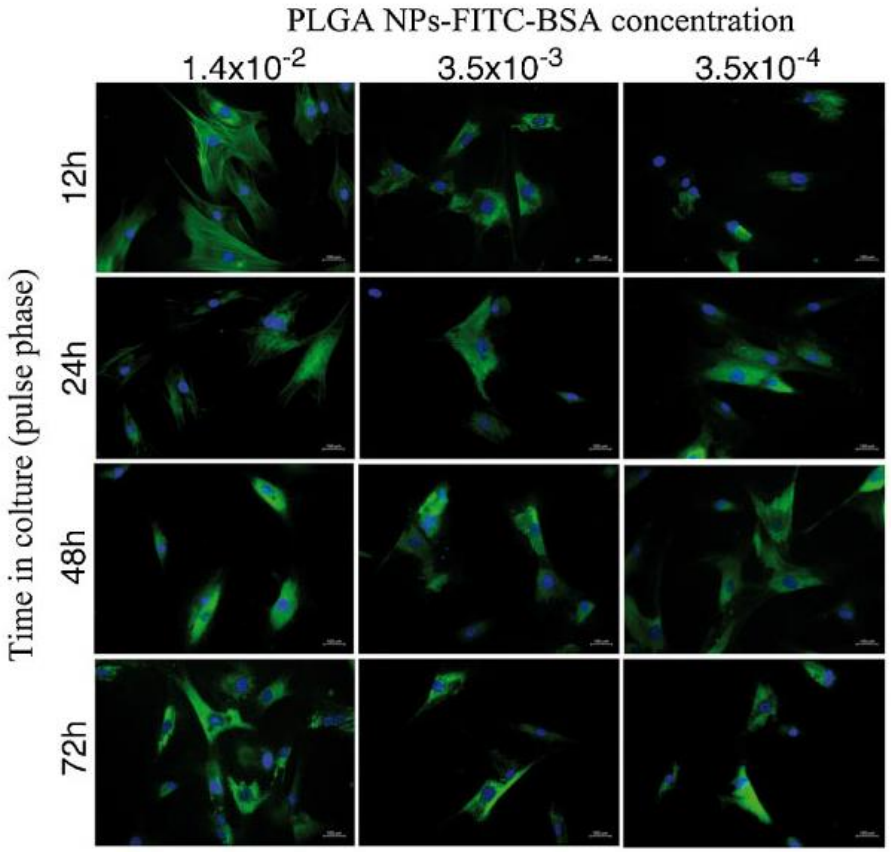
FESEM image of PLGA NPs loaded with BSA (a) and diameter size distribution by Nikon software Analyzer (b).

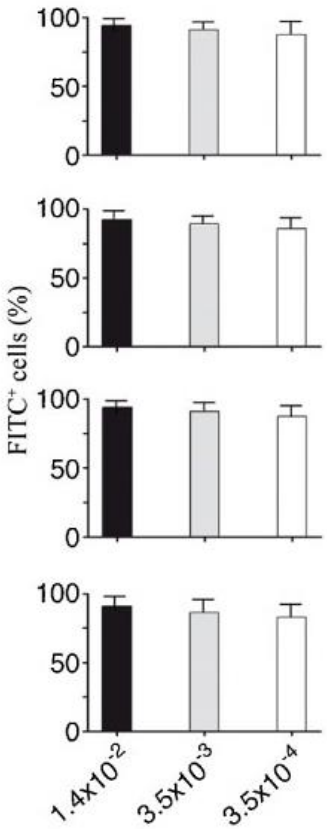
Protein Encapsulation in Biodegradable Polymeric Nanoparticles: Morphology, Fluorescence Behaviour and Stem Cell Uptake *Nicoletta Rescignano, Luigi Tarpani, Roberto Tiribuzi, Simona Montesano, Sabata Martino, Loredana Latterini, Jose Maria Kenny, Ilaria Armentano. 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim wileyonlinelibrary.com Macromol. Biosci. 2013, DOI: 10.1002/mabi.201300140*

BSA loaded PLGA NPs

Stem-Cells internalization

Total stem cells were PLGA NPs-FITC-BSA positive after 12 h of incubation, independent of the PLGA NPs-FITC-BSA concentration used.





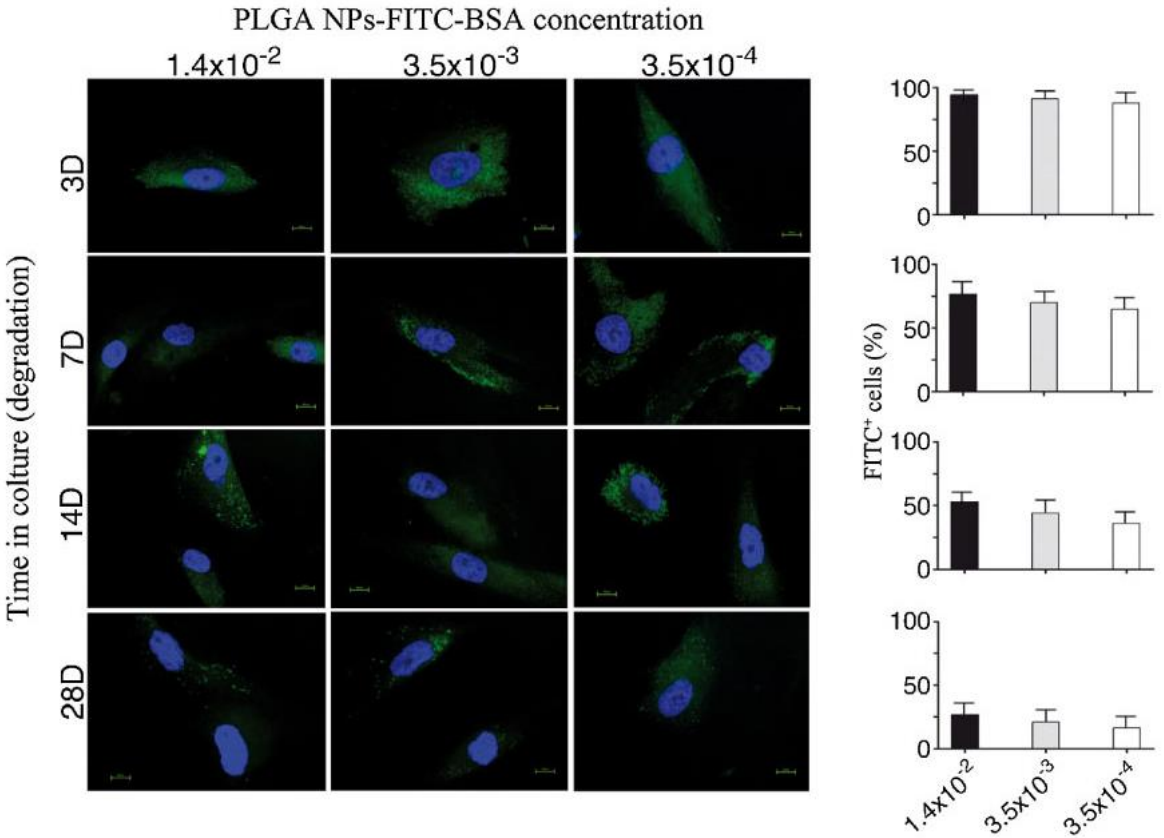
Quantification of the number of stem cells that have internalized the PLGA NPs-FITC-BSA

Protein Encapsulation in Biodegradable Polymeric Nanoparticles: Morphology, Fluorescence Behaviour and Stem Cell Uptake *Nicoletta Rescignano, Luigi Tarpani, Roberto Tiribuzi, Simona Montesano, Sabata Martino, Loredana Latterini, Jose Maria Kenny, Ilaria Armentano. 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim wileyonlinelibrary.com Macromol. Biosci. 2013, DOI: 10.1002/mabi.201300140*

BSA loaded PLGA NPs

Stem-Cells internalization

After 72 h of pulse, NPs-FITC-BSA were taken out, several washes with PBS were performed, and stem cells were maintained in culture in normal growth medium for four weeks in order to monitor the time dependent degradation (in terms of reduction intracellular fluorescence intensity) of NPs-FITC-BSA by hBM-MSCs



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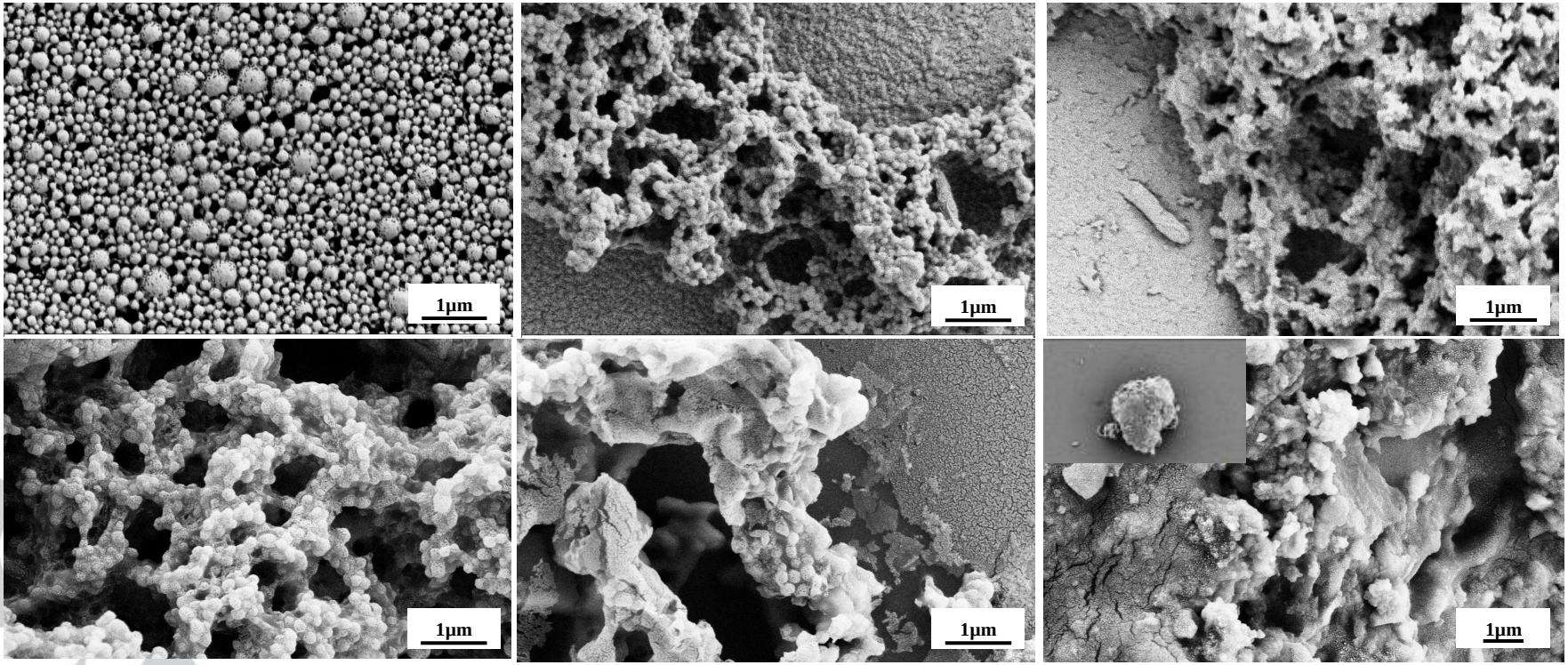
PLGA NPs

In vitro degradation

Morphological changes of PLGA nanoparticles taken 7, 14 and 21 incubation days in PBS at 37°C



Morphological changes of PLLA nanoparticles taken 21, 42 and 56 incubation days in PBS at 37°C

Polylysine loaded PLA/PEG-b-PLA MPs

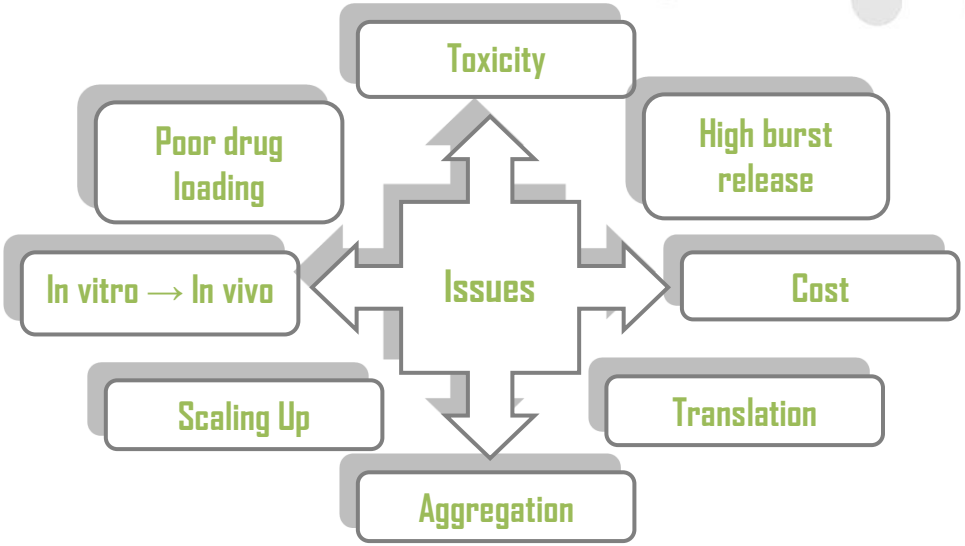
OBJECTIVE

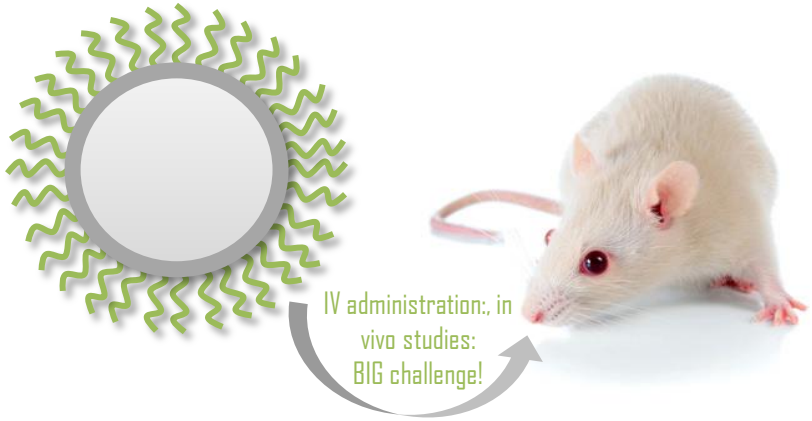
To study physicochemical and biopharmaceutical properties of **PLA/PEG-b-PLA** polylisine microparticulated carriers.

Polylysine as a molecule model for other peptides of interest.

PREPARATION OF NPs

Microparticles were obtained by a proprietary electrohydrodynamic technology (Bio-Target Inc., Chigaco, IL, USA; LNK Chemsolutions LLC, Lincoln, Nebraska, USA).

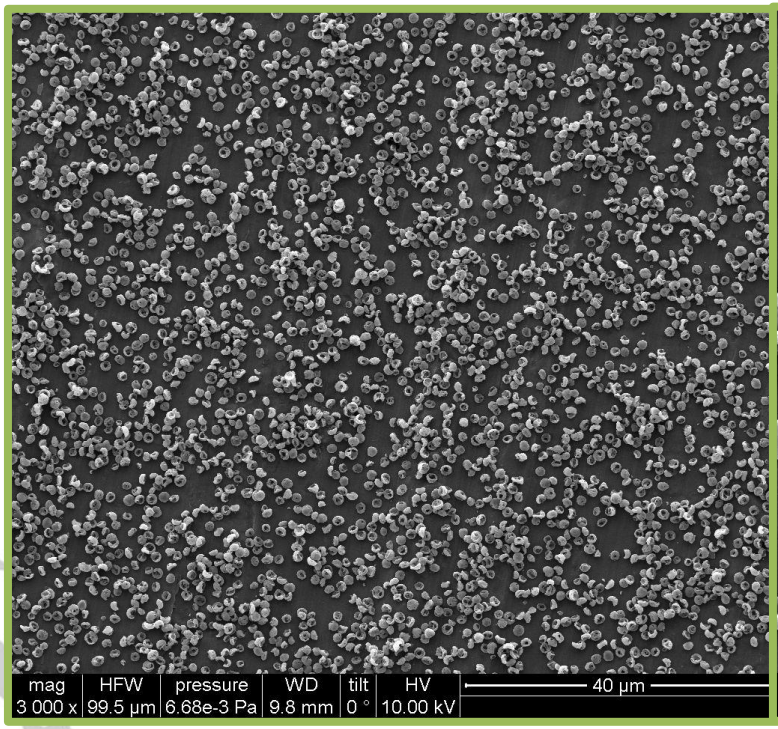


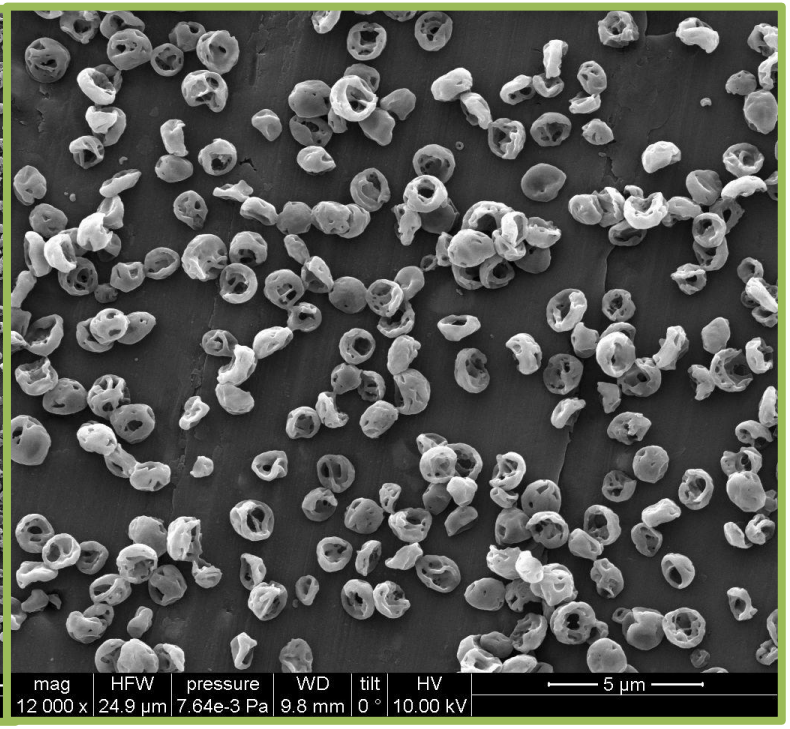


Polylysine loaded PLA/PEG-b-PLA MPs

Polymer Blend	Polilysine w/w% of Polymer	EE	Size (µm)
PLA/PEG-b-PLA (75%-25%)	1%	81%	1.2 ± 0.12

Scaning Electron Miroscopy



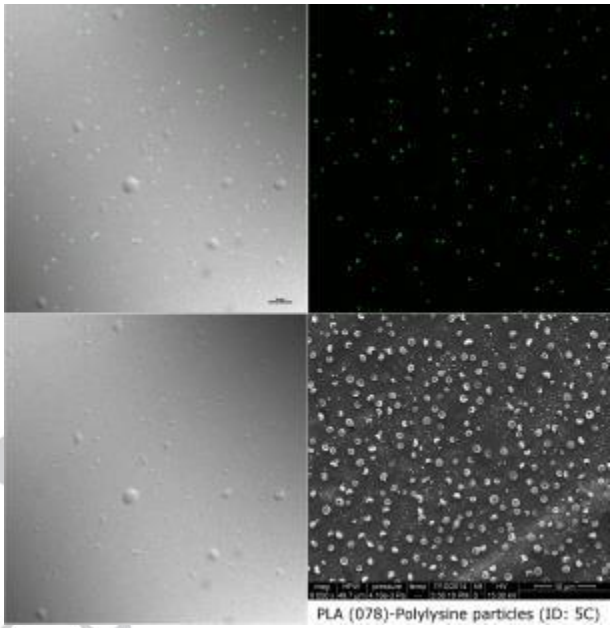


Polilisyne loaded PLA/PEG-b-PLA MPs

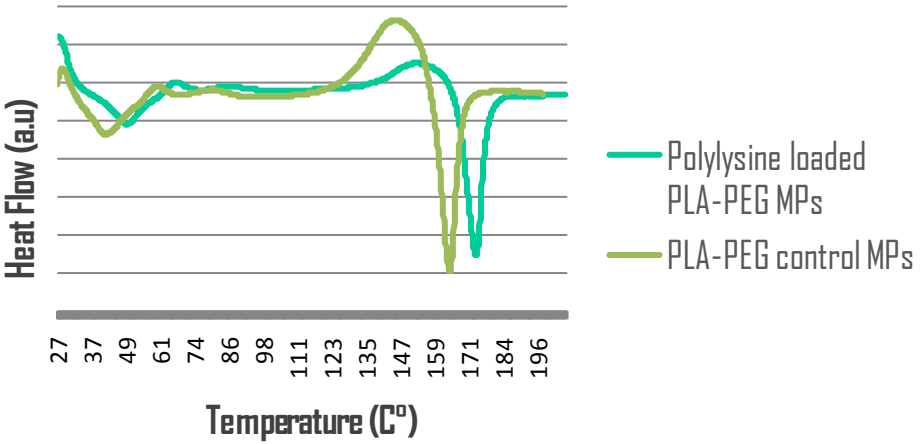
Thermal Analysis

The results obtained showed that the particles exhibit thermal stability. The TGA showed a thermal decomposition of the particles at 275 ºC. The DSC showed an endothermic event at 58 º C attributed to the glass transition temperature of PLA.

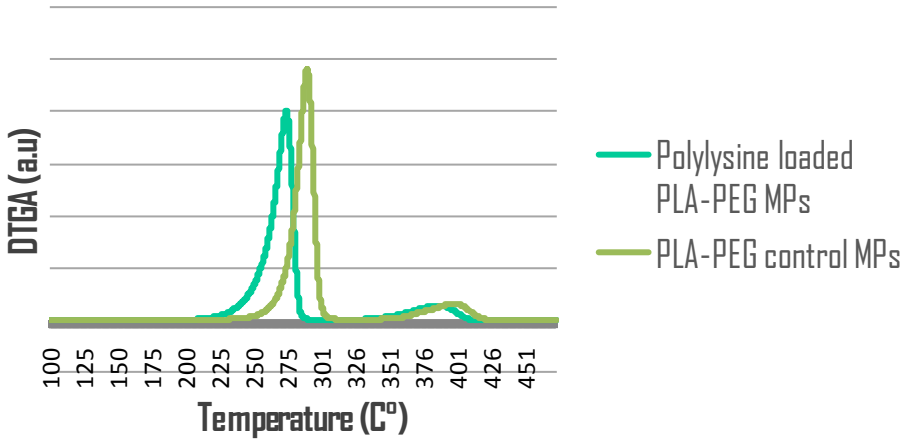
Confocal Microscopy



Differential Scanning Calorimetry (DSC)



Thermogravimetric Analysis (TGA)



Future Work

Polylysine loaded PLA/PEG-b-PLA MPs

- Release profile

The polylisine release from particles was performed in a bi-compartimental diffusion device (Franz´s cells) mounted with a semi synthetic cellulose membrane.

The amount of drug released will be conducted by high performance liquid chromatography (HPLC).

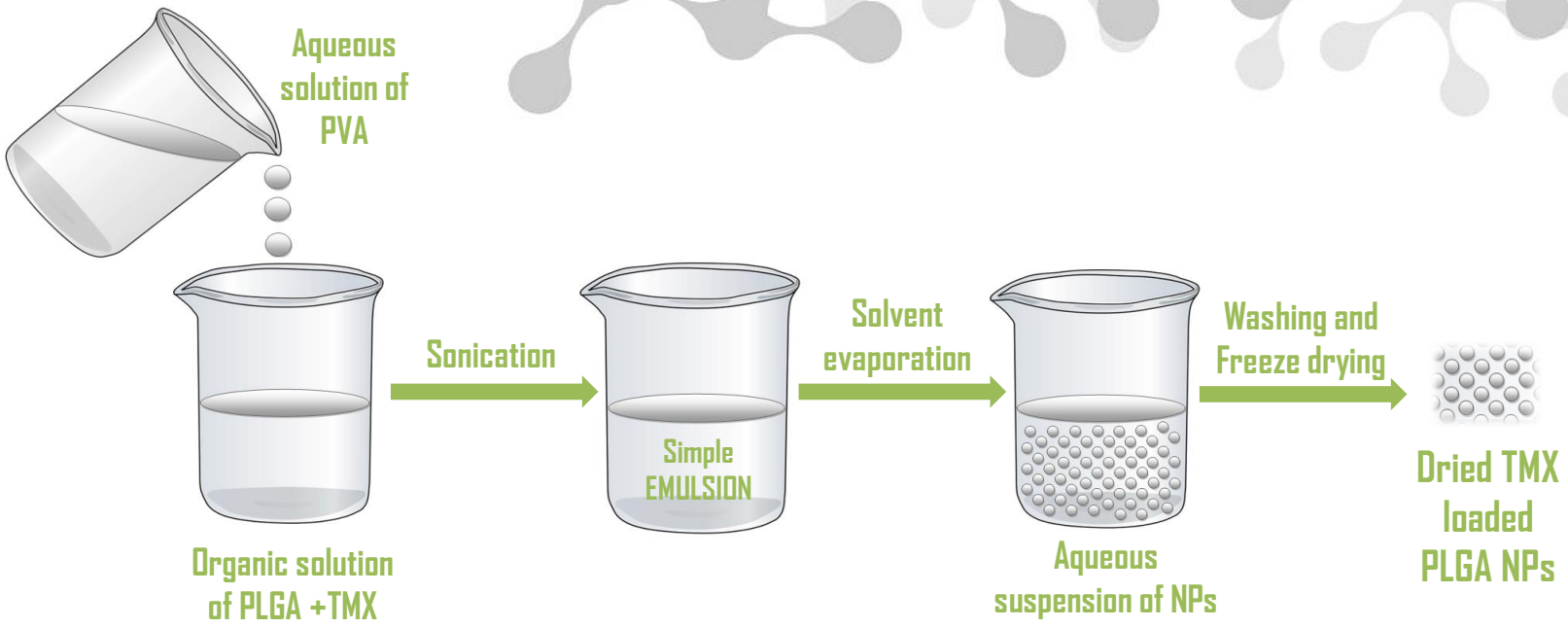
- In vitro

✓Citotoxicity

✓Internalization

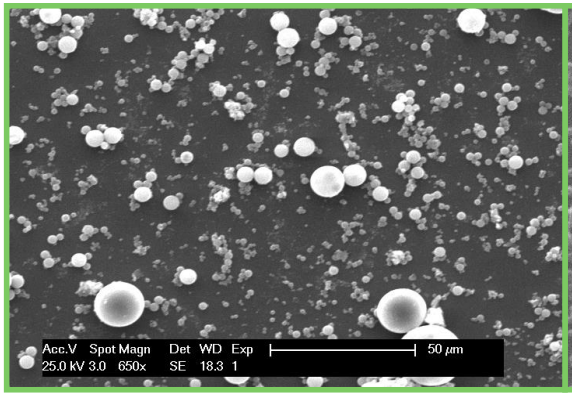
Extrapolation to innovative oncologic peptides for new cancer nanotherapies

TMX loaded PLGA NPs

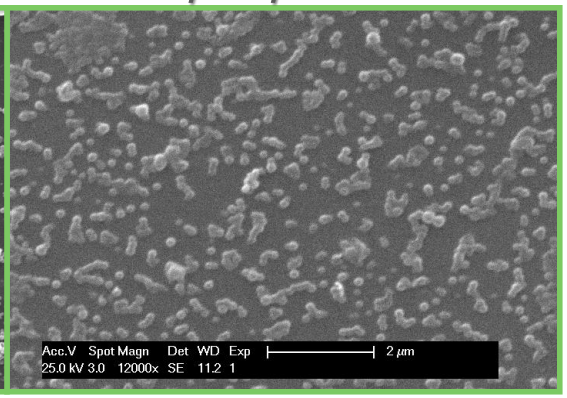


SEM

Poloxamer



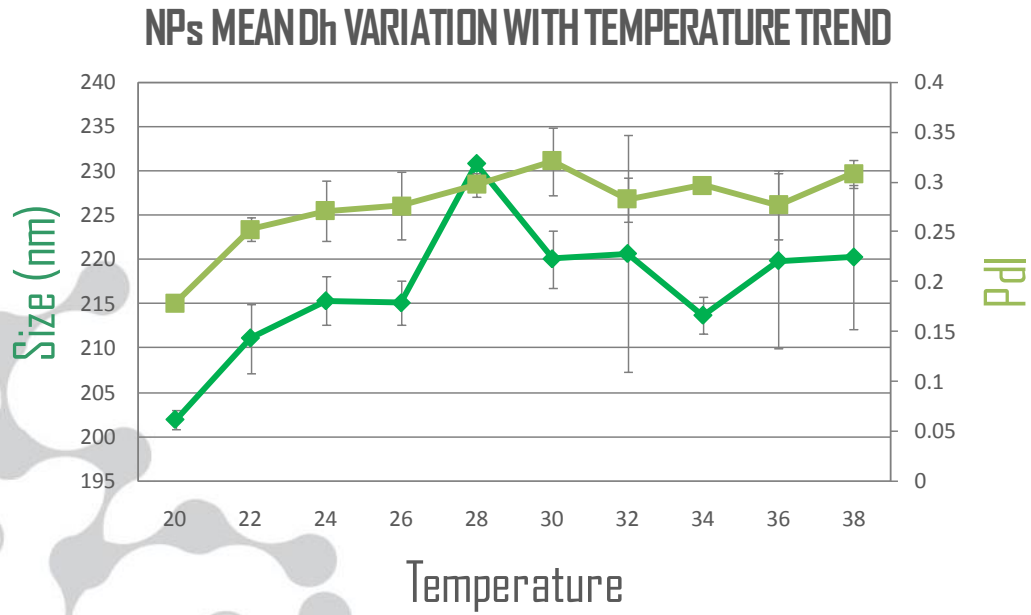
Polyvinyl alcohol

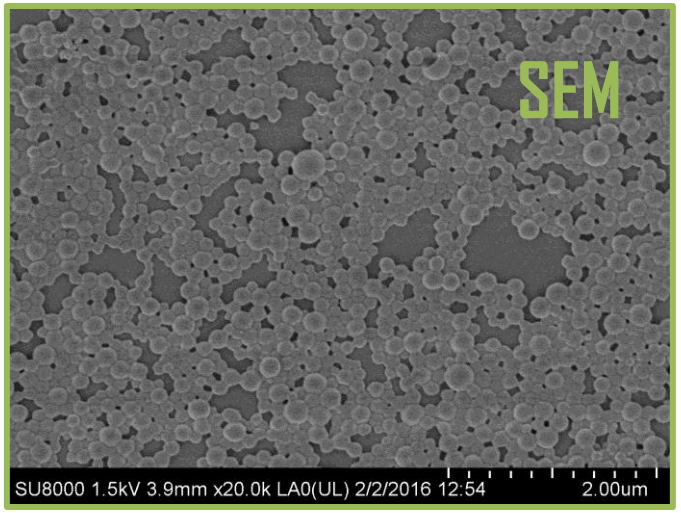


Surfactant effect

TMX loaded PLGA NPs

Polymer	TMX w/w %	Size (nm)	PdI	% EE
PLGA	10	201.7±3.1	0.04±0.01	65
PLGA	10	198.8±1.3	0.19±0.01	74

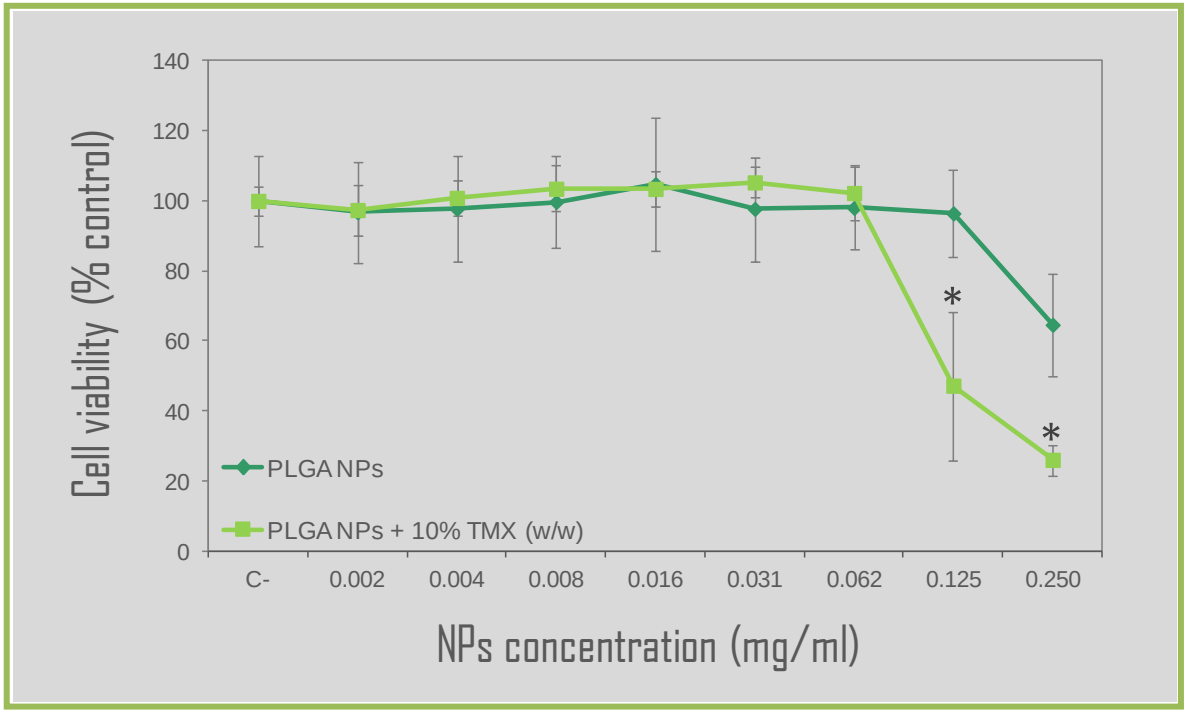




TMX loaded PLGA NPs

Cell Viability Assay

Performed in Human Umbilical Vein Endothelial Cells (HUVEC) and determined by Alamar Blue.
Briefly, lyophilized NPs were resuspended in PBS and different concentrations were tested over 24hs in cells with complete DMEM.



Colaboration with Biomaterials Group- ICTP-CSIC
Álvaro Gonzalez-Gomez
Julio San Roman

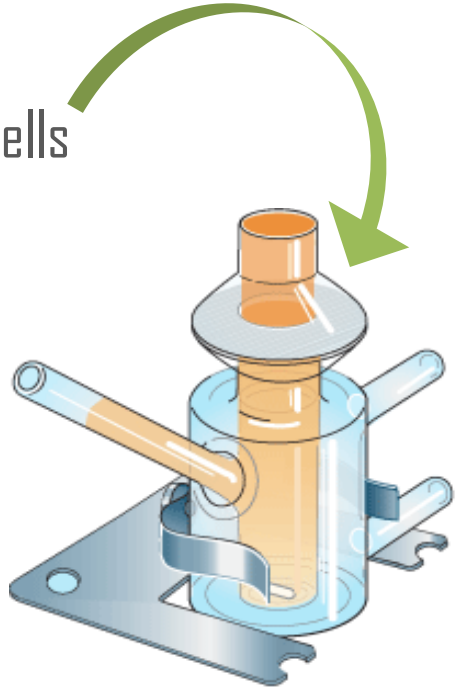
Future Work

TMX loaded PLGA NPs

- Release profile
- In vitro cell viavility assay in MCF-7 breast cancer cell line
- Test transdermal route
- Skin permeation test in a bi-compartimental Franz´s cells
- Active targeting using: PLA-PEG-COOH

in order to compare oral and transdermal route

- TMX loaded PLA nanofibers mat for transdermal route



Polymeric Matrix Composite Materials (CoMP)

INTEMA- CONICET-UNMdP

Argentina

CoMP

You are welcome!

Mar del Plata, Argentina

MUCHAS GRACIAS

THANK YOU VERY MUCH