LEITAT Technological Center
managing your technologies
member of TECNIO

Polymeric Nanocapsules as tools to face challenges in Regenerative Medicine

ETPN RegMed WG 23rd August 2016

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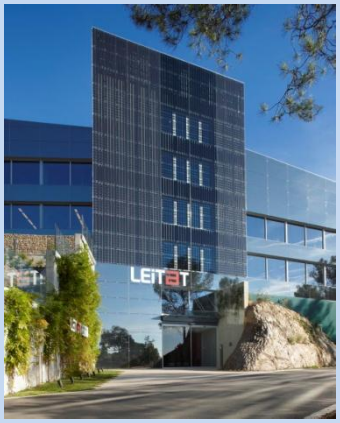
LEITAT

About us

Leitat is the brand of the institution **Acondicionamiento Tarrasense,** a private and non-profit organisation. It is recognised by the Catalan Government (TECNIO) and by the Spanish Ministry of Science and Innovation.

Since 1906

We develop and bank on development, expanding activities towards the knowledge generation and its transfer to the productive fabric.



MISSION

Create and transfer economic, social and sustainable value to companies and entities, through research and technology processes.

VISION

Be a Technology Partner to companies and Administration, by generating a corporate culture allowing sustained growth and efficient functioning.

CORPORATE CULTURE

PRINCIPLES:
We believe in

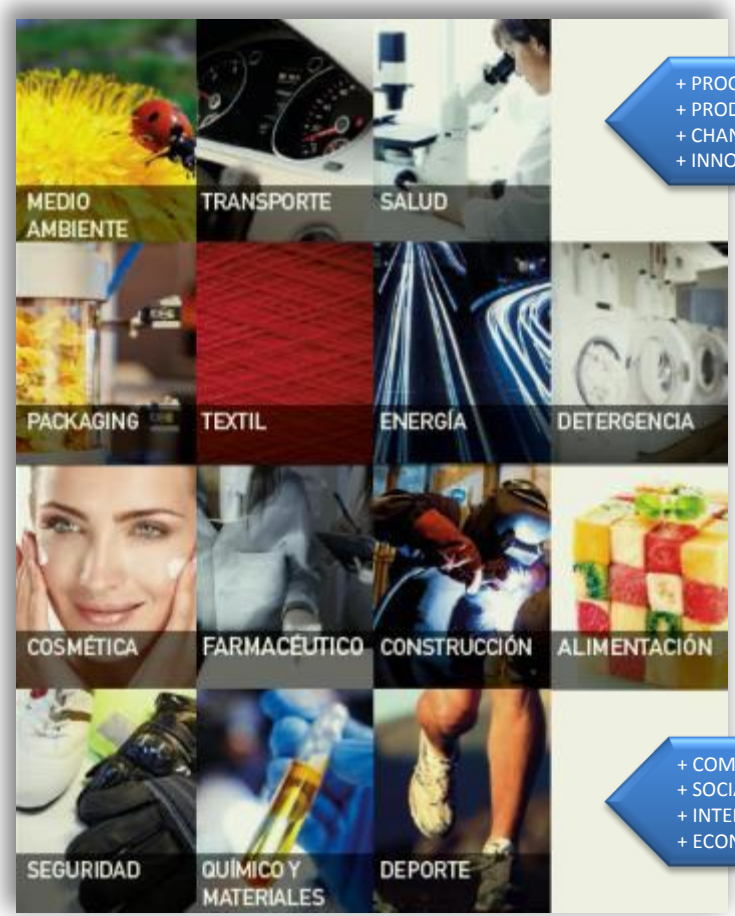
- Creativity
- Innovation
- Sustainability
- Environmental Awareness
- Diversity
- Efficiency
- Efficacy

VALUES:
We act with

- Dynamism
- Independency
- Commitment
- Confidentiality
- Market-orientation
- Global perspective
- Talent

LEITAT

Who we target?



+ PROCESS IMPROVEMENT
+ PRODUCTS IMPROVEMENT
+ CHANGE ADAPTATION
+ INNOVATION CAPACITY

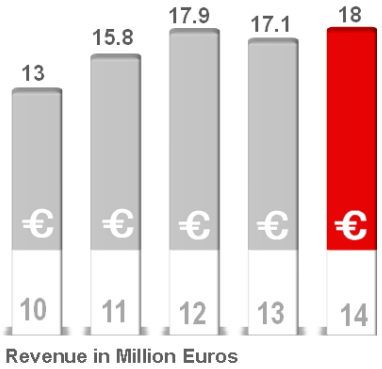
+ COMPETITIVE IMPACT
+ SOCIAL IMPACT
+ INTERNATIONALIZATION
+ ECONOMIC RETURN

MULTISECTORAL ANSWERS
to the technology needs of the companies and institutions

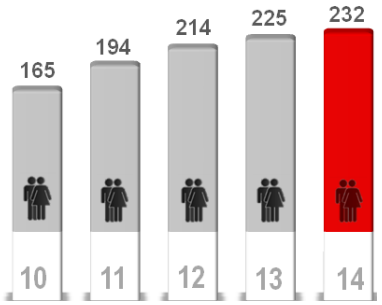
Large companies
Multinational companies
SMEs
Investment Funds
Public Entities
Sponsors

LEITAT

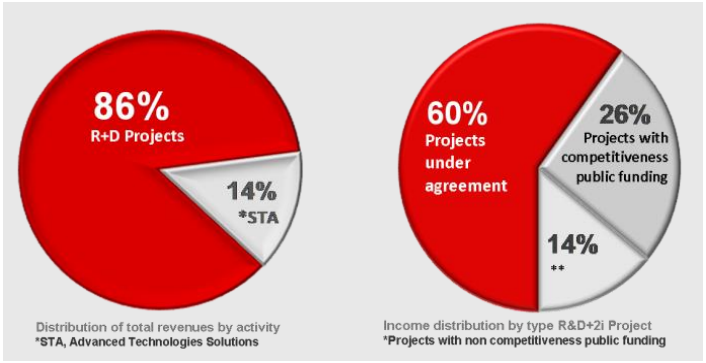
Results

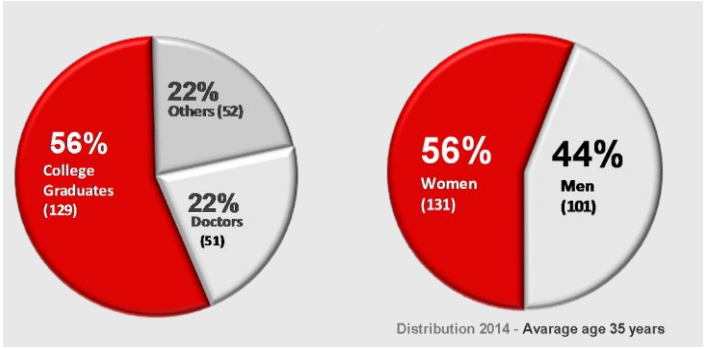


Revenue in Million Euros



Number of collaborators (average)





Figures 2014

265 Proposals managed
137 R+D+2i projects being executed*
4 Lead projects
240 Private R+D+2i projects
2.915 Advanced technology solutions
2 Patents

*We participate in European projects with **839** partners, overall budget of **432M €** and collaborating with **28** countries

\+ Corporate Social Responsibility

Our customers' value: ***Quality, personal contact, clarity of results***
Society value: ***Innovation, sustainability and environmental responsibility, market orientation***
Level of loyalty (future collaboration and recommendation) **> 97%**

LEITAT NMP Results – 2014-15

58 M€ secured (2014-2015)

EUROPE RTO Top list - 2014

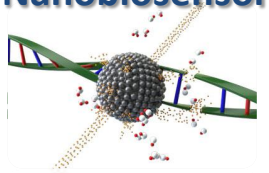
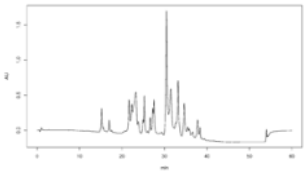
CEA	15	10.043.683,75 €
Fraunhofer	14	8.733.357,25 €
TECNALIA	7	4.698.132,50 €
LEITAT	7	4.518.352,75 €
CNRS	8	4.398.264,25 €
CIDETEC	4	4.306.997,50 €

LEITAT: 1st as NMP coordinator

EL PARTNER TECNOLÓGICO

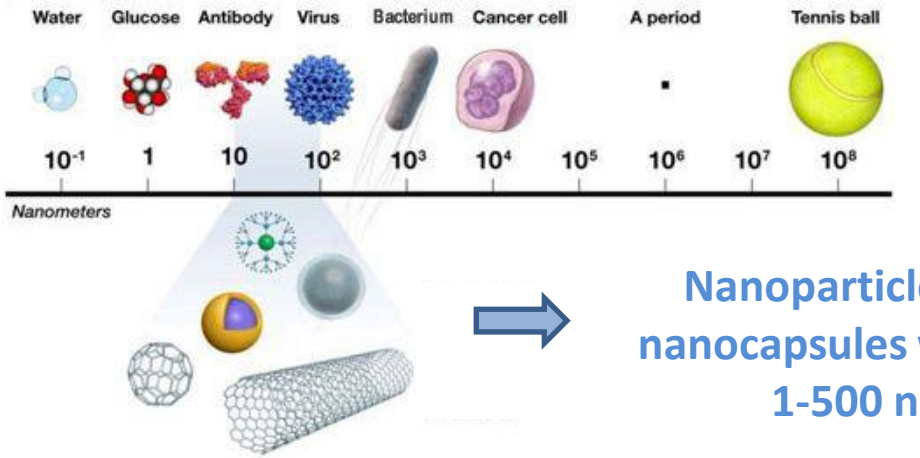
Human & Environmental Health & Safety

Group Leader: Socorro Vázquez Campos

Nanomedicine & Nanobiosensors 	**Materials Safety** 	**Nanotoxicology & Risk Assessment** 
Efficacy & Safety 	***In vitro* kits production** 	**Bioanalytics** 

Nanomed group

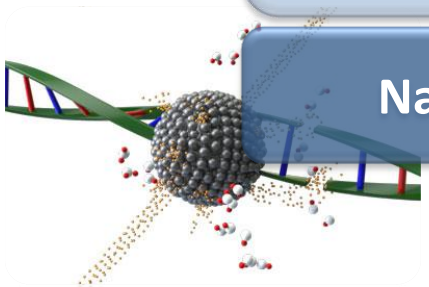
Nanomedicine & Nanobiosensors group



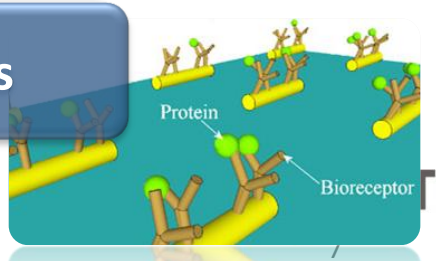
Nanoparticles and nanocapsules with size 1-500 nm

Therapy:
Biofunctionalization of NPs and nanoencapsulation for drug delivery purposes

Diagnostics:
Biofunctionalization of NPs for electrochemical and colorimetric nanobiosensor platforms



Nanomedicine & **Nanobiosensors**



Regenerative Medicine

Nanotechnology approaches (regenerative pharmacology)

- Drug delivery
  - Delivery and retention of complex mixtures of compounds: grow factors (GF) and other pharmacological agents in a specific organ/tissue/cell
- Stem Cells
  - Homing and fixing Stem Cells to their desired site for therapy
  - Stem Cells tracking

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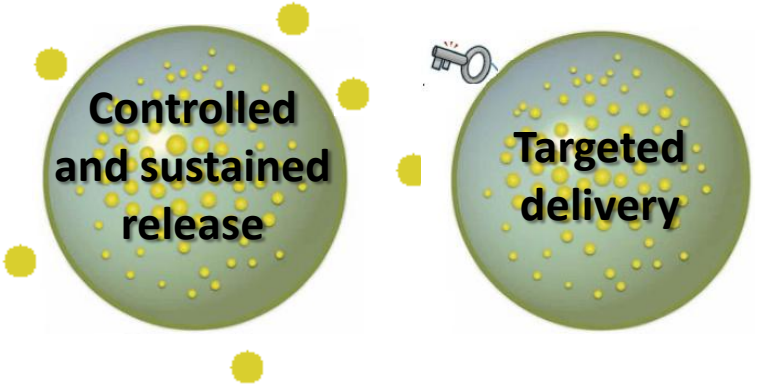
Curative therapeutics

What offer nanoencapsulation in curative therapeutics?

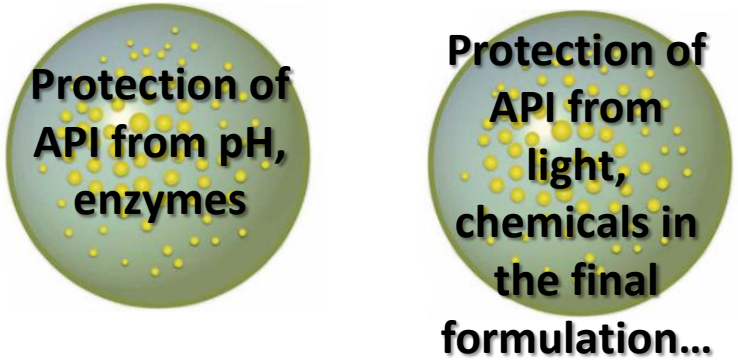
Some APIs produce **side effects** at high doses



No biological action:

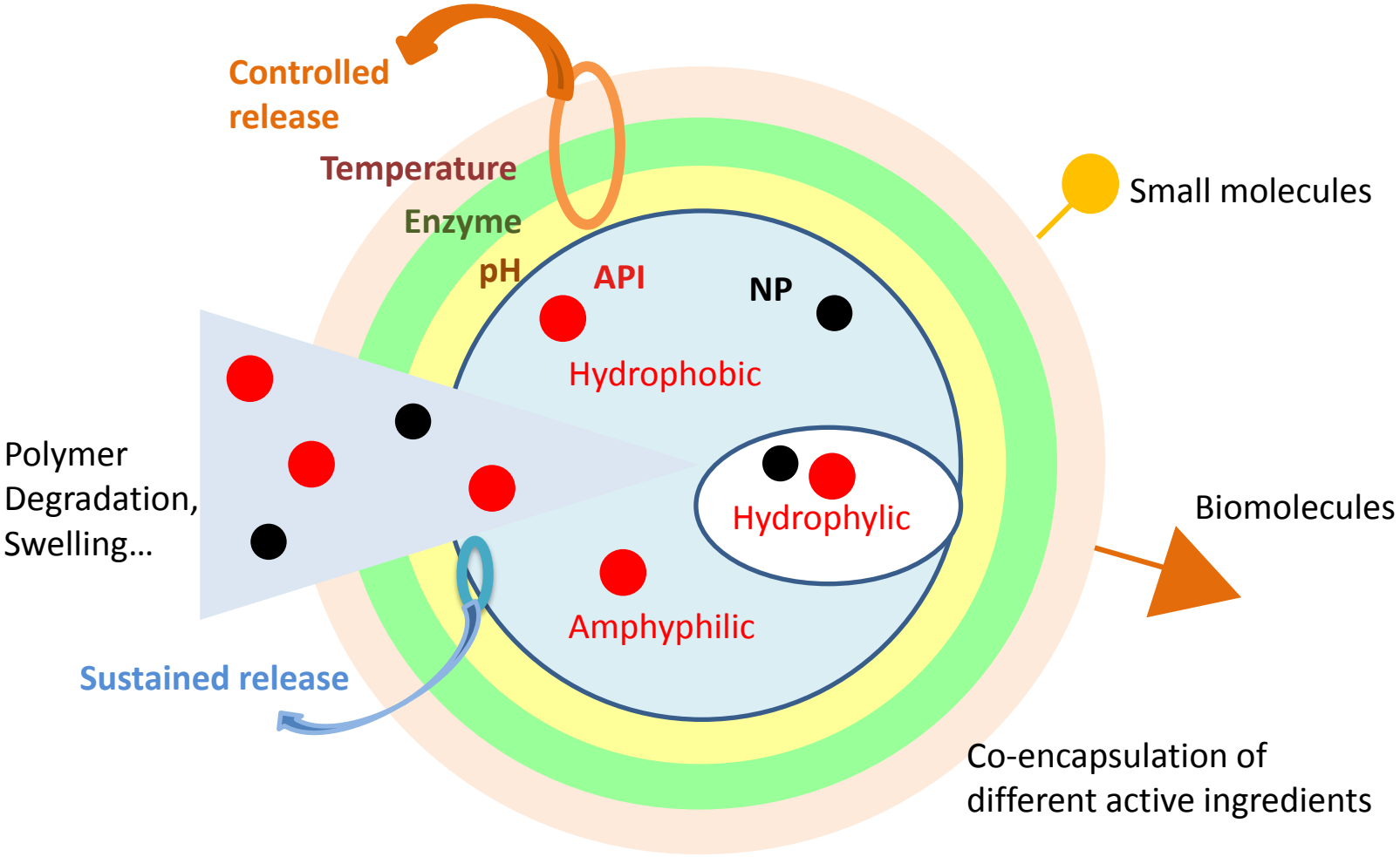
API **doesn't arrive to target cell**

Some APIs have **poor stability in the body**



Some APIs have **poor stability during storage**

Nano Toolbox



Systems for GF delivery

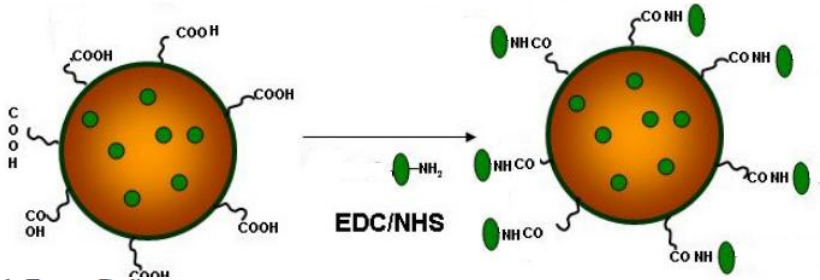
Table III. *In vivo* Investigations of NP Systems for Growth Factor Delivery for Tissue Induction or Regeneration

Growth factor	NP system	Regeneration	Targeted	Targeted tissue	Ligand/tissue interaction	Reference
bFGF (FGF-2)	PLGA	Arteriogenesis	Y (systemic)	Skeletal muscles	Ultrasound facilitated NP deposition	(45)
	Gelatin NPs	Nerve	N			(120)
	Peptide amphiphile	Angiogenesis	N			(127)
	Heparin-conjugated PLGA	Angiogenesis	N			(126)
	Mannan modified PCL-PEG-PCL	Anti-cancer effect	Y (systemic)	Dendritic cells	Mannan and lectin-like receptors on DCs	(52)
NGF	PBCA NPs coated with polysorbate-80	Nerve	N			(54)
	Streptavidin (strep-QDs)	Nerve	N			(118)
	DOPE-PEG-RMP-7 liposome	Nerve	Y (systemic)	Brain	RMP-7 and B2 receptor on BBB	(35)
	P80 coated PBCA	Nerve	Y (systemic)	Brain	P80-apolipoprotein E and BBB	(54)
HGF	DOPE-PEG-RGD liposome	anti-fibrotic effect	Y (systemic)	Liver	Cyclic RGD and hepatocytes	(34)
PDGF	Calcium sulfate	Bone	N			(81)
BMP-2	Heparin conjugated PLGA NPs	Bone	N			(46, 110)
	PLGA/F-127 /heparin NPs	Bone	N			(108)
	Magnetic EPC liposomes	Bone	Y (local)	Bone	Magnetic induction	(31)
	HA/collagen nanocomposite	Bone	N			(83)
	PLGA/HA NPs composite	Bone	N			(102)
	Peptide amphiphile	Bone	N			(103)
EGF	DPPC and LPC liposome	Teeth	N			(30)
	PEG coated liposome	Gastric ulcer healing	N			(29)
BMP-7	PLGA NPs	Bone	N			(44)
TGF-β1	Heparin/PEI NPs	Cartilage	N			(109)
	Magnetic EPC liposome	Cartilage	Y (local)	Bone	Magnetic induction	(32)

Y Yes, *N* No

Sufeng Zhang and Hasan Uludağ. Nanoparticulate Systems for Growth Factor Delivery. Pharmaceutical Research, Vol. 26, No. 7, July 2009.

Targeted NPs systems



Sufeng Zhang and Hasan Uludağ. Nanoparticulate Systems for Growth Factor Delivery. Pharmaceutical Research, Vol. 26, No. 7, July 2009.

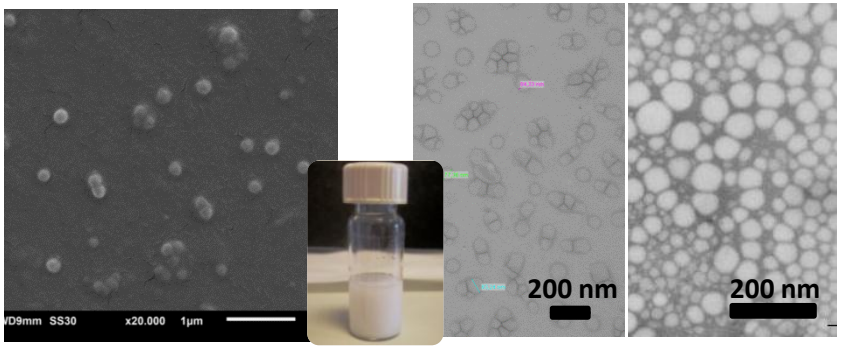
Table II. Summary of Studies on Targeted NP Systems for Growth Factor Delivery

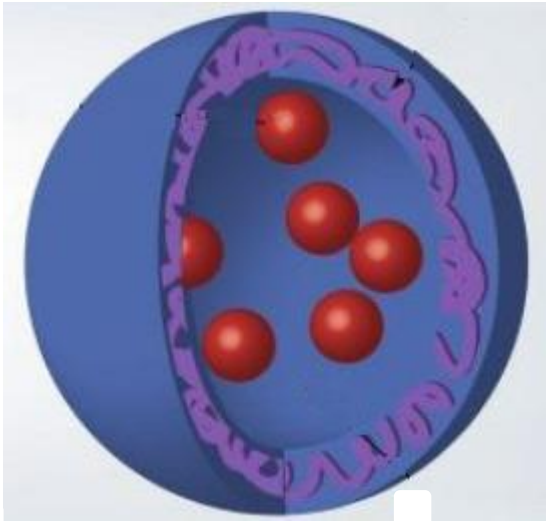
Growth factor	NP system	Route	Study outcome	Reference
bFGF (FGF-2)	Mannan modified PCL-PEG-PCL	SC	bFGF-specific autoantibody titer in mice was significantly higher when bFGF was delivered with mannan-bearing NPs.	(52)
	PLGA	IV	>80% enhancement in diameter of the posterior collateral arterial vessel and an ~11-fold increase in flow capacity of this vessel as compared to BSA NPs-treated control.	(45)
NGF	PBCA coated with polysorbate-80	IP	Injection of PS-80 coated NGF-NPs showed 1.8~2.9-fold higher capacity in the restoration of motor activity than the control (MPTP injected, but no NPs) 7 days after injection. The motor activity was completely restored till day 21 in the NPs treated group, but not in the control.	(54)
	DOPE-PEG-RMP-7 liposome	IV	The targeting efficiency of RMP-7 guided liposome was ~2.1 times higher than the non-targeted liposomes.	(35)
HGF	DOPE-PEG-RGD liposome	IP	HGF encapsulated DOPE-PEG-RGD liposomes stimulated the remission of liver cirrhosis to a significantly higher extent than HGF in liposome without RGD or HGF alone.	(34)
BMP-2	Magnetic EPC liposomes	Topical injection	Magnetic liposomes with BMP-2 showed 1.5~1.7-fold higher radiographic scores and bone formation areas at the defect site than BMP-2 liposomes without magnetite 9 weeks post-operation.	(31)
TGF-β1	Magnetic EPC liposomes	Topical injection	Abundant chondrocyte-like cells by histological analysis and clear positive immunohistological staining around the chondroncyte-like cells at the defect site 8 weeks after treatment with magnetic liposomes containing TGF-β1, but not in other groups.	(32)

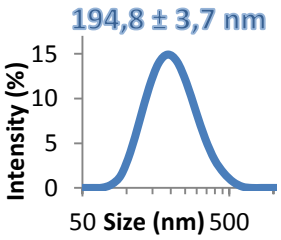
SC subcutaneous injection; *IV* intravascular injection; *IP* intraperitoneal injection; *PCL* poly(ε-caprolactone); *PEG* polyethylene glycol; *PLGA* poly(DL-lactide-*co*-glycolide); *PBCA* poly(butylcyanoacrylate); *MPTP* a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; *DOPE* 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; *RMP-7* receptor mediated permeabilizer-7; *RGD* arginine-glycine-aspartate peptide; *EPC* egg phosphatidyl-choline

Sustained release

~ 150-200 nm







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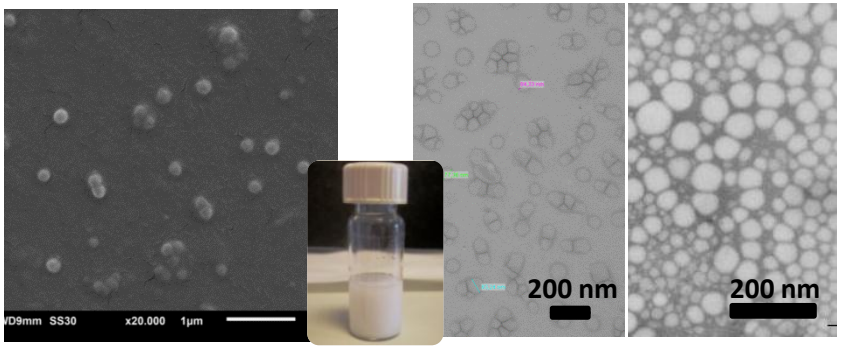
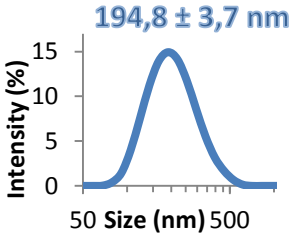
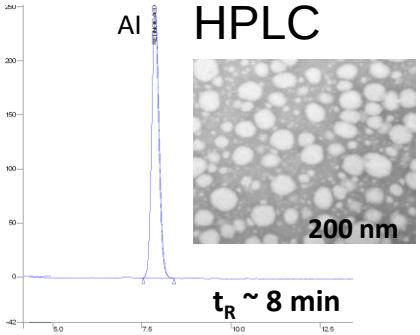
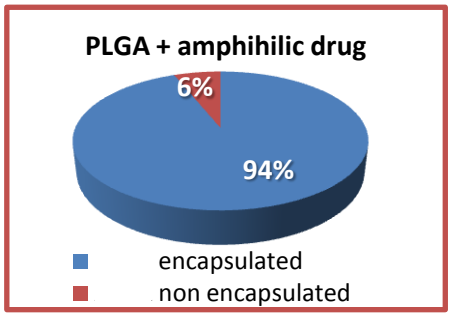
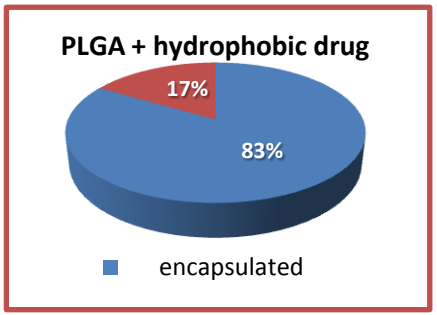


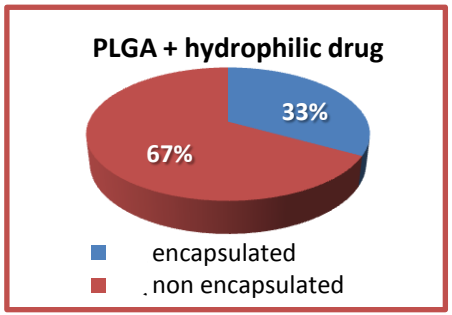
Versatile nanocapsules: **APIs with different physical and chemical nature**





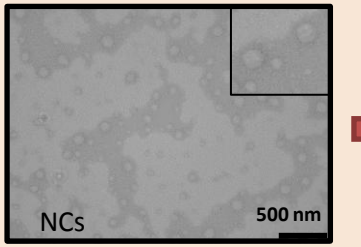


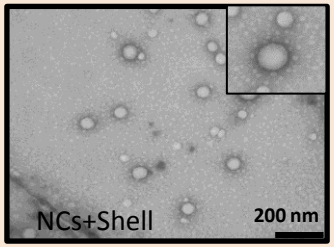


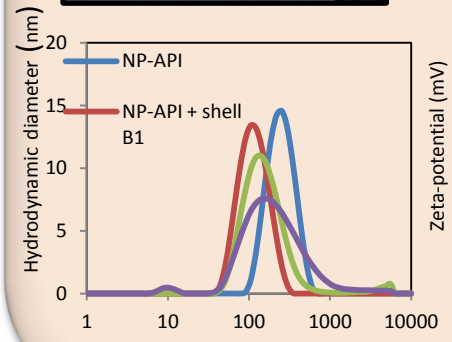


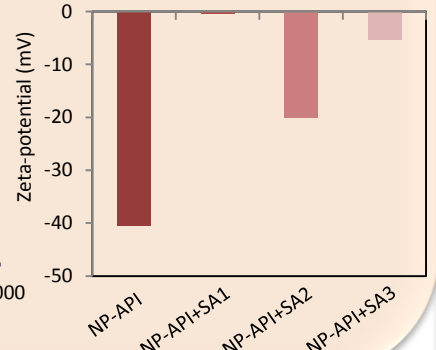
On switch (smart systems) + sustained release

pH sensitive nanocapsules





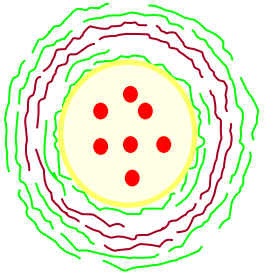




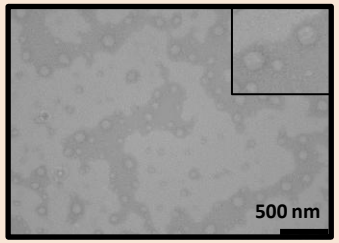
Labile to pH 5.5

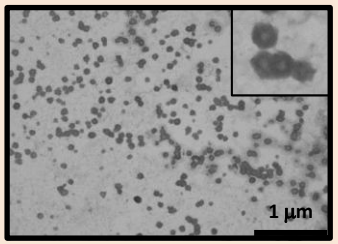
Labile to proteases

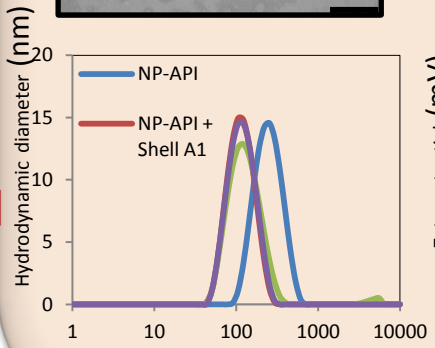
- Negative polyelectrolyte
- Positive polyelectrolyte
- API
- Biodegradable polymer

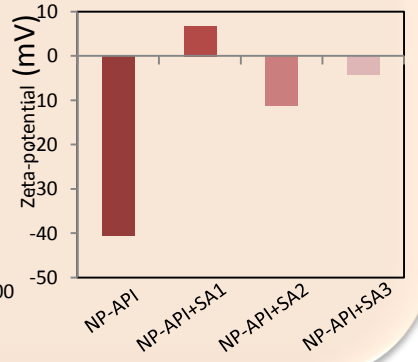


Enzyme sensitive nanocapsules

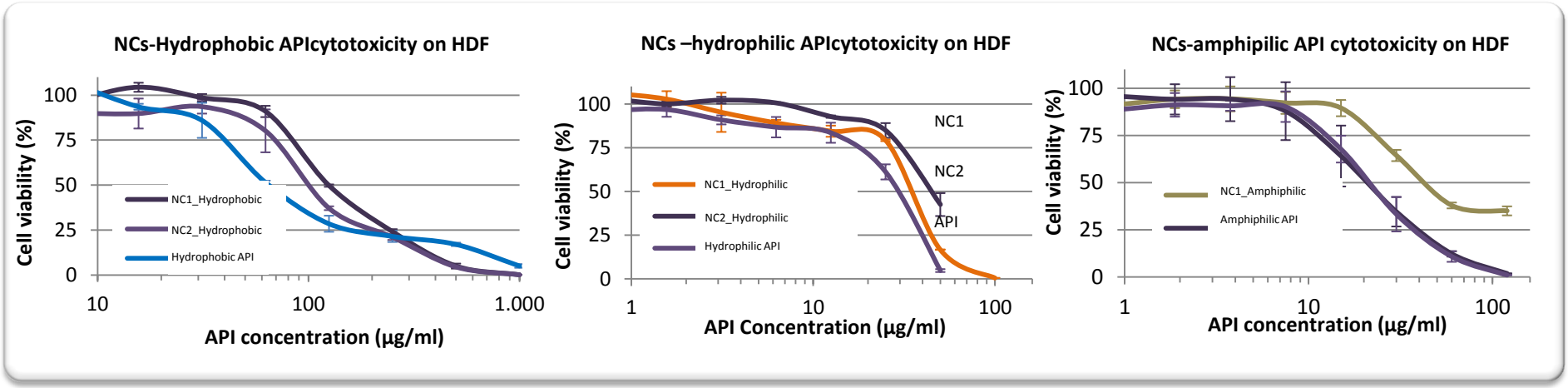








Toxicity & Efficacy

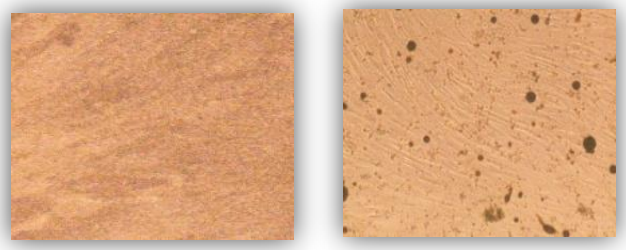


Encapsulated active is less toxic than free on human dermal fibroblasts (HDF)

Toxicity & Efficacy

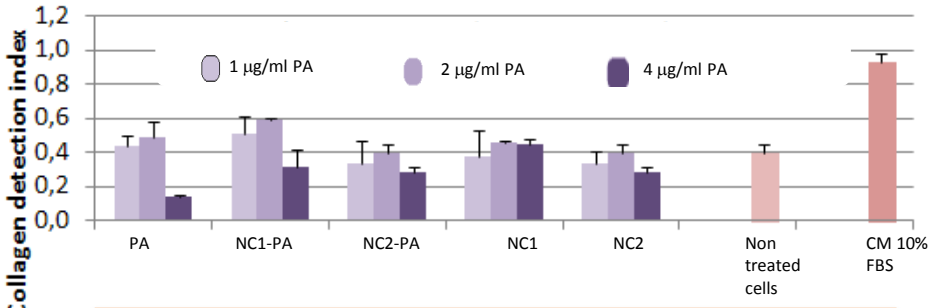
HUMAN DERMAL FIBROBLASTS

NC+PA (120µg/ml) PA(120µg/ml)



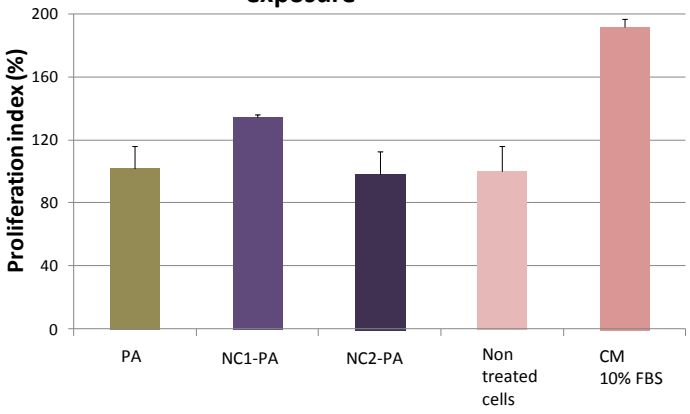
Nanoencapsulation offers **solubility and bioavailability** of AI

Collagen I production on HDF after 72h of treatment



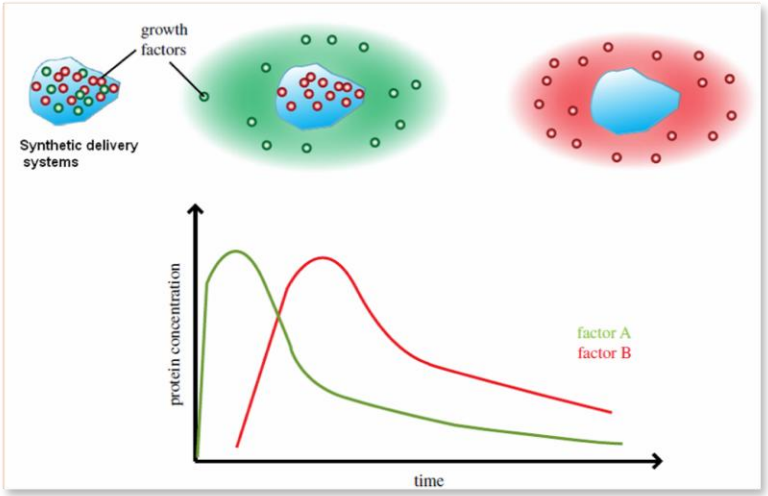
The **efficacy** is increased with nanoencapsulation

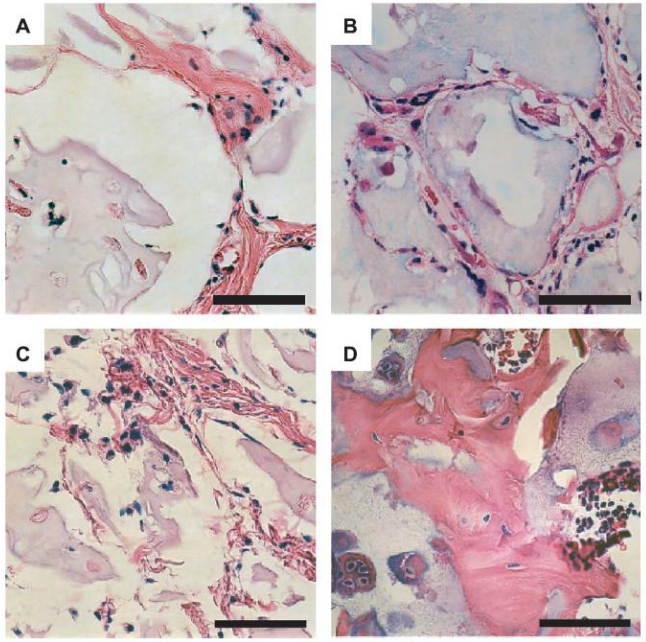
Proliferation index of HDF after 24h of exposure



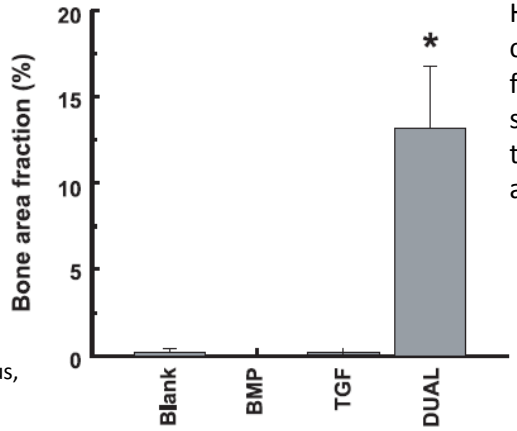
Co-encapsulation. Synergic effect

Bone regeneration





(A) blank, (B) BMP2 only, and (C) TGF-h3 only conditions, the new tissue was primarily fibrous, with little evidence of bone formation. However, in the implants with BMP + TGF (D), there was significant bone formation (pink).



Histomorphometric analysis confirmed the dual growth factor condition had significantly more bone tissue than any other condition analyzed

Craig A. Simmons, Eben Alsberg, Susan Hsiong, Woo J. Kim, and David J. Mooneya. Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. Bone 35 (2004) 562– 569

Tendon and muscle tissue

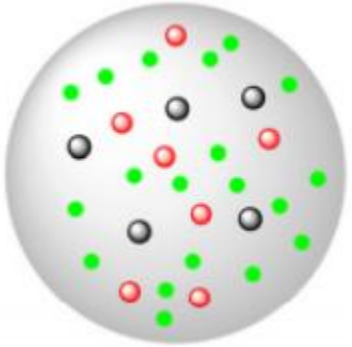
GENERATION OF A NEW MODEL OF SKELETAL MUSCLE LESION IN RATS

GENERATION OF A NEW MODEL OF ACHILLES TENDON INJURY IN RATS

MuscleTech Network

Most research network in muscle and tendon

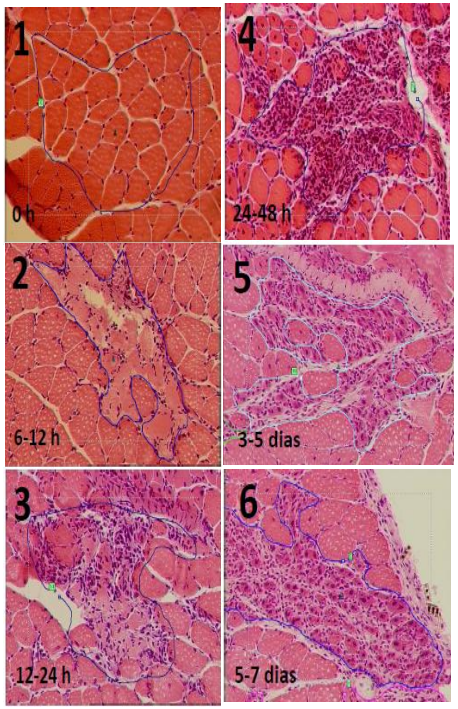
Co-encapsulation growth factors
→ Synergic effect



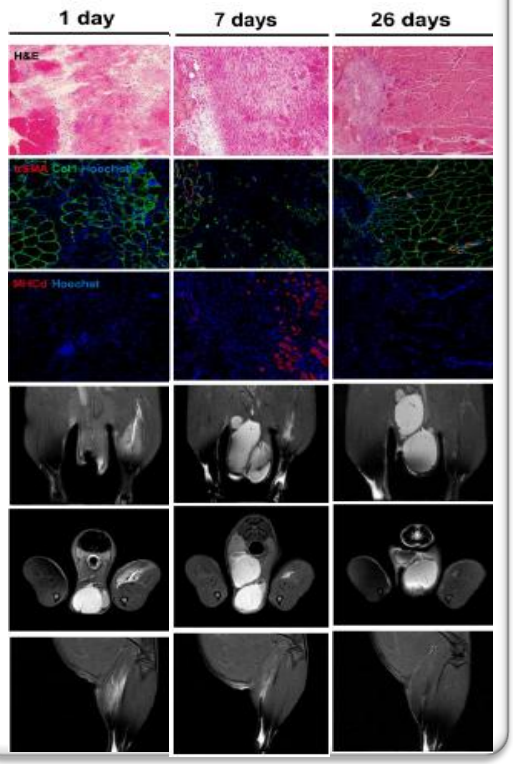
http://muscletechnetwork.org

Tendon and muscle tissue

LESION BY RUPTURE OF MUSCLE SARCOLEMA: NECROSIS-REGENERATION PROCESS



HISTOLOGY, IMMUNOFLUORESCENCE AND MRI



ULTRASOUND INTRAMUSCULAR ANALYSIS

http://muscletechnetwork.org

Regenerative Medicine

Nanotechnology approaches (regenerative pharmacology)

- Drug delivery
  - Delivery and retention of complex mixtures of compounds: grow factors (GF) and other pharmacological agents in a specific organ/tissue/cell
- Stem Cells
  - Homing and fixing Stem Cells to their desired site for therapy
  - Stem Cells tracking

Horizon 2020 H2020 website
Pillar: Industrial Leadership
Work Programme Year: H2020-2016-2017
Work Programme Part: Innovation in SMEs
Call : H2020-SMEInst-2016-2017 Call budget overview

Topic Description

- Less

Specific Challenge:

The healthcare biotechnology sector offers huge business and commercial opportunities; however it also requires heavy and risky investments which are often lacking in Europe, hampering the development of the industry.

The challenge includes either:

a) Cell technologies in medical applications (phase 1 only for 2016 deadlines and phase 2 for all deadlines in 2016 and 2017)

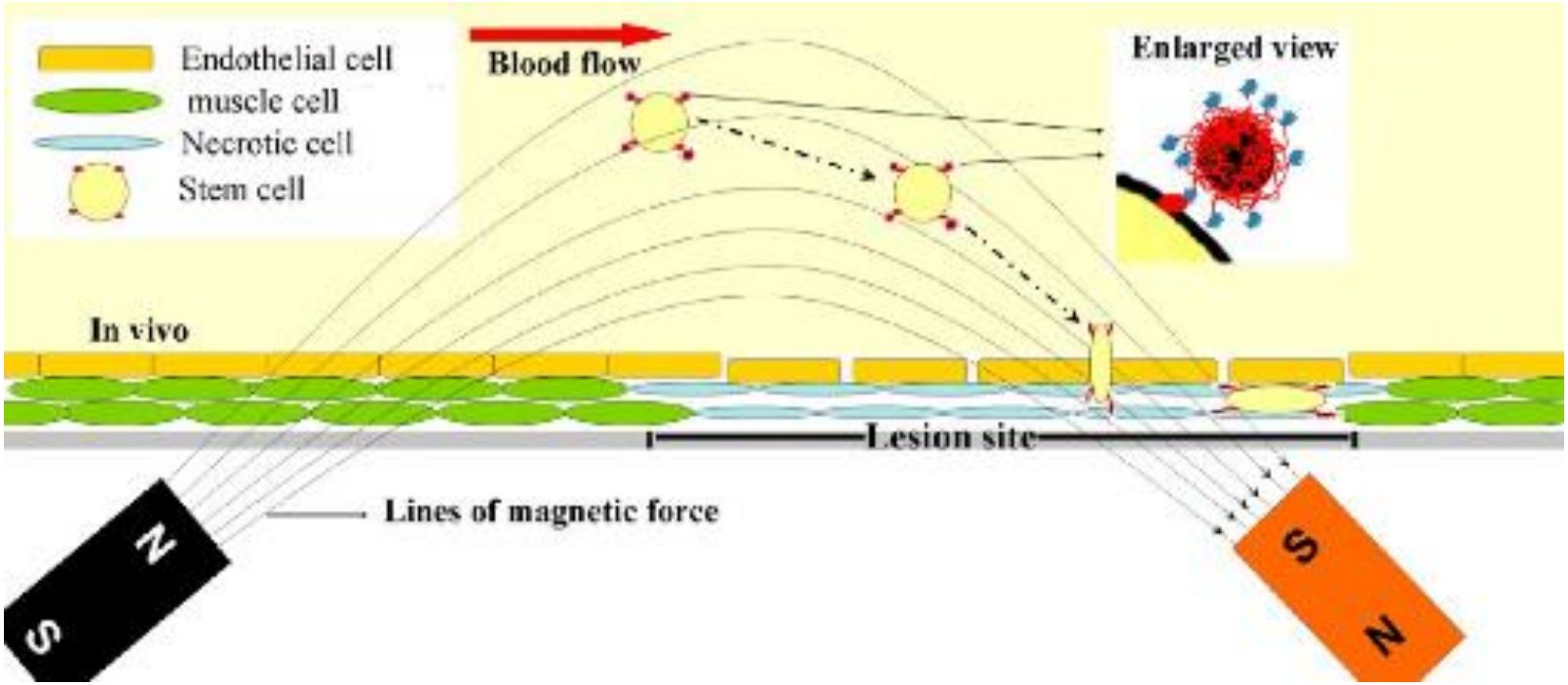
Cell technologies include cell manufacturing (culture, multiplication, scale-up and automation), preservation, banking and transport; identification, cell sorting and delivery, imaging, tracking, process and quality control; genetic engineering and gene editing; production of therapeutic biomolecules. The medical applications of cell technologies include diagnostics and biosensors; cell and gene therapy, tissue engineering, bio-artificial organs, haematology, immunotherapy, and vaccine and antibody production; predictive toxicology, synthetic biology, and modelling development and disease processes.

However, the diversity, complexity and variability of living cells pose challenges for bringing safe, reliable, regulatory-compliant and cost-effective products to the market and to the patient. SMEs developing cell-based products and processes have limited financial resources to take the critical steps to move from proof of concept to practical application while at the same time addressing considerations such as scale-up/scale-out, automation, logistics, regulatory pathways and business models.

Particular attention should be given to dialogue with regulators and compliance with safety and regulatory requirements, such as those pertaining to cell procurement, GMP, ethics, clinical trials, ATMPs and medical devices.

The challenge addresses cells from any eukaryotic source though their eventual application must be to human medicine.

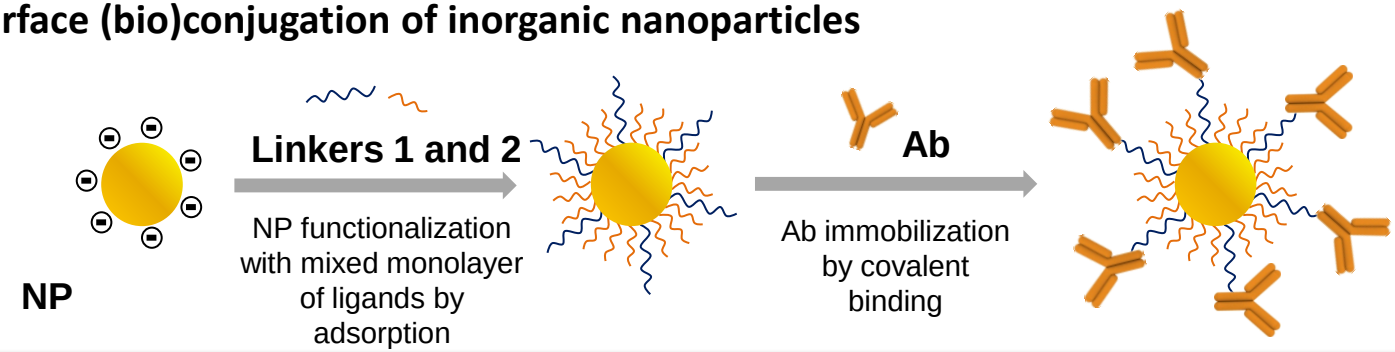
Homing and fixing SC SMEInst



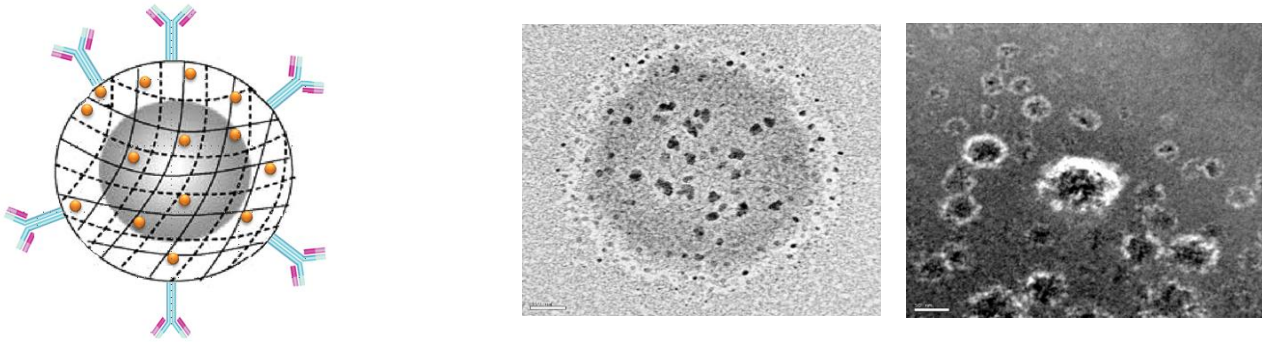
Chen, J.; Huang, N.; Ma, B.; Maitz, M.F.; Wang, J.; Li, J.; Li, Q.; Zhao, Y.; Xiong, K.; Liu, X. Guidance of stem cells to a target destination in vivo by magnetic nanoparticles in a magnetic field. ACS Appl. Mater. Interfaces 2013, 5, 5976-5985

Nanosystems

Surface (bio)conjugation of inorganic nanoparticles



Encapsulation of inorganic NPs in polymeric NPs



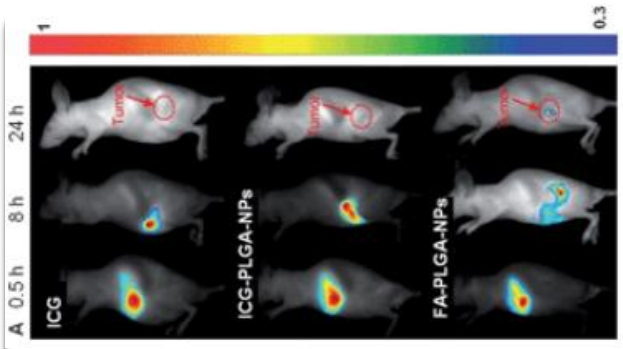
Topics of interest

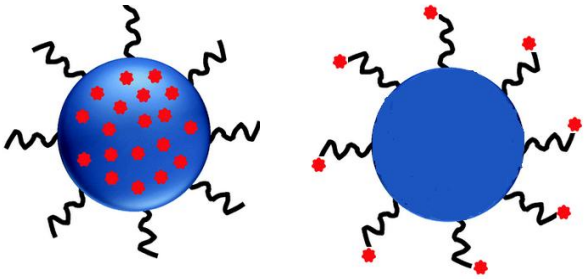
Topic	Title	Type of action
1.1 Understanding health, well-being and disease		
PM 02 – 2017	**New concepts in patient stratification**	**RIA**
PM 03 – 2017	Diagnostic characterisation of rare diseases	RIA
1.3 Treating and managing diseases		
PM 08 – 2017	New therapies for rare diseases	RIA
PM 11 – 2016/2017	**Clinical research on regenerative medicine**	**RIA**
1.4 Active ageing and self-management of health		
PM 15 – 2017	**Personalised coaching for well-being and care of people as they age**	**RIA**
1.5 Methods and data		
PM 16 – 2017	In-silico trials for developing and assessing biomedical products	RIA
PM 17 – 2017	Personalised computer models and in-silico systems for well-being	RIA
ADVANCED MATERIALS AND NANOTECHNOLOGIES FOR HEALTHCARE		
NMBP-12-2017	Development of a reliable methodology for better risk management of engineered biomaterials in Advanced Therapy Medicinal Products and/or Medical Devices	RIA
NMBP-13-2017	**Cross-cutting KETs for diagnostics at the point-of-care**	**RIA**
NMBP-14-2017	Regulatory Science Framework for assessment of risk benefit ratio of Nanomedicines and Biomaterials	RIA
NMBP-15-2017	**Nanotechnologies for imaging cellular transplants and regenerative processes in vivo**	**RIA**

Nanotechnology approaches (regenerative pharmacology)

- Drug delivery
  - Delivery and retention of complex mixtures of compounds: grow factors (GF) and other pharmacological agents in a specific organ/tissue/cell
- Stem Cells
  - Homing and fixing Stem Cells to their desired site for therapy
  - Stem Cells tracking

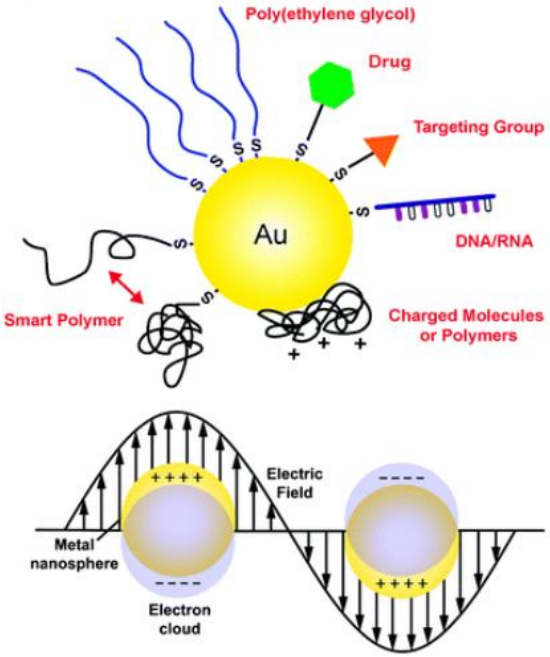
SC tracking



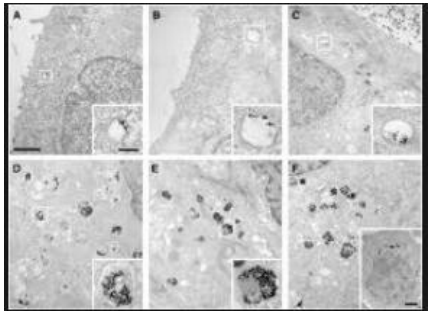


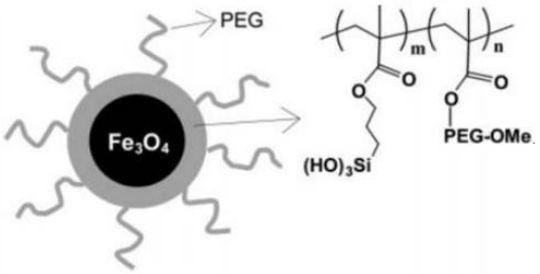
Fluorescent organic dyes

Fluorescent organic dyes could be either physically entrapped in the polymer interior during the preparation of NPs or covalently bound to the polymer chain



The strongly enhanced surface plasmon resonance of Au NPs optical frequencies makes them excellent scatterers and absorbers of visible light

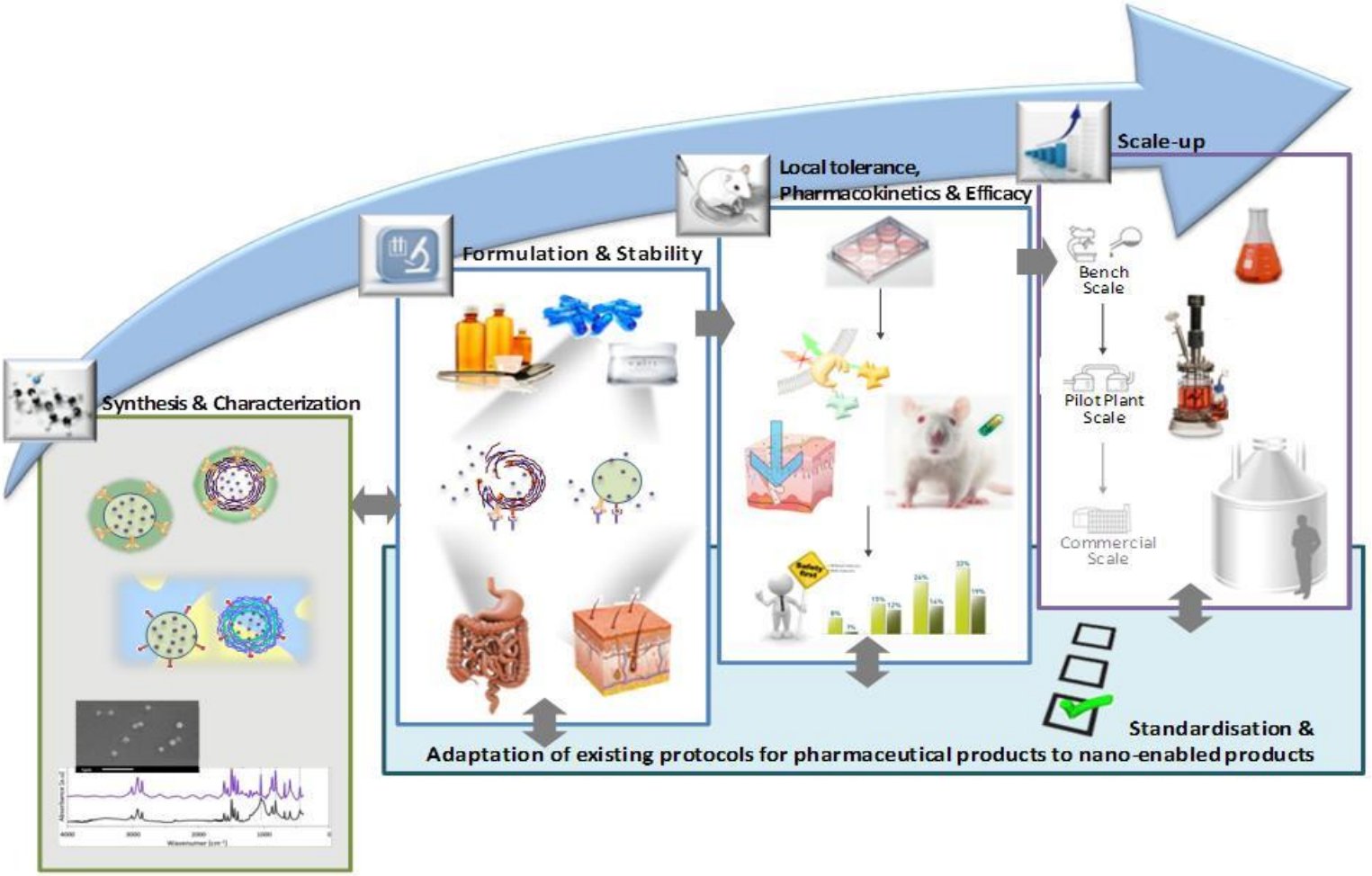




They act as good contrast agents in MRI, enhancing the contrast between different tissues present by inducing a darker area (negative contrast).

Complete development of nanosystems

Nanomedicine: Complete development of nanosystems



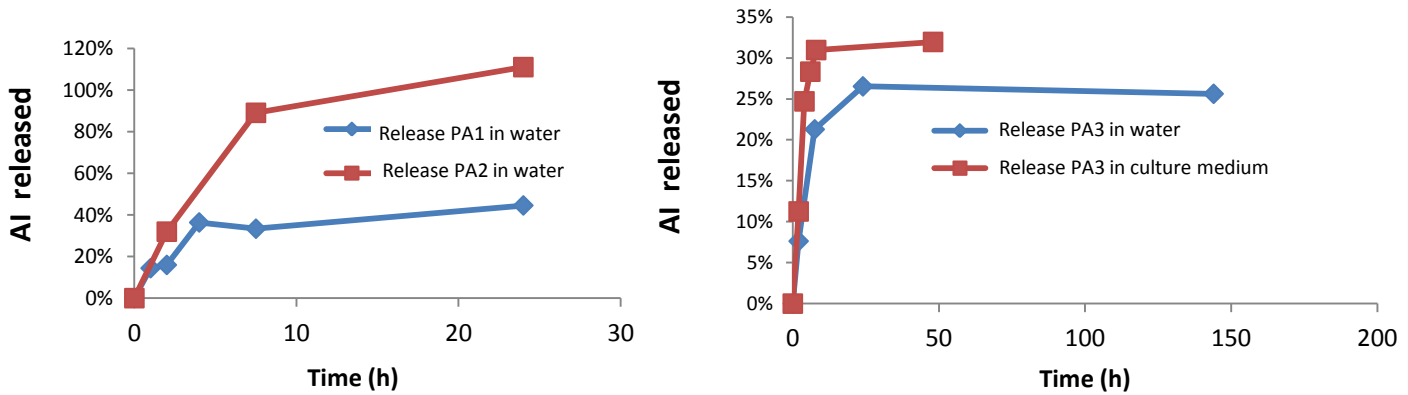
Other services:
Formulation & Stability

Release and stability in final application and storage

Final application

Transformation studies:
-Aggregation state
-NM size and shape
-Release kinetics of the AI
-Chemical degradation

Release studies at **37 °C**



Formulation & Stability

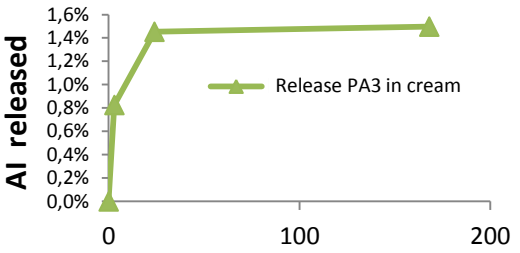
Release and stability in final application and storage

Storage

Parameters affecting stability: T, light, pH, final formula composition

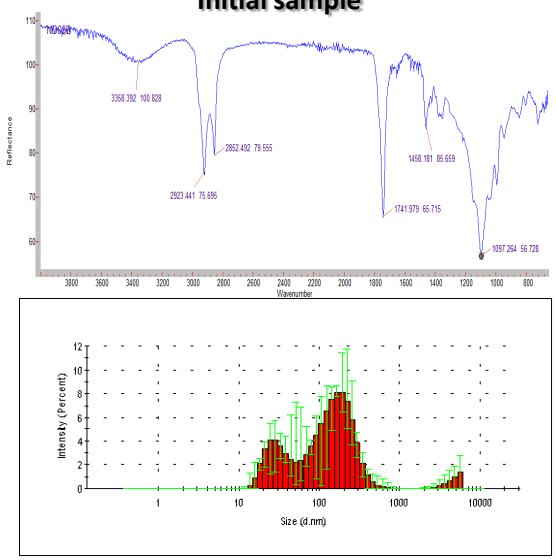
Transformation studies:
-Aggregation state
-NM size and shape
-Release kinetics of the AI
-Chemical degradation

Release studies at **4 and 25 °C**

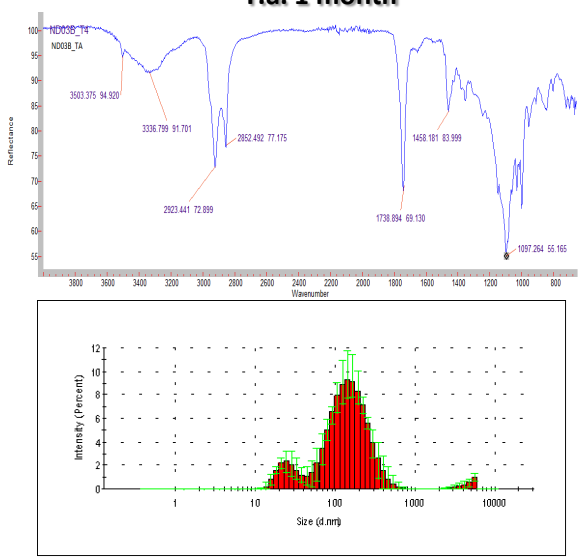


Physical and chemical structure

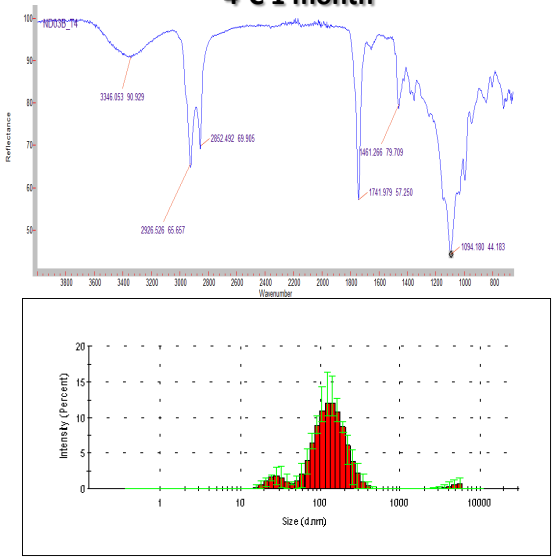
Initial sample



T.a. 1 month



4°C 1 month



Local tolerance, Pharmacokinetics & Efficacy

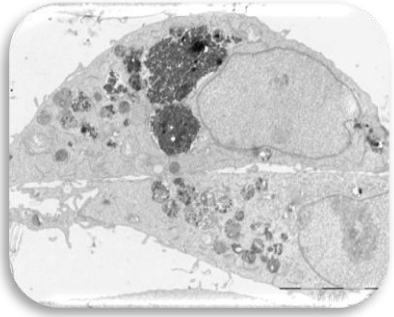
Nanotoxicology

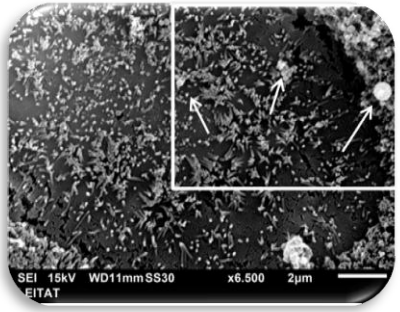
In-vitro toxicity

- Viability, proliferation, apoptosis
- Oxidative stress
- Inflammatory responses
- Phagocytic activity
- Phototoxicity
- Genotoxicity/Photogenotoxicity
- Membrane integrity

In-vivo toxicity / biokinetics

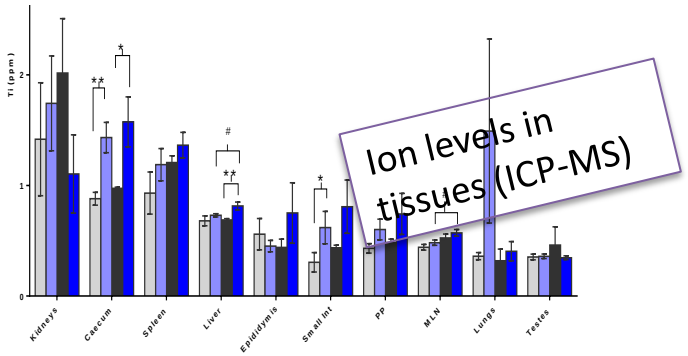
- Animal models: Rats and mice
- Administration Routes: Oral, dermal, parenteral
- OECD-like evaluations + Nanospecific focus





In-vitro biokinetics

- Cell uptake and intracellular trafficking
- Intestinal barrier permeability
- ICP-MS, confocal microscopy, fluorescent microscopy, TEM, SEM.



Nanotox and Efficacy

ADME

Metabolism and Tox

- Hepatotoxicity
- Stability
- Clearance
- Metabolic profiling
- Enzyme mapping
- Panel Screening
- Bio-analytics

Absorption

- Intestinal
- Kidney
- Brain barriers
- Gastric epithelium
- Cell transport

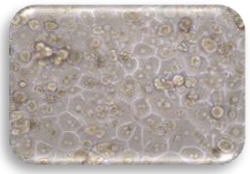
Skin biology and topical Nc

Safety

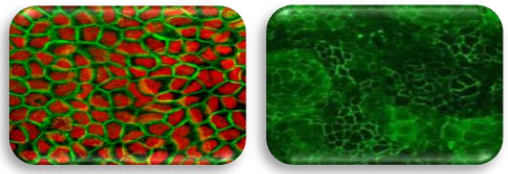
- Skin permeation
- Percutaneous absorption
- In vitro skin irritation
- In vitro ocular irritation
- Skin sensitisation
- Skin inmuno and neuro inflammatory responses
- Skin disorders models

Efficacy

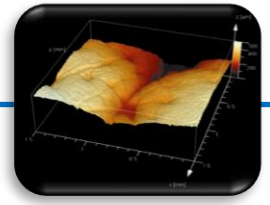
- Anti-aging and vitalizing
- Anti-oxidant
- Anti-inflammatory
- Firmess and elasticity
- Moisturizing
- Skin and DNA repair/protection
- Photoprotection

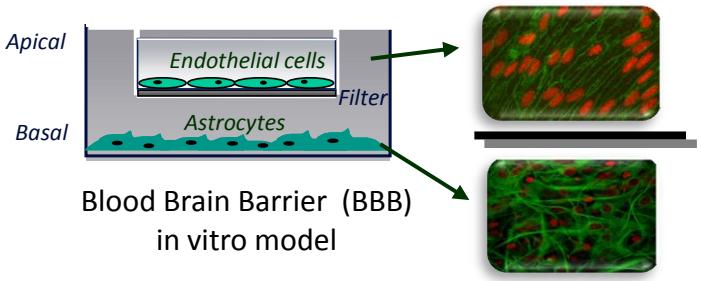


Human Hepatocytes

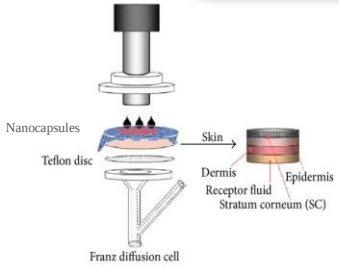


In vitro intestinal models



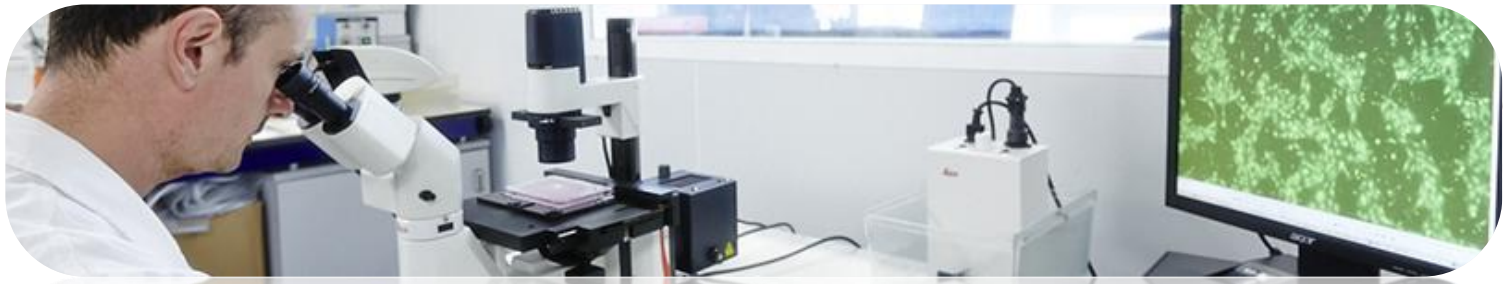


Blood Brain Barrier (BBB) in vitro model

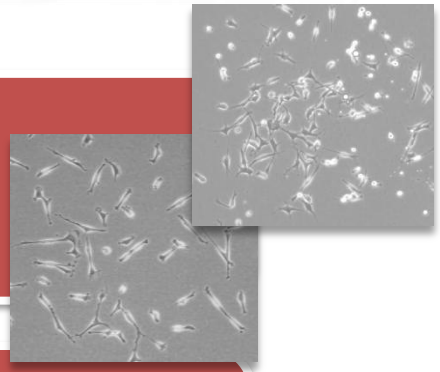


Human skin samples

Skin explant culture



Other Capacities - RegMed

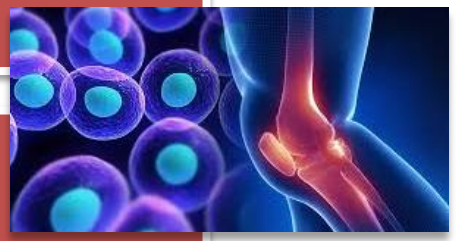


Stem cells Biology

- Primary cell cultures from human or animal models
- Isolation and characterization of stem cells

Cell Therapy

- Stem cell transplantation *in vivo*. Advanced surgical techniques or ultrasound-guided cell implantation.
- Small (mice, rat) and large (rabbit, pig, sheep) animal models



Advanced surgical techniques in experimental *in vivo* models

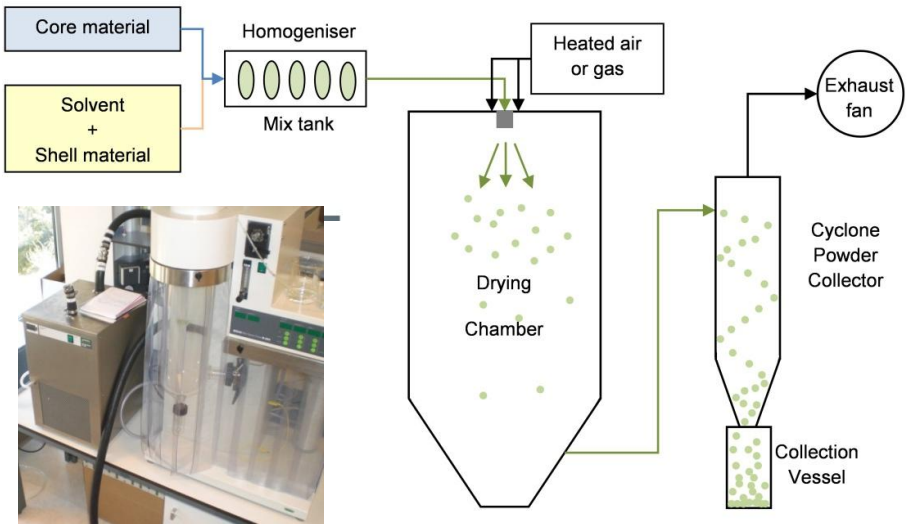
- Skeletal muscle injuries *in vivo* models.
- Tendon injuries (Achilles and patellar) *in vivo* models
- Surgically-induced congenital malformations. Myelomeningocele or diaphragmatic hernia in fetuses in large animal models.

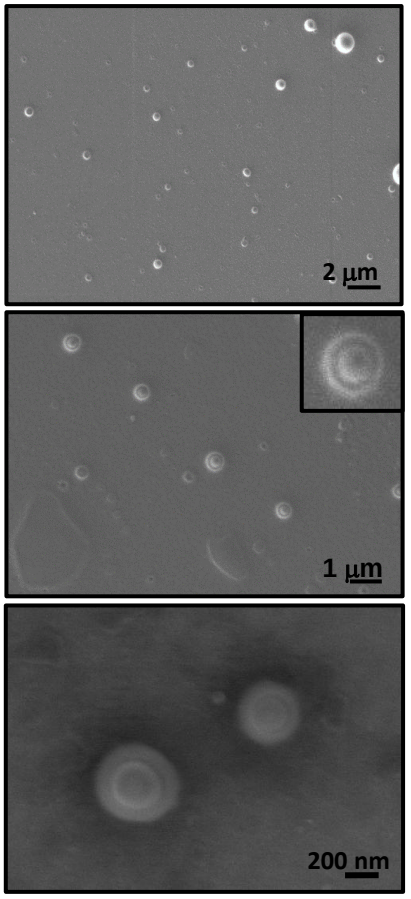
Introduction

Scale-up

Spray dryer tecnique

pH-sensitive nanocapsules





- Relatively uniform spherical particles
- Large production (up to Kg)
- Continuous operation and automatic control
- Sample purification

EL PARTNER TECNOLÓGICO

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