PLA/PLGA nanocarriers designed for tissue engineering application and fabricated by Supercritical Emulsion Extraction

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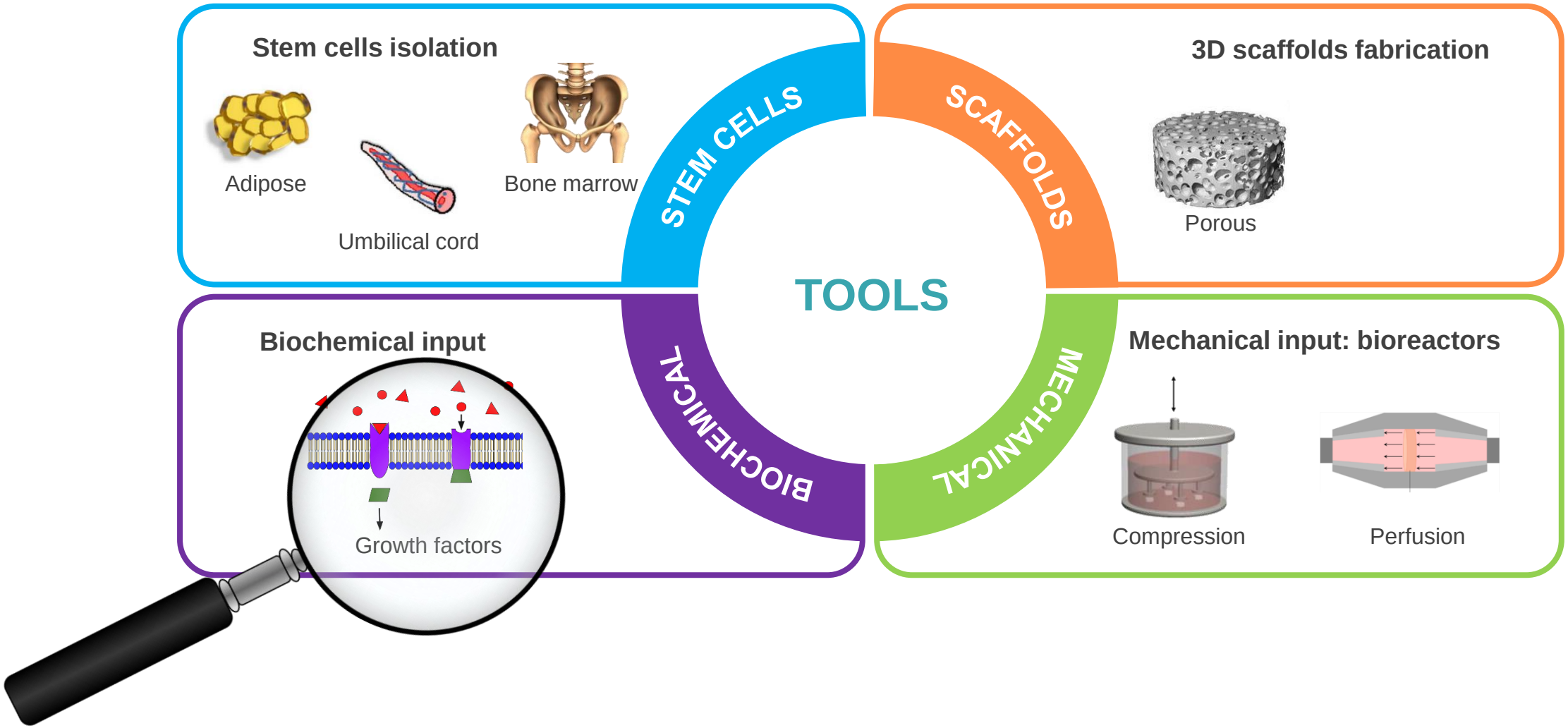
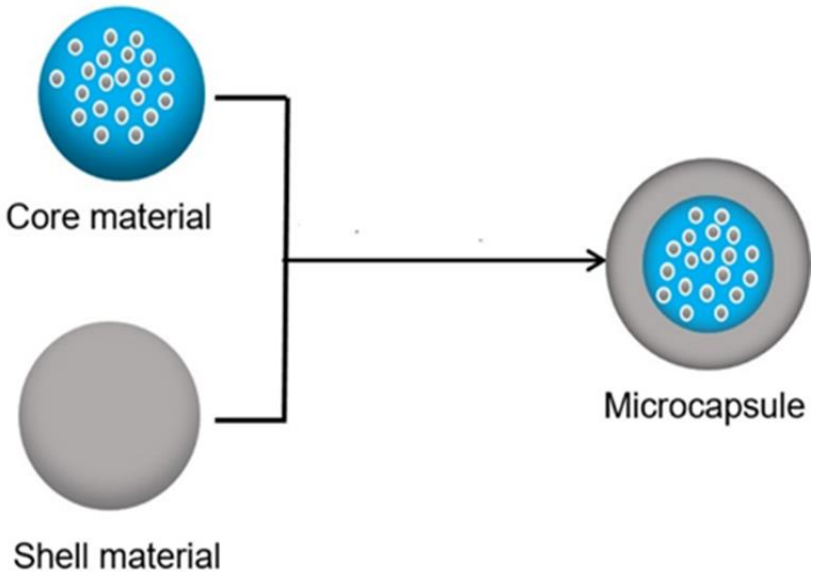
INTRODUCTION

The controlled release of growth factors to promote differentiation of human stem cells, during their tridimensional cultivation into a synthetic matrix, is a major challenge for modern tissue engineering



INTRODUCTION

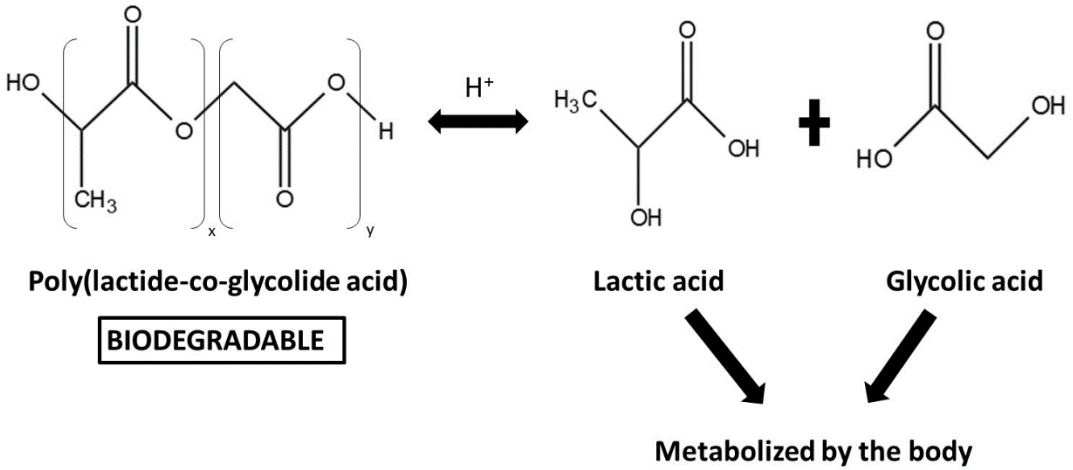
Is it better to use growth factor free or encapsulated ?



The benefits of encapsulation in tissue engineering:

- Confers stability to growth factor
- Improves biodistribution and efficacy
- Provide a controlled release

Among biopolymers, suitable for protein encapsulation, poly-lactic acid (PLA) and poly-lactic-co-glycolic acid (PLGA) seem most promising, even if the preliminary step for their application in tissue engineering protocols is the kinetic and cytotoxic characterization



AIMS OF THE STUDY

1. Comparative analysis between empty nanocarriers obtained with Solvent Evaporation (SE) and Supercritical Emulsion Extraction (SEE) technologies;

2. Microencapsulation of transforming growth factor beta 1 (TGF-β1) and growth differentiation factor 5 (GDF-5) used respectively to promote chondrogenic and tenogenic commitment;

3. In vitro evaluation of the potential cytotoxic effect of bio-polymeric nanocarriers on different cell lines;

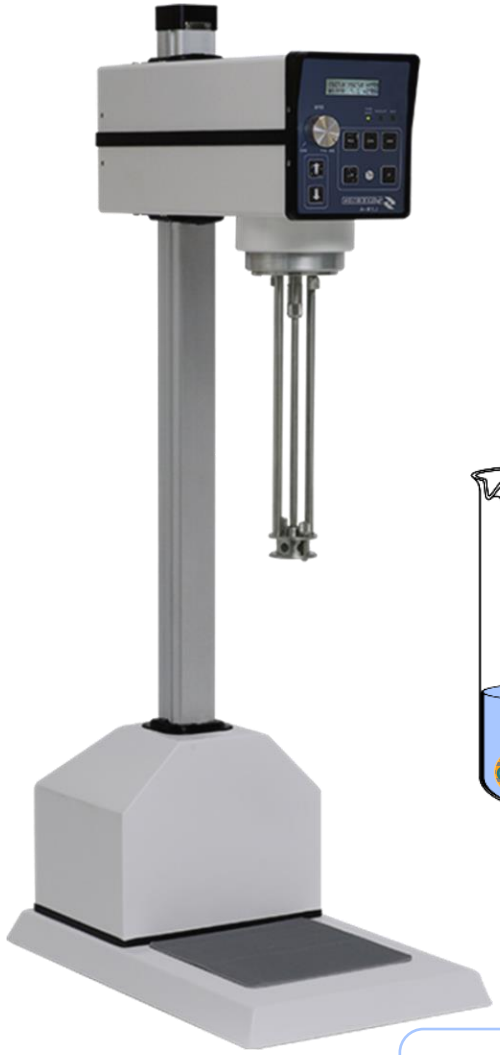
DOUBLE EMULSIONS PREPARATION

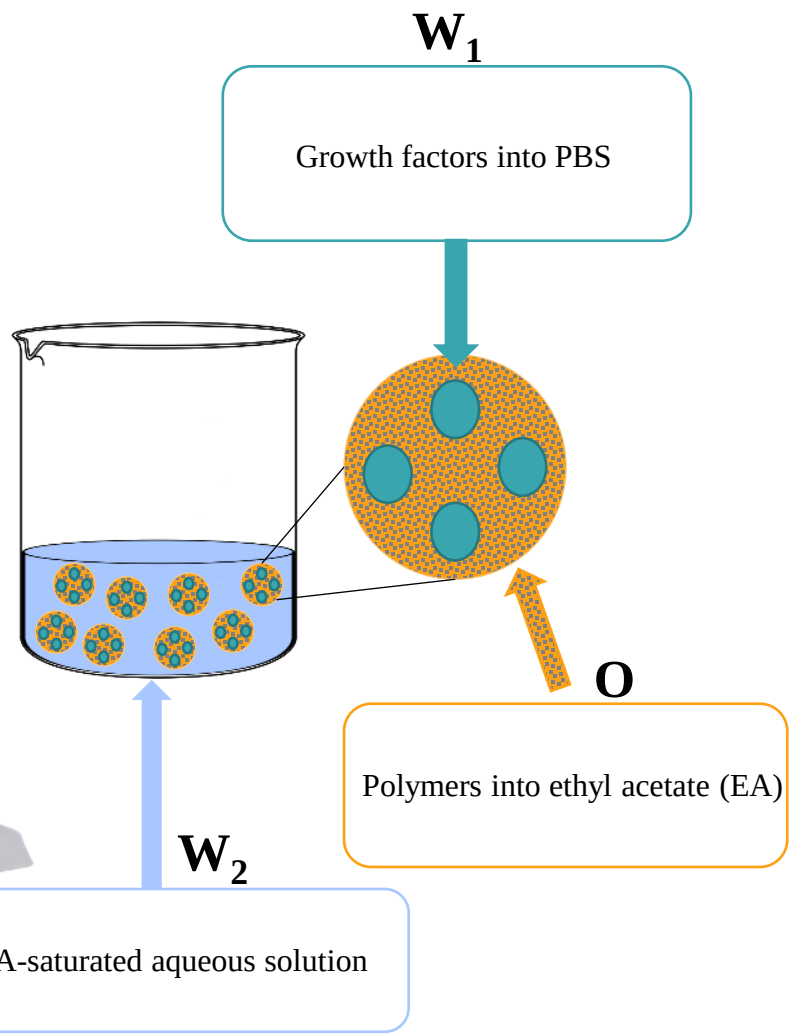
Vortex vs Sonicator

To avoid growth factors denaturation, the primary emulsion was obtained by vortex instead of ultrasound. Indeed, the sonication can induce changes in the structural and thermal properties of proteins.

(Chandrapala et al., 2011)

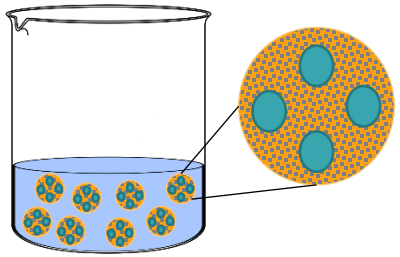
Water/Oil/Water ($W_1/O/W_2$)

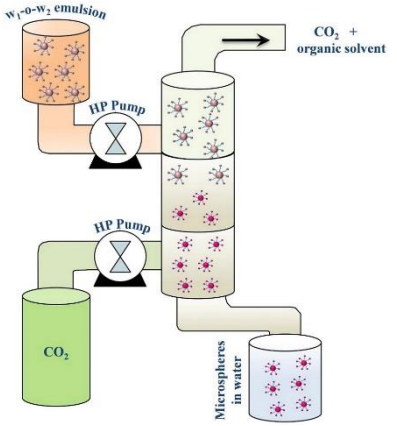




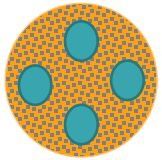
PRODUCTION TECHNOLOGIES

Water/Oil/Water ($W_1/O/W_2$)





SOLVENT EVAPORATION

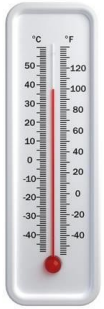


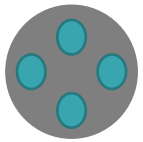
Droplets

organic solvent

SE PROCESS

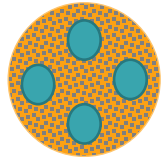
HEATING





Particles

SUPERCRITICAL EMULSION EXTRACTION

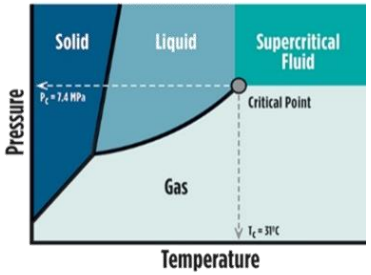


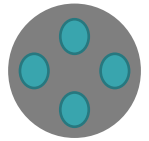
Droplets

scCO2
+
organic solvent

SEE PROCESS

sc CO_2





Particles

Immediately after production, emulsions were stirred at 100 rpm for 3 hours using a temperature of 38 °C in a sterile controlled environment, to allow the solvent elimination by evaporation.

Pressure and temperature conditions used were of 8 MPa and 38 °C, respectively, with a SC-CO_2 flow rate of 1.4 kg/h and a liquid/gas flow rates ratio (L/G) of 0.1 on mass based. SC-CO_2 was fed at the bottom of the extraction column whereas the double emulsion from the top.

NANOCARRIERS CHARACTERIZATION

Morphological analysis

Field emission-scanning electron microscopy (FE-SEM)

Granulometric analysis

Zetasizers nano

Kinetic analysis

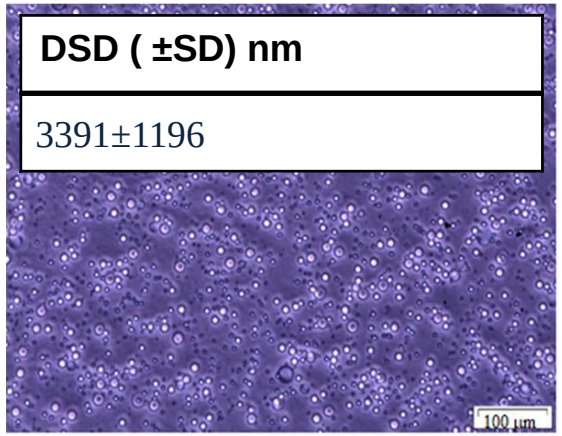
Enzyme-linked immunosorbent assay (ELISA)

MORPHOLOGICAL AND GRANULOMETRIC ANALYSIS

Optical images of double emulsions and FE-SEM images of related carriers loaded with growth factors.

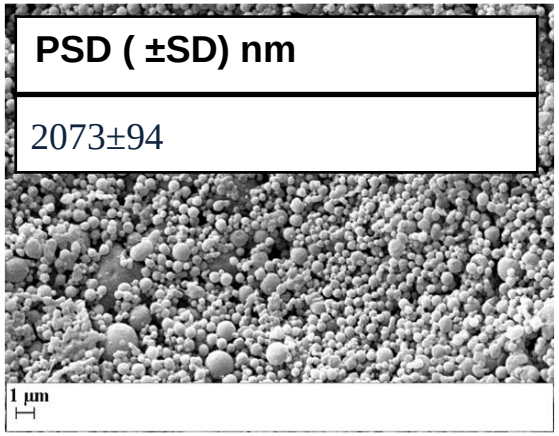
PLGA loaded with hGDF-5

DSD (±SD) nm
3391±1196



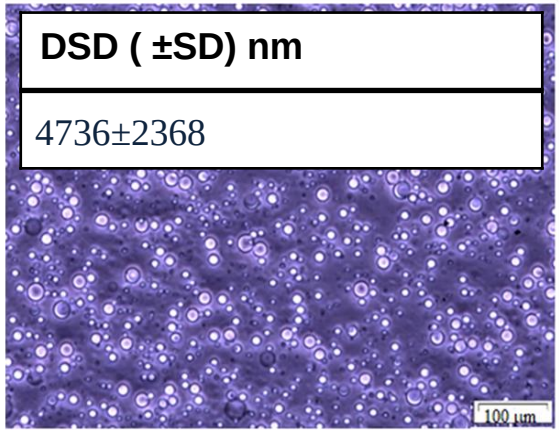
SEE

PSD (±SD) nm
2073±94



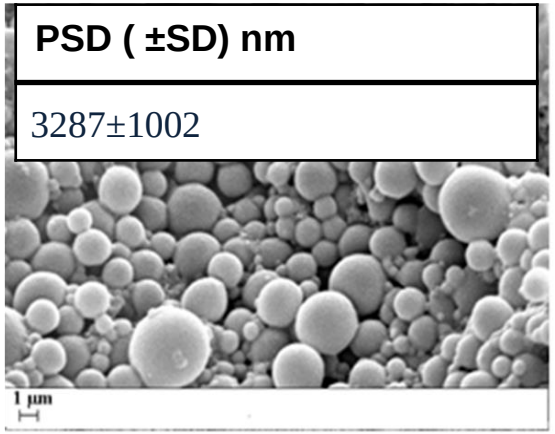
PLGA loaded with hTGF-β1

DSD (±SD) nm
4736±2368



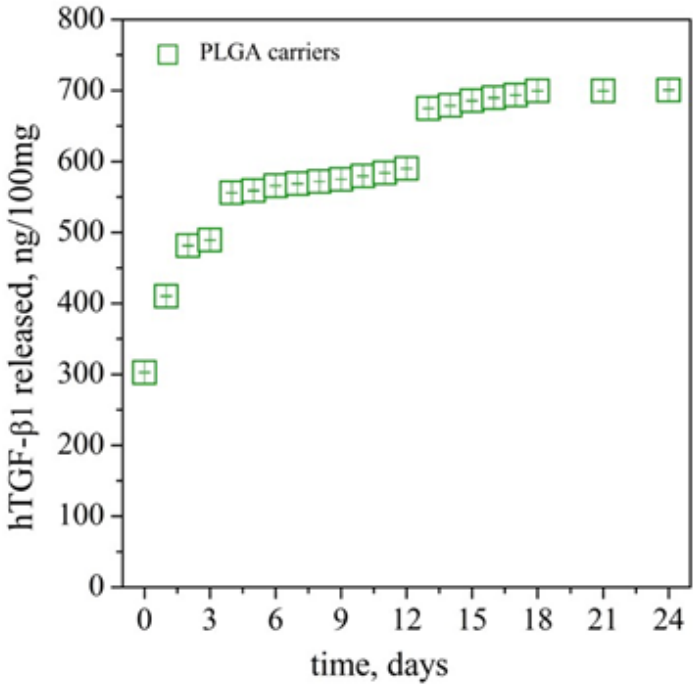
SEE

PSD (±SD) nm
3287±1002



KINETIC ANALYSIS

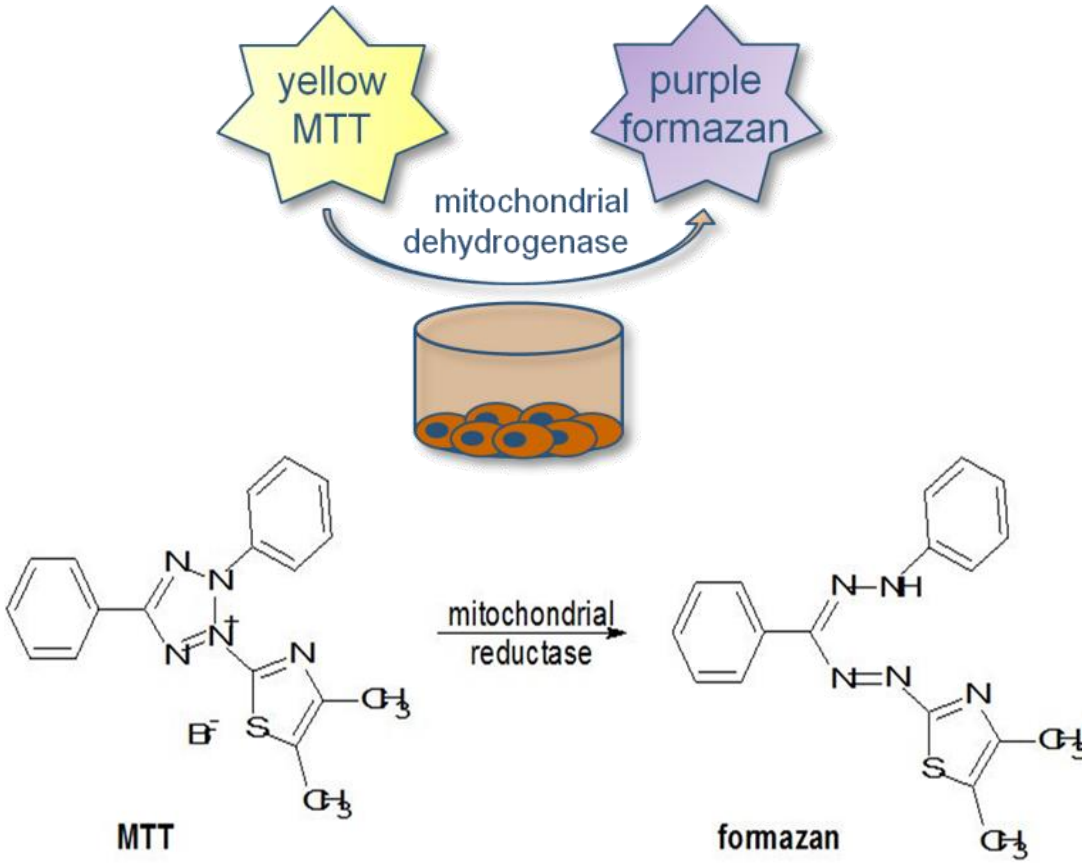
The release profile of growth factors from PLGA particles was monitored in vitro at 37°C using an enzyme-linked immunosorbent assay (ELISA).



Collected data demonstrated a significant burst effect followed by more linear kinetics for hTGF-β1

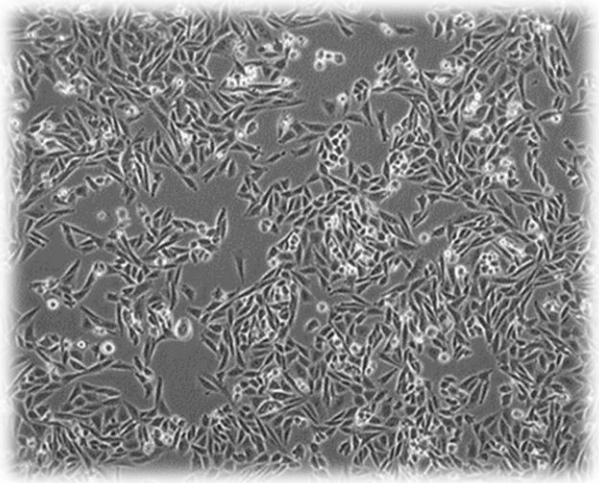
CYTOTOXICITY EVALUATION

Carriers cytotoxicity was evaluated through MTT assay both on ovary cell line from hamster (CHO-K1 cells) and human peripheral blood mononuclear cells (hPBMC) in order to obtain more information about the toxic effects of carriers on replicating and terminally differentiated cells.



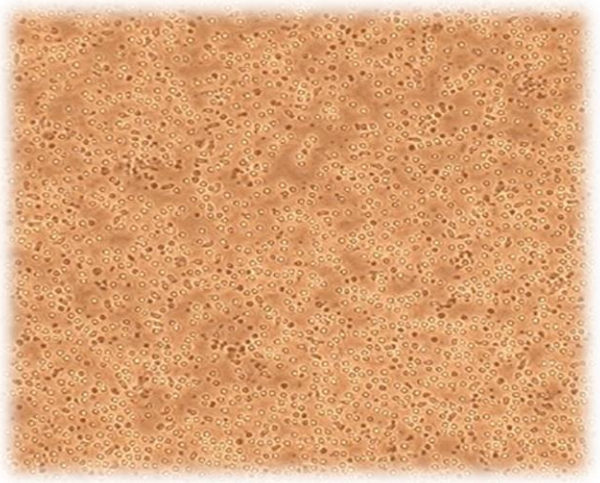
CYTOTOXICITY EVALUATION

CHO-K1s and PBMCs were treated for 24h and 48h with decreasing concentrations of nanocarriers obtained both Solvent Evaporation and Supercritical Emulsion Extraction



Chinese Hamster Ovary Cells (CHO-K1)

Replicating cells

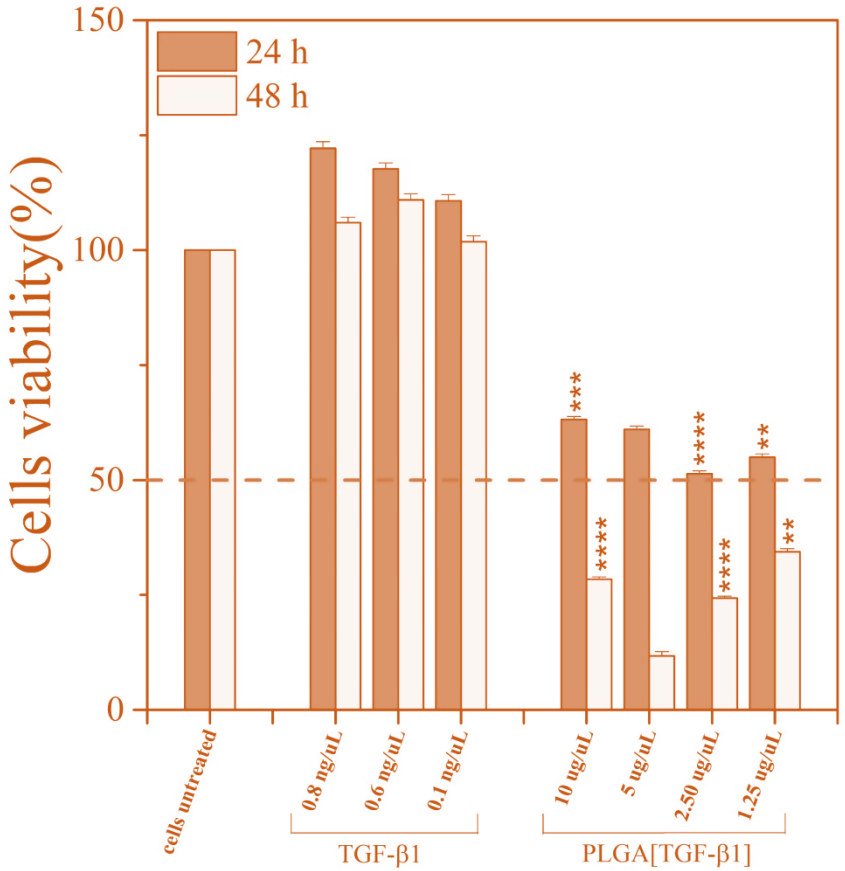


Peripheral Blood Mononuclear Cells (PBMCs)

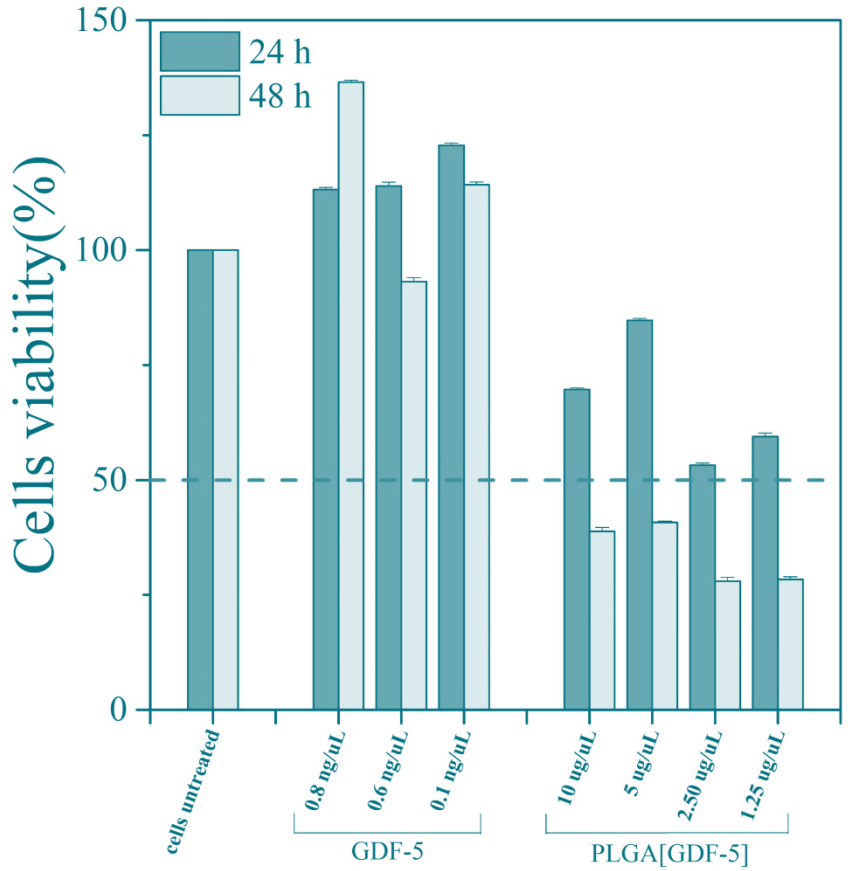
Terminally differentiated cells

CYTOTOXICITY EVALUATION ON CHO-K1

Evaluation of cell viability following treatment with decreasing concentrations of free TGF-β1 growth factor and PLGA[TGF-β1] carriers made with the SEE technique.



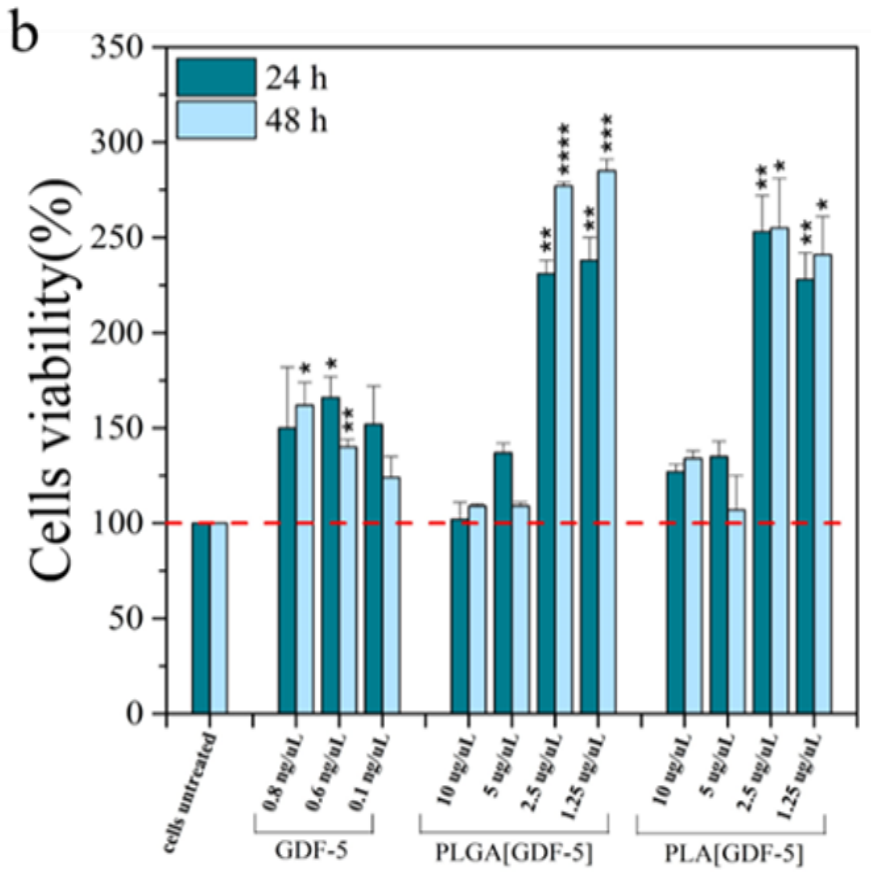
Evaluation of cell viability following treatment with decreasing concentrations of free GDF-5 growth factor and PLGA[GDF-5] carriers made with the SEE technique.



The histograms shows the average percentage of viability of the treated cells compared to the control (untreated cells, 100%).The results were analyzed through the bilateral t-test, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p \leq 0.0001$; $n = 3$.

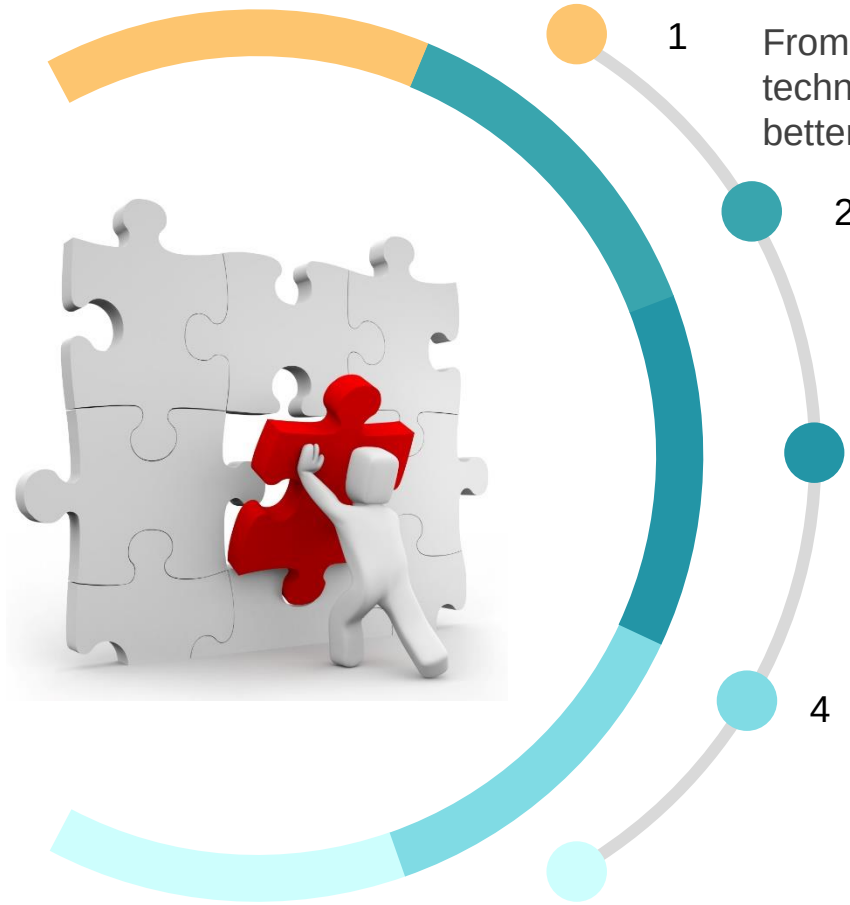
CYTOTOXICITY EVALUATION ON PBMC

Evaluation of cell viability following treatment with decreasing concentrations of free GDF-5 growth factor, PLGA[GDF-5] and PLA[GDF-5] carriers made with the SEE technique.



The histograms shows the average percentage of viability of the treated cells compared to the control (untreated cells, 100%).The results were analyzed through the bilateral t-test, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p \leq 0.0001$; $n = 3$.

CONCLUSIONS AND PERSPECTIVES



1 From the comparative analysis between the two production technologies, it emerged that SEE products, in addition to having a better morphology, induce lower cytotoxicity.

2 Biopolymer carriers showed cytotoxicity in CHO-K1 only at the lowest concentrations.

3 No cytotoxic activity was found in PBMCs.

4 These studies confirmed the reduced toxicity of biomedical devices produced using SEE technology.

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Thank you
for your attention!