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

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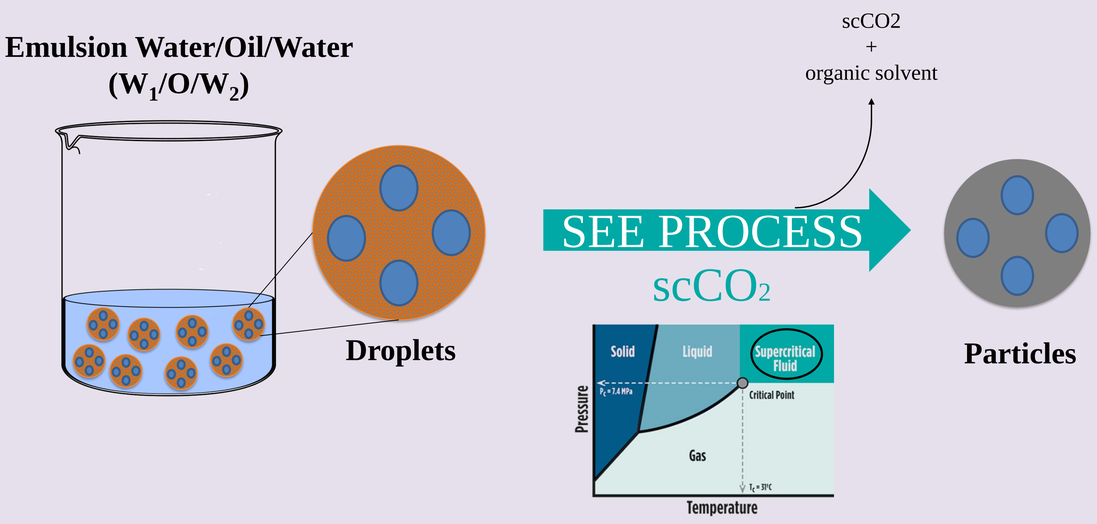
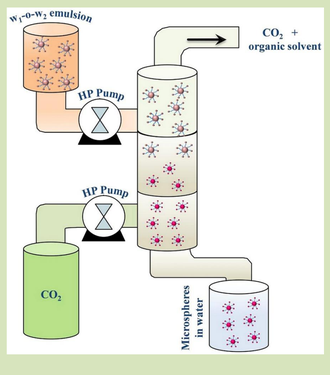
INTRODUCTION

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 [1, 2, 3, 4]. 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 [5,6].

The microencapsulation of human growth differentiation factor-5 (hGDF-5) and human transforming growth factor β1 (hTGF-β1), used respectively to induce tenogenic and chondrogenic commitment, was tested using an innovative process based on emulsions and supercritical fluids. Specifically, this technique, known as Supercritical Emulsion Extraction (SEE), employs supercritical CO_2 to remove the organic solvent from emulsions, obtaining carriers with suitable sizes and low solvent residual.



METHODS



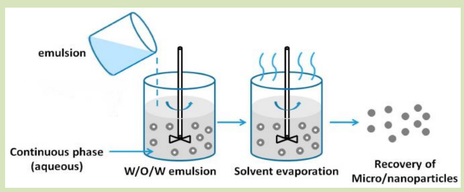
Several double emulsions have been processed, using both solvent evaporation (SE) and supercritical emulsion extraction (SEE) technology, to establish the better composition in terms of polymer molecular weight, surfactant amount and phases mixing rate in order to develop carriers with a suitable loading.

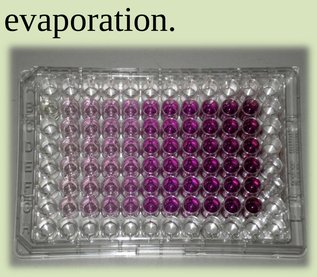
Supercritical Emulsion Extraction (SEE)

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. Particles suspension is continuously collected at the bottom of the column by decompression, whereas the extracted solvent phase is recovered in a separator, located downstream the top of the column.

Solvent Evaporation (SE)

In the case of SE, 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.





MTT assay

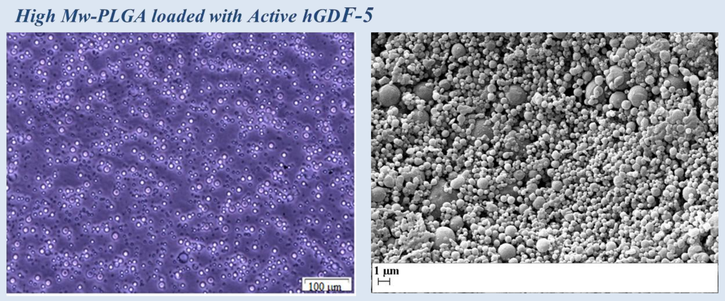
Carriers cytotoxicity was evaluated through MTT assay both on ovary cell line from hamster (CHO-K1 cells) and human peripheral blood mononuclear cells (hPBMC) in order to obtain more information about the toxic effects of carriers on replicating and terminally differentiated cells.

RESULTS

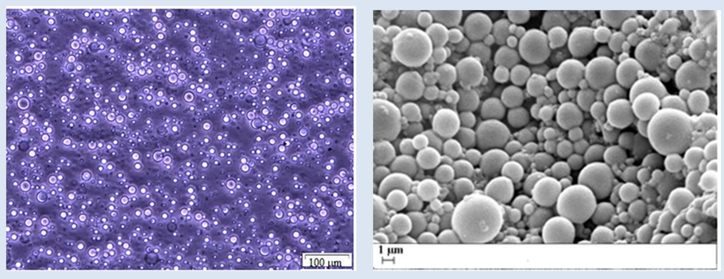
CARRIERS CHARACTERIZATION

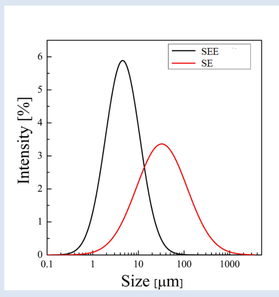
Spherical hGDF-5 and hTGF-β1 loaded carriers with a mean size of 2073±94 nm and 3287±1002 nm were produced using SEE process.
Particles are regular and not aggregated.

High Mw-PLGA loaded with Active hGDF-5

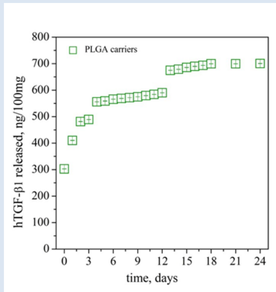


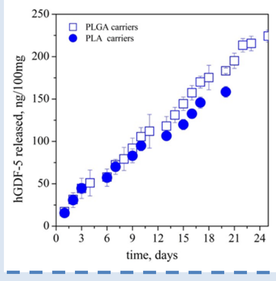
High Mw-PLGA loaded with Active hTGF-β1





Comparison of particle size distributions between carriers fabricated using the same emulsion by traditional solvent evaporation (SE) or Supercritical Emulsion Extraction (SEE) technique.



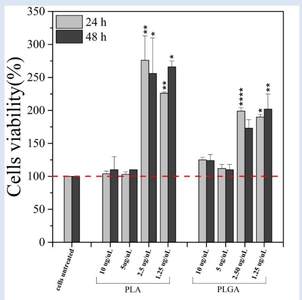


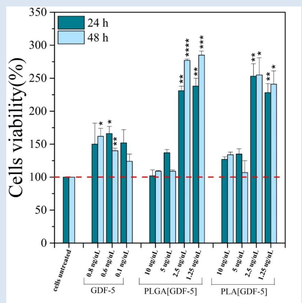
The release profile of growth factors from PLA and PLGA particles was monitored in vitro at 37°C using an enzyme-linked immunosorbent assay (ELISA).

Collected data demonstrated a linear release rate per day in the case of hGDF-5, and more significant burst effect followed by more linear kinetics for hTGF-β1

MTT assay on human peripheral blood mononuclear cells (hPBMC)

The cytotoxicity of carriers loaded with hGDF5 was also established with human PBMCs in order to provide further evidence on their toxicity in human differentiated cells

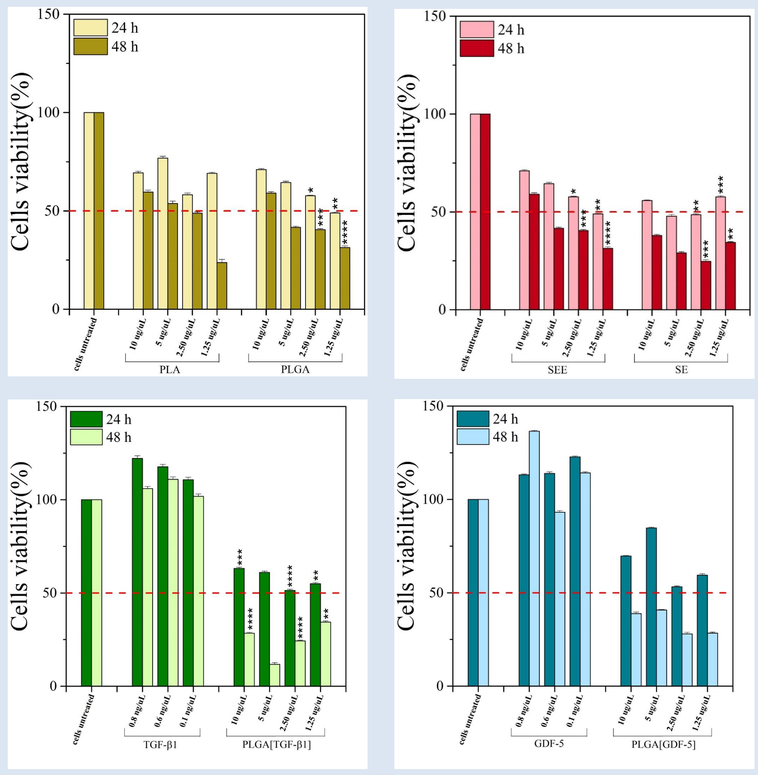




CYTOTOXICITY EVALUATION

MTT assay on Chinese Hamster Ovary Cells (CHO-K1)

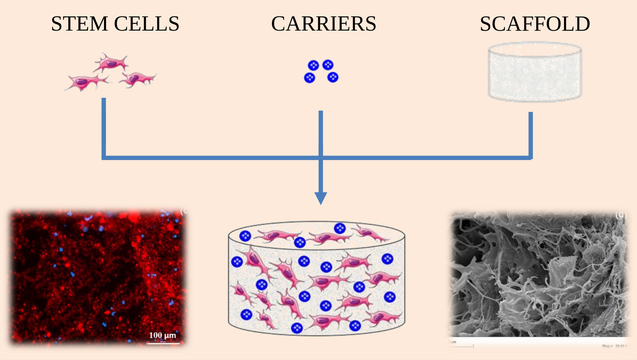
Cells were incubated for 24 and 48h using 10ug/uL, 5ug/uL, 2.50ug/uL and 1.25ug/uL concentrations of carriers (unloaded and loaded with growth factors).



Cell viability was determined by MTT assay. Histograms report the percentage of viable cells compared to controls (untreated cells, 100%)

CONCLUSIONS & PERSPECTIVES

The results indicated a suitable loading coupled with reduced toxicity for SEE carriers and suggested Supercritical Emulsion Extraction as an effective technology for both micro/nano systems formulation and their use for controlled delivery in a 3D synthetic scaffold.



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