SUPERCRITICAL EMULSION EXTRACTION TECHNOLOGY FOR THE PRODUCTION OF "SMART" MICRO AND NANO CARRIERS

Della Porta G.*, Campardelli R. and Reverchon E.
Department of Industrial Engineering, University of Salerno, Via Ponte Don Melillo 84084 Fisciano (SA), Italy. gdellaporta@unisa.it Tel.: +39 089964104; Fax: +39 089964057

.

ABSTRACT

Supercritical Emulsion Extraction technology (SEE) is proposed for the production of "*smart"* biopolymer carriers. The supercritical carbon dioxide (SC-CO_2) is used to extract selectively the organic solvent from the droplets of multiple emulsions generating solid micro- or nano-devices. The carrier dimension can be directly related to the droplet ones due to a continuous and fast unit operation by using a counter-current extraction tower.

The technique has been demonstrated to be effective for the production of complex smart devices of several biopolymers, such as PLGA, PLA and PCL loaded with multiple bioactive compounds and able to sustained or pulsed release of the entrapped molecules. The operating parameters, such as pressure, temperature and flow rate ratio has been also optimized to engineer carrier size distribution and their encapsulation efficiency. For example, PLGA microdevices loaded with serum insulin or growth factors are produced with different sizes and/or loading (encapsulation efficiency between 60-70%) to assure a bioactive molecule release for 15-21 days; whereas, PLA nanocarriers loaded with gold nanospheres are produced to obtain a pulsed light-sensitive release. PLGA microdevices are also successfully loaded with multiple molecules, such as hydroxyapatite, teriparatide and gentamicin to be used as osteoinductive bone material.

INTRODUCTION

Biopolymer nanocarriers of biopolymer, such us poly-caprolactones, poly-amminoacids, poly-lactic–co-glycolic acids are particularly actractive in nanomedicine because able to improve biological therapies such as vaccination, cell therapy and gene therapy. Particularly, smart nano carriers able of target an active molecule at the appropriate site and release it at an adjustable rate in response to the progression of the disease are particularly appealing; as well as, light-responsive devices activated by an electromagnetic radiation [1]. Microcarriers of the biopolymers previously described, have also a great potential to be used for tissue engineering; indeed, they can be processed using bioreactor methods that are more akin to highly scalable fermentation methods than the somewhat-constrained monolayer culture methods typically adopted for human cell expansion. The use of stimulus-responsive smart devices is effective for controlled release of drugs and bioactive molecules, and the compositional variation with ECM molecules confers further support either to early cell responses such as attachment, migration, and proliferation, or later responses such as directed differentiation. Furthermore, microdevices encapsulating into

hydrogel provides 3D internal environment that will support the cells differentiation. In both cases, the particle size of the micro and nano carriers is another of the key factor because acts on the surface area/volume ratio and, the production of monodisperse “systems” lead to more uniform behaviour avoiding any side effect [2-4].

Recently, our research group has proposed the use of supercritical fluids for the solvent extraction of emulsions, as a new technique for the production of biopolymer microspheres with a controlled and narrow particle size. The proposed technology can overcome several disadvantages of the conventional emulsion extraction technology, such as high processing temperatures and long extraction times. Particularly, above to the critical point, small changes in temperature or pressure can produce large changes in the density/solvation ability of supercritical fluids. This property can be fruitfully exploited for the extraction of organic solvents in the emulsion droplets. In addition, lower viscosity and higher diffusivity of a supercritical fluid with respect to the liquid solvent improve mass transfer, which is often a limiting factor for the solvent elimination in such emulsions. Among all the possible supercritical fluids, carbon dioxide (SC-CO_2) is largely used. Recent studies confirmed that the SC-CO_2 is an excellent solvent for oily phase upon contact with the aqueous phase of the emulsion leading to rapid diffusion of solvent from the emulsion droplets; the -process is also faster than the conventional solvent evaporation of emulsion, resulting in the precipitation of spherical monodisperse nano and micro devices, due to the prevention of any droplets coalescence or aggregation. Moreover, any batch to batch reproducibility problems are overcame with continuous operation by counter current extraction tower and encapsulation efficiency higher than 80% are assured by the fast emulsions processing [5-7].

In this work, several examples of the obtainable results will be described, such as PLGA microcarriers loaded with serum insulin or growth factors. The smart device action will be also monitored when loaded in a complex bioactive “scaffold” for tissue engineering application. Other devices, such as PLA nanocarriers loaded with gold nanospheres to assure a light-sensitive target release and PGA microdevices carring hydroxyapatite, teriparatide and gentamicin to be used as osteoinductive bone material will be proposed.

SEE APPARATUS

SEE apparatus based on a high pressure packed tower capable of working under pressure has been designed and constructed on purpose. The bench scale-plant consists of at around 2000 mm long column with an internal diameter between 10-15 mm. The column is packed with stainless steel packing with a specific surface and high void degree and is thermally insulated and controlled. Carbon dioxide is fed from the bottom of the column by a high-pressure diaphragm pump at a constant flow rate, whereas, the emulsion is formulated in continuous and fed to the column by a high pressure pump at the column top. A separator located downstream the top of the column is used to recover the extracted oily phase solvent and the pressure in the separator is regulated by a backpressure valve. Before starting the emulsion delivery, the column is wetted and the flooding conditions have been evaluated. The microcarriers suspension is collected at the bottom of the column, washed several times by centrifugation with distilled water and, then, recovered by membrane filtration and dried in a controlled air. To obtain an overall process operating in continuous mode, a membrane emulsifier is also designed and set up on the top of the packed tower for the continuous delivery of mono-disperse emulsions that will be continuously formed and processed to also produce mono-dispersed nano-structured devices. The operating parameters, such as

pressures, temperatures, and flow rates ratio are optimized by several trials [8] and GMP operations allow the production of several batches for in vitro and in vivo trials. A detailed description of the SEE apparatus is reported in Figure 1.

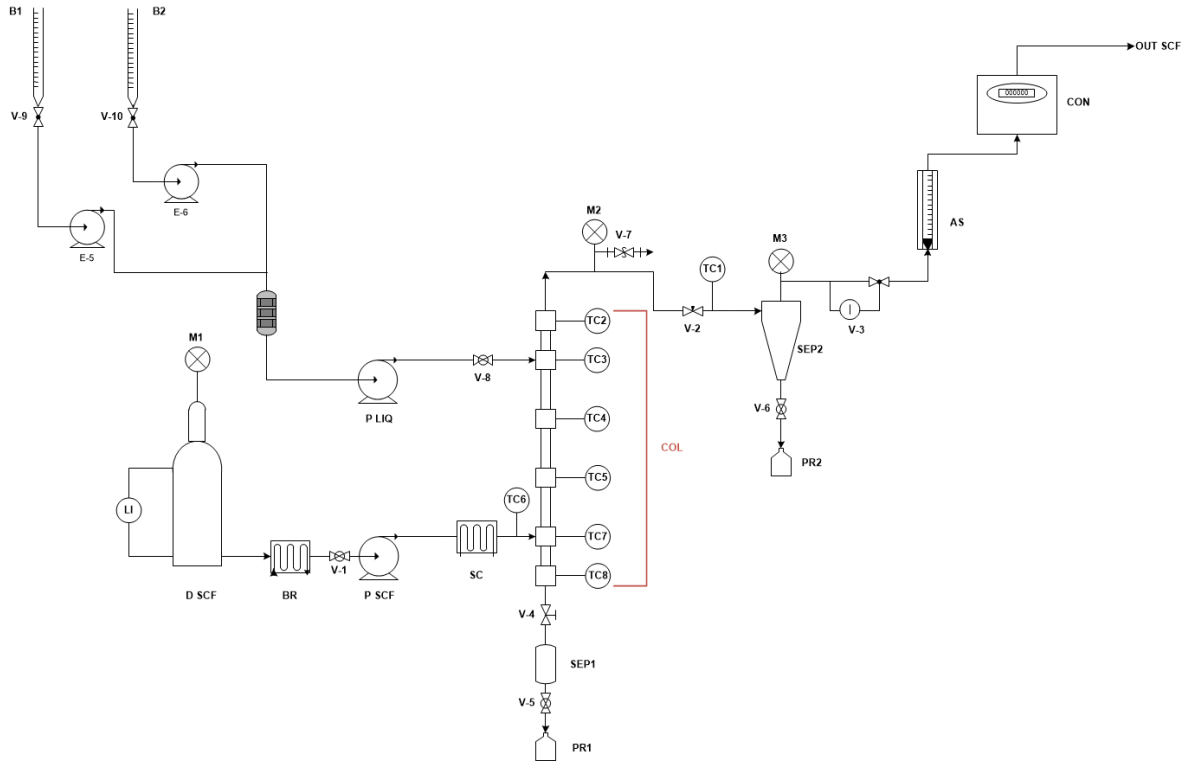
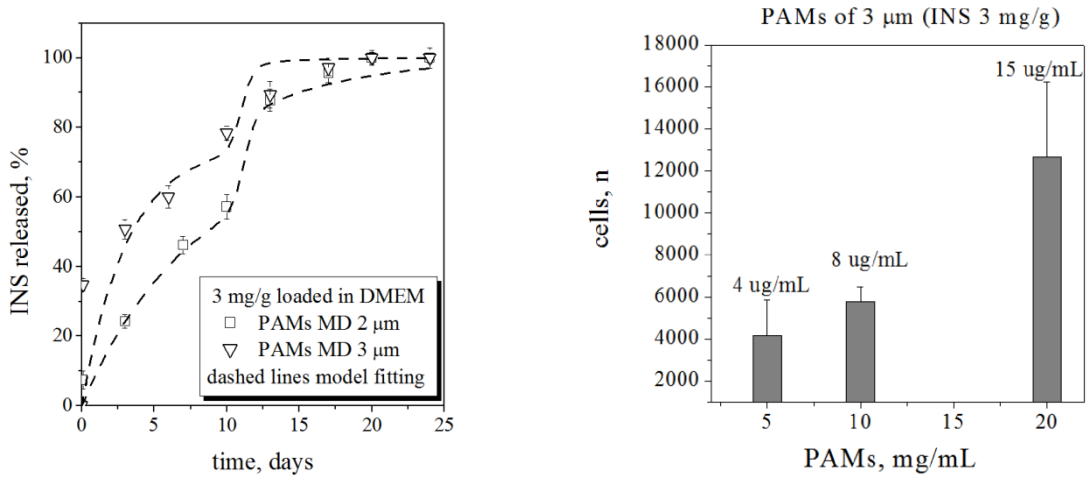


Figure 1. SEE continuous tower diagram: D-SCF, CO_2 supply; B1-2, liquids supply; M, pressure gauges; SCF_P, diaphragm pump used for high pressure SC-CO_2; Liq_P, piston pump used for the emulsion; TC, thermocouples/controllers; SEP, separator; AS, rotameter; SC, heat exchangers; V, valves.

RESULTS & DISCUSSION

Tissue engineering

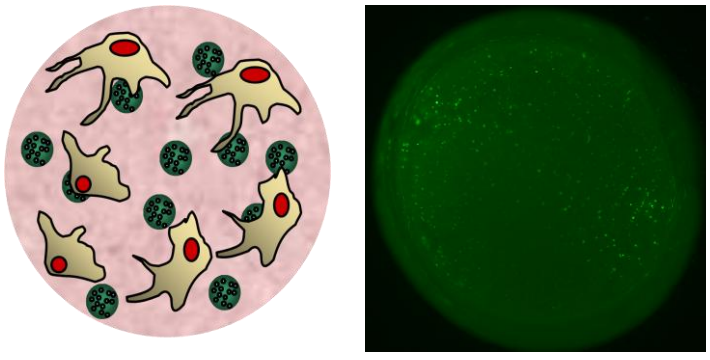
PLGA microcarriers loaded with bovine serum insulin with different sizes (2 and 3 µm) and insulin charges (3 and 6 mg/g) were successfully produced by SEE technology. The insulin release profiles were monitored for 21 days in DMEM medium at 37°C (see Figure 2a) and their activity was tested in a static cultivation of embryonic ventricular myoblasts (cell line H9c2 from rat) with a FBS serum free medium to monitor cell viability and growth in dependence of insulin released. Good cell viability and growth were observed on 3 µm microdevices loaded with 3 mg/g of insulin (see Figure 2b).



(a) *(b)*

Figura 2. Insulin (3mg/g) release profiles from 2 and 3 µm devices in DMEM (a). Number of cells growth after 72 h of incubation with different PLGA microspheres amounts (mean size of 3 µm, insulin loading of 3 mg/g) vs. the insulin delivered in a serum-free medium (b), adapted from [9].

PLGA microdevices loaded with growth factors (GFs) were also produced by SEE with very high encapsulation efficiency (80%) and loaded into alginate scaffold with human Mesenchymal Stem Cells (hMSC), that are promising cell source for bone tissue engineering. These "living" 3D scaffolds (see Figure 3a-b) were incubated in a direct perfusion tubular bioreactor to enhance nutrient transport and exposing the cells to a given shear stress. Different GFs such as, h-VEGF, h-BMP2 and a mix of two (ratio 1:1) were loaded into alginate beads. Samples were recovered from dynamic and static culture at different time points (1st, 7th, 21st days) for the analytical assays as, live/dead and Van Kossa staining for calcium deposition. Alkaline phosphatase, osteocalcin and osteopontin immunoassay were also performed. The immunoassay confirmed always a better cells differentiation in the bioreactor with respect to the static culture and revealed a greater influence of the two growth factor mix on cell differentiation [10].

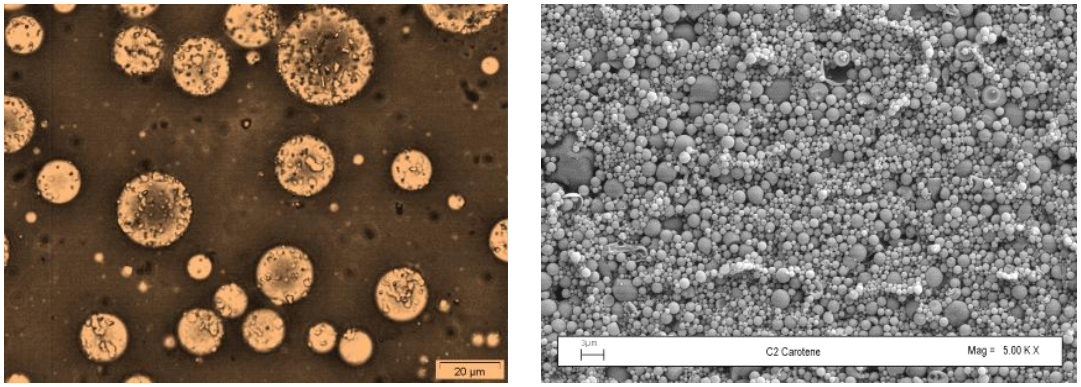


(a) *(b)*

Figure 3. A schematic representation of the bioactive hydrogel structure with cells and the bioactive microdevices (green). Live & dead image of hydrogel taken from 28 days of cultivation with an enlargement of 10 X. Live cells are stained in green.

Nanomedicine

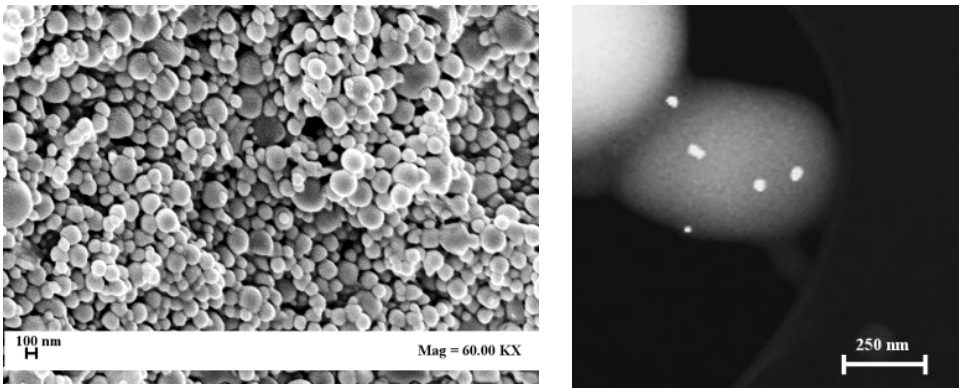
SEE technology also produces microdevices of different biopolymers such as, PLGA, PLLA, PCL, PMMA high efficiency loaded with thermolabile compounds such as β-carotene (see an emulsion optical image and the SEM image of particles in Figure 4a-b, respectively). Indeed, thanks to the mild temperature condition of the SEE process, sensitive molecules are not damaged during their processing. Optimizing also the gas to liquid ratio in order to obtain the maximum extraction efficiency, very low solvent residues (< 10 ppm) are obtained in the final microparticles suspensions. The size of the particles is tuneable by changing the emulsion formulation and production conditions.



(a) ***(b)***

Figure 4. Optical microscope image of emulsion (a) and SEM image (b) of PLGA microparticles encapsulating β-carotene.

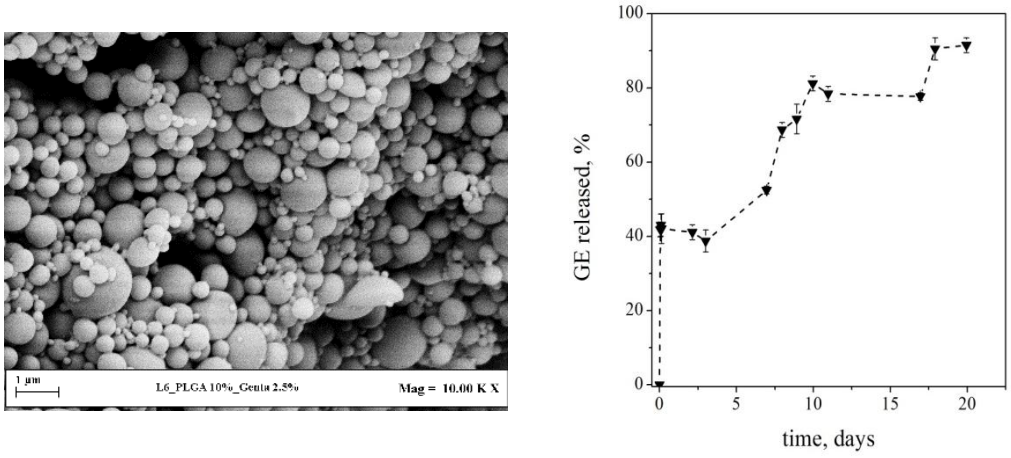
The SEE-C process were applied for the encapsulating of gold nanoparticles to generate smart photosensitive carriers of PLA; these devices can be activated by NIR irradiation timing the release kinetic of co-loaded drug (see SEM and TEM images of the devices in Figure 5a-b, respectively) [11].



(a) ***(b)***

Figure 5. SEM (a) and TEM (b) images of PLA nanospheres encapsulating hollow gold nanospheres.

The SEE-C process was recently applied to the production of "smart" systems for bone repairing applications. In the same PLGA device, hydroxyapatite (HA), gentamicin (GE) and teriparathyde hormone (TH) were loaded (see SEM image in Figure 6a). HA because of its structural similarities to the mineral bones and because of its diagnostic radiopacity; GE to prevent infection phenomena and TH because its effective anabolic action in the treatment of osteoporosis. These multi drugs devices are able to release their charge in 21 days (see GE release profile in Figure 6b) and will be in vivo tested, as bone cement.



(a) *(b)*

Figure 6. SEM image of PLGA devices encapsulating HA/GE/TH (a); GE (10 mg/g) release profile from 1.3 μm PLGA carriers in saline solution.

REFERENCES

[1] BOISSELIER, E., ASTRUC, D., Chem. Soc.Rev., Vol., 38, **2009,** 1759-1782.

[2] TATARD, V.M., VENIER-JULIENNE, M.C., BENOIT, J.P., MENEI, P., MONTERO-MENEI, C.N., Cell Transplant. Vol. 13(5), **2004**, pp. 573-583.

[3] HOLLAND, T.A., BODDE, E.W., CUIJPERS, V.M., BAGGETT, L.S., TABATA, Y., MIKOS, A.G., JANSEN, J.A., Osteoarthritis Cartilage. Vol. 15(2), 2007, pp.187-197.

[4] PARK J.H., CHOI, S.J., PEREZ, R.A., KIM, H.W., JIN, G.Z., WALL, I.B., Tissue Engineering: Part B, Vol. 19 (2), **2013**, pp. 172-190.

[5] DELLA PORTA, G., FALCO, N., REVERCHON, E. Biotechnology & Bioengineering J., Vol. 108 (3), **2011**, pp. 676-86.

[6] DELLA PORTA, G., FALCO, N., REVERCHON, E., Pharmaceutical Sciences J., Vol. 99 (3), **2010**, pp. 1484-99.

[7] DELLA PORTA, G., CAMPARDELLI, R., REVERCHON, E., Supercritical Fluids J., Vol. 76, **2013**, pp. 67-73.

[8] FALCO, N., REVERCHON, E., DELLA PORTA, G.,. Ind. Eng. Chem. Res. Vol. 51, **2012**, pp 8616-23.

[9] DELLA PORTA, G., FALCO, N., GIORDANO, E., REVERCHON, E., Biomaterial Science J. Poly Ed., Vol. 24 (16), 2013, pp. 1831-47.

[10] DELLA PORTA, G., NGUYEN, B., CAMPARDELLI, R., REVERCHON, E., FISHER, J., Biomed Mater Res. J., submitted **2014**.

[11] CAMPARDELLI, R., DELLA PORTA, G., GOMEZ, L., IRUSTA, S., REVERCHON, E., SANTAMARIA, J., Journal of Materials Chemistry B Vol. 2 (4), **2014**, pp. 409-417.