Development and optimization of two processes (conventional fluidic batch and continuous millifluidic one) for producing nanosized liposomes using supercritical CO_2 - DNA encapsulation for the treatment of Hutchinson-Gilford Progeria Syndrome (HGPS)

Mathieu MARTINO[1]; Adil MOUAHID[1]; Camille DESGROUAS[2,3]; Catherine BADENS[2,3]; Michelle SERGENT[4]; Elisabeth BADENS[1]

[1] Aix Marseille, CNRS, Centrale Marseille, M2P2, Marseille, 13451, France
[2] Laboratoire de Génétique Moléculaire, Hôpital d'enfants de la Timone, APHM, Marseille, 13005, France
[3] MGG, Inserm UMR 1251, Aix-Marseille Université, Marseille, 13385, France
[4]Aix Marseille Univ, Univ Avignon, CNRS, IRD, IMBE, Marseille, France

RNA interference therapy consists in using deoxyribonucleic acid (Antisense oligonucleotides, ASOs) or ribonucleic acids (small interfering RNA, siRNA) to specifically modulate a targeted gene expression. Indeed, ASOs or siRNAs make it possible to either degrade messenger RNAs (encoding genetic information for protein synthesis in cells) or inhibit their translation into proteins. ASOs can be used to treat viral diseases or diseases linked to the abnormal expression of a gene. One possibility to encapsulate these compounds is the use of liposomes, which are soft matter vesicular carriers, non-toxic and biodegradable which can protect ASOs and improve their delivering. Their phospholipidic bilayers that mimic the cell membranes make liposomes the first choice for encapsulating matrices in therapy. The main limitation for the use of liposomes as drug carriers for gene or cancer therapy is their size. Indeed, the particle size is a key characteristic for the particle cellular internalization. Particles size up to 5 microns can undergo a cell internalization, but the process is more rapid for particles with a size smaller than 150 nm. It is commonly stated that the recommended diameter of particles for treating cancer is in the range 10-150 nm, and nanoparticles in the low tens of nanometer range enter more effectively using pathways of cell internalization limiting degradation [1].

In this work, two processes using supercritical CO_2 for the elaboration of liposomes smaller than 150 nm have been developed and optimized by experimentation according to an optimal Design Of Experiments. The two liposome elaboration processes developed are first a conventional fluidic batch process and then a continuous millifluidic process allowing to obtain reduced quantities of liposomal suspension ideal for DNA encapsulation. The optimization focused on studying the influence of pressure (from 90 to 150 bar), temperature (from 35 to 45°C) and egg yolk lecithin concentration in the feed solution (from 0.1 to 1.9 % w/w) on liposome size. The results obtained show that the liposomes formed are smaller than 203 nm with a minimum size of 61 nm (observed at 120 bar, 45°C and 0.1% w/w) for batch process and smaller than 166 nm with a minimum size of 124 nm for millifluidic process. The most significant parameter on liposome size and size distribution variations appears to be the lecithin concentration. The repeatability of the process was also studied. For conditions giving the smallest liposomes, a deviation of ± 0.7 nm and 0.8 nm were observed on liposome size for the same size distribution (PID at 0.266 ± 0.005 and 0.30 ± 0.03) for batch process and millifluidic process, respectively. The liposomes formed can therefore serve as drug matrices for gene or cancer therapy. These results demonstrate that supercritical technology addresses liposome use limitations. Furthermore, the liposome size and size distribution can be adjusted tuning on the operating conditions.

In order to test the process efficiency for encapsulating therapeutic molecules, an ASO was encapsulated in liposomes by the implemented millifluidic supercritical process under optimized operating conditions (150 bar, 35°C and 0.1% w/w). This ASO is a synthetic, single-stranded oligodeoxynucleotides of 25 nucleotides that can target *LMNA* transcripts resulting in DNA:RNA heteroduplex inducing cellular RNase H recruitment and cleavage of the RNA by RNase H. This technology could constitute a promising strategy for the treatment of Hutchinson-Gilford Progeria Syndrome and milder forms of premature ageing involving the gene *LMNA* that encodes for lamin A protein [2]. The efficacy of the formulations obtained will be tested *in vitro* on control and patient cells using different tests addressing pre-lamin A and lamin A expression level by western-blot, nuclear abnormalities rate by immunofluorescence, senescence associated to beta-galactosidase and cell replication by BrdU incorporation. The results will be compared to other commercial solutions commonly used *in vitro* to transfect cells for gene modulation (Interferin®).

[1] J. Wang, J.D. Byrne, M.E. Napier, J.M. DeSimone, More Effective Nanomedicines through Particle Design, Small. 7 (2011) 1919–1931. https://doi.org/10.1002/smll.201100442.

[2] K. Harhouri, C. Navarro, C. Baquerre, N. Da Silva, C. Bartoli, F. Casey, G. Mawuse, Y. Doubaj, N. Lévy, A. De Sandre-Giovannoli, Antisense-Based Progerin Downregulation in HGPS-Like Patients' Cells, Cells. 5 (2016) 31. https://doi.org/10.3390/cells5030031.