MICROFLUIDIC PLATFORMS BASED ON CHAOTIC MIXING AND DIFFUSION FOR COMPLEXATION OF CATIONIC LIPOSOME AND pDNA

Ismail Eş, Leonardo José Montebugnoli, Lucimara Gaziola de la Torre*

bioengineerx@gmail.com; leojmonte@gmail.com; latorre@feq.unicamp.br*

Abstract: *Microfluidics, engineering of fluids at micro-scales, appears as an emerging technology that enables the continuous production of non-viral vector systems to use in gene therapy. The present study aims the technological development of microfluidic platforms to incorporate pDNA into liposomes. For this purpose, two different microfluidic devices were fabricated via soft lithography: (1) Hydrodynamic focusing micromixer (HFM); (2) Staggered herringbone micromixer (SHM). SHM was designed to have 40 sub-mixing inside of the main channel to provoke chaotic mixing instead of only hydrodynamic focusing, which the diffusion is predominant factor. It was observed that SHM, comparing to HFM, could allow the complexation of genetic material and liposomes at higher flow rates and with better efficiency. The complexation between CLs and genetic materials (pDNA) occurred by the electrostatic interaction. Important physico-chemical properties of CLs and lipoplexes such as size, polydispersity, zeta potential were evaluated and the results showed promising characteristics for liposomes.*

Key-Words*: pDNA, Cationic liposomes, microfluidics, chaotic mixing, hydrodynamic flow focusing*

Introduction: Several comprehensive studies have led to the discovery of efficient treatment or prevention methods against genetic diseases. For this purpose, gene therapy, which is the introduction of suitable genetic material into cells to correct the abnormal genes, appeared as a critical approach (Ginn et al. 2013). However, the success of gene therapy mostly relies on safe and competent delivery of genetic material into target cells, which, in most cases is hard to overcome. Numerous viral and non-viral vectors have been employed in clinical trials of gene therapy. The use of non-viral vectors exhibits great potential to overcome the gene therapy limitations. Among different non-viral vectors, cationic liposomes (CLs), which are amphipathic lipid systems, are important candidates. Several techniques have been employed in order to produce liposomes. Comparing to conventional methods, microfluidics exhibit great advantage due to their economic and technical facilities such as low reagent use, low-cost design, high control, reduced reaction time, minimization of the effect of mass and heat transfer, low energy consumption, real time data acquisition and easy automation (Halldorsson et al. 2015). Hence, the evaluation of this carrier system for gene delivery is also essential for studies in gene therapy. The number of studies with great novelty on complexation of liposomes and genetic material has been increasing recently. The novelty of these studies is attributed mostly to chemical modifications on liposome structure, type of genetic material and above all, the geometrical design of microfluidic device and fluid dynamics that determine complexation efficiency. As it is mentioned before, the lack in optimization of microfluidic systems mostly causes needless waste of drug or genetic material. Considering the high cost of these specific materials with promising pharmaceutical importance (e.g. siRNA with specific target), this significant material loss might result in increase of overall production cost. Therefore, the optimization of these systems in terms of molar charge ratio, geometry, physical conditions and engineering aspects like fluid dynamics is crucial to achieve high productivity.

Experimental: Fabrication of PDMS/glass microfluidic devices was carried out according to the method previously described by Moreira et al. 2009. Prior to microfabrication, microfluidic devices were geometrically designed and projected using AUTOCAD software (version 2015) then printed in form of photolite, which serves as positive template for the process of soft lithography. After photolithography, the mask layouts were photo-plotted with 8000 dpi resolution, and the UV exposures were made in a MJB-3 UV300 contact mask aligner (Karl-Suss, Garching, Germany) in order to obtain negative template. Using negative template, Sylgard® 184 Silicone Elastomer Kit was used as material precursor of Polydimethylsiloxane (PDMS) layers to form microchannels. PDMS channels and glass were irreversibly sealed by O_2 plasma surface activation sealing techniques using applying potential of 70 W during 20 seconds exposure time (Plasma Technology PLAB SE80

plasma cleaner, Wrington, England). All steps of microdevice fabrication were conducted in Microfabrication Laboratory of "The Brazilian Center for Research in Energy and Materials" (CNPEM).

CL production was performed using hydrodynamic flow focusing microfluidic device according to method described by Balbino et al. 2013. EPC, DOTAP and DOPE at molar ratio of 50/25/25 (%) were dispersed in anhydrous ethanol at a lipid concentration of 25 mM. Prior to CL production, lipid solution was sonicated for 15 min at 35 ºC (Ultrasonic Cleaner 8890, Cole-Parmer). Lipid solution and deionized water were loaded into a 1 mL and two 2.5 mL glass syringes (Hamilton, NV, USA), respectively. Syringe pumps (PHD Ultra, Harvard Apparatus) were used to maintain constant infusion rates of each solution into 3-inlet microchannels. Lipid solution dispersed in ethanol was introduced into systems through the center inlet while deionized water was introduced through 2 side inlet channels. Flow rate ratio (FRR) of 10 and average fluid flow velocity of 143 mm/s were set for CL production. The final concentration of cationic liposomes obtained at the exit of microfluidic device was 2.27 mM. For the CL production on Herringbone Micromixer with two inlets, lipid solution and deionized water were introduced through each inlet at same flow rates. Average hydrodynamic diameter and polydispersity of cationic liposomes and lipoplexes were measured using dynamic light scattering technique (DLS) (Zetasizer NanoZS, Malvern).

Results and discussion: As the first part of evaluation, we produced cationic liposomes using hydrodynamic flow focusing microdevice and herringbone micromixer and as the second part, we complexed CLs with pDNA using given micromixers. The physico-chemical properties of liposomes produced in HFM and SHM were favorable (Z-average: 140-160 nm; Polydispersity index: 0.08-0.13 and Zeta Potential: 40-60 mV). It should be noted that it could be more possible to achieve high productivity using SHM since this microdevice allowed the introduction of liquids at higher flow rates. The maximum flow rate on SHM that offered good liposome properties was 800 µL/min; while this value was around 150 µL/min in HFM. The same microdevice was used for complexation study (pDNA and CLs). Since the laminar flow is imposed in microscale, the mixing process through microchannels is poor due to predominance of diffusion process. Therefore, it becomes a challenging process to improve mixing in microchannels. The absence of turbulence, most of the time, reduces the mass transfer and consequently this situation will not allow the efficient particle formation due to the lack of mixing. In this case, it is important to manufacture novel microchannel systems to improve mixing. This can be achieved by using chaotic micromixers. In order to achieve an efficient complexation, we evaluated the complexation study using SHM with sub-channels at different flow rates. The flow characteristics of solutions entering the microchannels show great influence of the physicochemical properties of CLs. In our study, the flow velocity and flow rate ratio for CL production in hydrodynamic focusing device were determined to be 143 mm/s and 10, respectively. Using these parameters, we produced cationic liposomes with average size, PdI and ζ of 164 nm, 0.117 and 49.1 mV, respectively. There have been many techniques employed for cationic liposome production; however, novel techniques, principally microfluidics, are mostly proved to be more effective than conventional methods. On microfluidic platforms, mostly hydrodynamic focusing technique has been assessed for CL production. Different from hydrodynamic focusing systems, staggered herringbone micromixer (SHM) resulted in efficient formation of liposomes.

Conclusion: The complexation of pDNA and CL was performed using two different micromixers in order to evaluate their efficiency in terms of physico-chemical properties of liposomes and lipoplexes. Both micromixer offered favorable properties of liposomes and lipoplexes. However, the complexation was more rapid on SHM since this device allowed a more chaotic mixing, while HFM could not achieve high flow rates. This situation is crucial to analyze since studying the flow performance at higher velocities allows improved process productivity.

References and acknowledgements:

References

[1] Ginn, S.L., Alexander, I.E., Edelstein, M.L., Abedi, M.R. and Wixon, J., 2013. "Gene therapy clinical trials worldwide to 2012–an update". *The Journal of Gene Medicine*, **15(2)**, pp.65-77.

[2] Halldorsson, S., Lucumi, E., Gómez-Sjöberg, R. and Fleming, R.M., 2015. "Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices". *Biosensors and Bioelectronics*, **63**, pp.218-231.

Acknowledgments

I. Eş thanks the Sao Paulo Research Foundation (FAPESP) for the scholarship (Grant No. 2015/14468-0).