Production of nanoliposomes using *in-house* 3D printed microfluidic chips

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Background: In recent years, 3D printing (3DP) and microfluidics have gained a leading role in the field of nanomedicine. These techniques are suitable for the production of innovative formulations, such as lipid structure nanosystems. More specifically, the 3DP of microfluidic devices is proving to be a synergetic approach that could potentially revolutionise the world of nanotechnology. 3DP is being applied to the production of devices that are customisable through *in-house* design, fast to manufacture, with a very high accuracy of internal geometry. In addition, microfluidics allows novel formulations to be obtained quickly and safely, removing batch-to-batch variability and challenges associated with classical production methods. Both techniques ensure low production costs and fast processing times.

Methods: The design of the devices has been made by TinkerCad software. For the 3DP process, three devices with square channels and an internal geometry designed to implement fast passive mixing were designed. Two of these prepared with different angle (45° and 90°) at which the two inlets (aqueous phase and lipid phase) meet, the third has an internal geometry characterised by a fast-mixing phase and a linear channel with wedges inside. PlasCLEAR was the chosen material using an AsigaMax 3DP. Once the devices were obtained were tested in the microfluidic setup and their resistance to working pressure was evaluated. Empty liposomes were produced using PBS as the aqueous phase and DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine):Chol (Cholesterol) in ethanol as the organic phase. The particle size, polydispersity index (PDI), and ζ- potential was measured.

Results: The 3D printed chips were purposely designed to improve the mixing between the aqueous phase and the lipid phase. In addition to consistent mixing, the presence of an interconnected internal geometry made it possible to create a passive chaotic advection. Interestingly, the printed devices are able to tolerate total flow rates of 10 mL min^{-1}, exceeding the mechanical resistance of many commercial ones. For each device, various PBS: lipid phase fluid ratios (e.g., 5:0.5, 5:1, 3:1, 5:2, 5:3) were tested to optimise the production process, and all provided successful results. In particular, the formulated liposomes have been less than 200 nm in size with a PDI < 0.25. The ζ- potential is negative and stable, ranging between -14 and -8 mV.

Conclusions: This explorative work has shown very promising initial results, and further studies are underway to investigate its potentials. Although the advantages of using 3DP and microfluidics are well known, this research has highlighted the great potential of the synergy of the two techniques. In this way, by producing the devices in-house and applying them via microfluidics, it is possible to decide the type of geometry to be printed with very high accuracy, reduce production times and costs, in order to obtain formulations suitable for nanomedicines.