A 3D PRINTED REACTOR-IN-A-CENTRIFUGE (RIAC) FOR THE PRODUCTION OF THERAPEUTICALLY RELEVANT LIPOSOMES

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Background: Liposomes have been extensively investigated and clinically employed for the delivery of bio-active compounds, including chemotherapy drugs and vaccines, demonstrating improved pharmacokinetic behaviour and therapeutic efficacy. Traditional bulk production methods often suffer from limited control over liposome size dispersity and lamellarity, and may rely on laborious multi-step procedures. Microfluidic-based methods have been introduced to provide greater control over the end-product characteristics; however, their widespread adoption is often hindered by complexities and costs associated with device manufacturing and operation, as well as the relatively short device lifetime and low production rates. In this study, we demonstrate production of therapeutically relevant liposomal formulations, using a novel reactor-in-a-centrifuge (RIAC) device. RIACs rely on a cost-effective and single-step manufacturing process, and are actuated by conventional laboratory centrifuges to drive reagents through the reactor. The reactor concept developed in this study has the potential to simplify production of organic and inorganic nanomaterials over more complex flow-reactor technologies.

Methods: RIACs were manufactured in polylactic acid (PLA) using a fused deposition modelling (FDM) printer (Ultimaker S5). The device architecture included two reservoirs connected to a spiral-shaped mixing channel. Channels were printed within a cylindrical body, which could be hosted in a standard 50 mL centrifuge tube. Liposomes were produced through a 'solvent exchange' mechanism, which was driven by advective mixing between ethanol (where liposome constituents were solubilized) and deionized water. The formulations investigated in this study contained distearoylphosphatidylcholine (DSPC) and cholesterol, as the core liposome constituents. Liposome average size, size dispersity (or polydispersity index, PDI) and surface charge were measured as a function of different production- and formulation-related parameters. These included temperature, centrifugation time, relative centrifugal force, total lipid concentration, and presence of a PEG-moiety. Formulations containing cationic and biotinylated lipids were also investigated, in order to demonstrate RIAC's ability to synthesize functionalised liposomes with potential application in targetable delivery of genetic materials.

Results: The mean size of the produced DSPC:cholesterol liposomes could be tuned in the range of 140 nm to 200 nm, by varying the RIAC actuation parameters. The optimised actuation method (centrifugal force: 2000 rcf, centrifugation time: 4 min) resulted in the production of liposomes with a therapeutically relevant mean size of 174 nm and a narrow size distribution (PDI = 0.10), at a production rate of ~6 mg/min. Notably, PEGylated liposomes were stable up to one month, at a storage temperature of 4°C. The versatility of the developed production method was also demonstrated by successful synthesis of cationic and biotinylated liposomes, with a mean size of ~120 nm and relatively low size dispersity (PDI < 0.2). Experiments are currently being performed to further evaluate liposome morphology through fluorescence microscopy imaging.

Conclusions: The flow-through production method proposed in this study has the potential to be an effective and versatile approach to simplify the synthesis of therapeutically relevant liposomal formulations, due to the single-step and pump-free nature of the process. Future studies could investigate scaling-up strategies to achieve greater production rates and improve continuity of device operation.