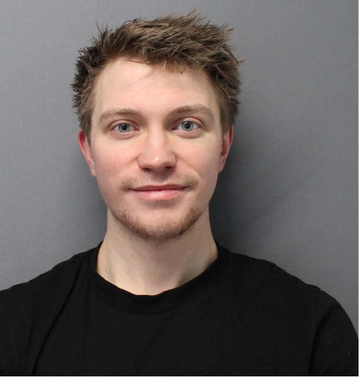

Implementation of a novel microfluidic structure to transition protein loaded liposome production from bench to GMP

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Introduction

Microfluidics is a flexible process that offers scale-independent manufacturing processes for liposomes. Microfluidics offers higher drug loading and better physio-chemical attributes compared to traditional liposomal production processes. These processes are often time-consuming and complex which can be circumvented using microfluidics allowing for easier up-scaling. The aim of this study was to compare different microfluidic architectures and test liposome production from the lab bench to GMP scale.

Objectives

- Define liposome characteristics manufactured by two different laminar flow microfluidic cartridge designs.
- Assess the scalability of liposome production from the lab bench (12 mL/min) through to GMP production (> 20 L/h) using the same standardised operating procedures.

Materials and Methods

- 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholestrol (Chol) were dissolved in methanol (MeOH) or ethanol (EtOH) at 2:1 wt/wt (initial lipid concentration 4 – 16 mg/mL).
- Liposomes were produced using the staggered herringbone mixer (SHM) or a toroidal micromixer mixer (TrM). Liposomes produced using the Blaze or GMP system used the MF-60 or NxGen 500 cartridges from Precision NanoSystems Inc. using the TrM planar design.
- The flow rate ratio (FRR) used was 3:1 aqueous:lipid phase at a total flow rate (TFR) 12-60 mL/min.
- Ovalbumin (OVA) was dissolved in PBS and added to the aqueous inlet (250 - 1000 µg/mL).
- The phospholipid assay was purchased from Sigma Aldrich Company Ltd., Poole, UK
- Liposomes were purified using tangential flow filtration (TFF; Krosflow Research Iii with a 750 KDa mPES column).
- Liposome size, charge and PDI was measured using a Malvern Zetasizer Nano ZS.
- Protein encapsulation was quantified using reversed-phase high performance chromatography (RP-HPLC, Shimadzu 2010-HT, Milton Keynes, UK) connected with a UV detector at 210 nm using a Jupitar 5 µm C5 300A 4.6 mm i.d x 250 mm length or micro-BCA protein assay (Thermo Fisher Scientific, Waltham, MA, USA).

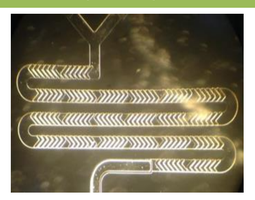
Staggered Herringbone Micromixer (SHM)	Toroidal Mixer (TrM)
	
Flow rate capacity 1 – 20 mL/min	Flow rate capacity 1 mL to > 20 L/h
Platform used: • NanoAssemblr Benchtop®	Platform used: • Ignite™ • NxGen Blaze™ • GMP system
Parallelisation of multiple SHM required to achieve GMP scalability	One chip required to achieve GMP scalability

Figure 1: *Micromixer cartridge designs used within these studies. Schematics illustrate the staggered herringbone micromixer (SHM) with embossed chevrons allowing consistent fluid mixing and the toroidal mixer (TrM) with planar geometry employing centrifugal forces to encourage uniform mixing facilitating greater fluid stream velocities. The flow rate capacities for each of the microfluidic mixers and the microfluidic platforms that are used are listed.*

Results

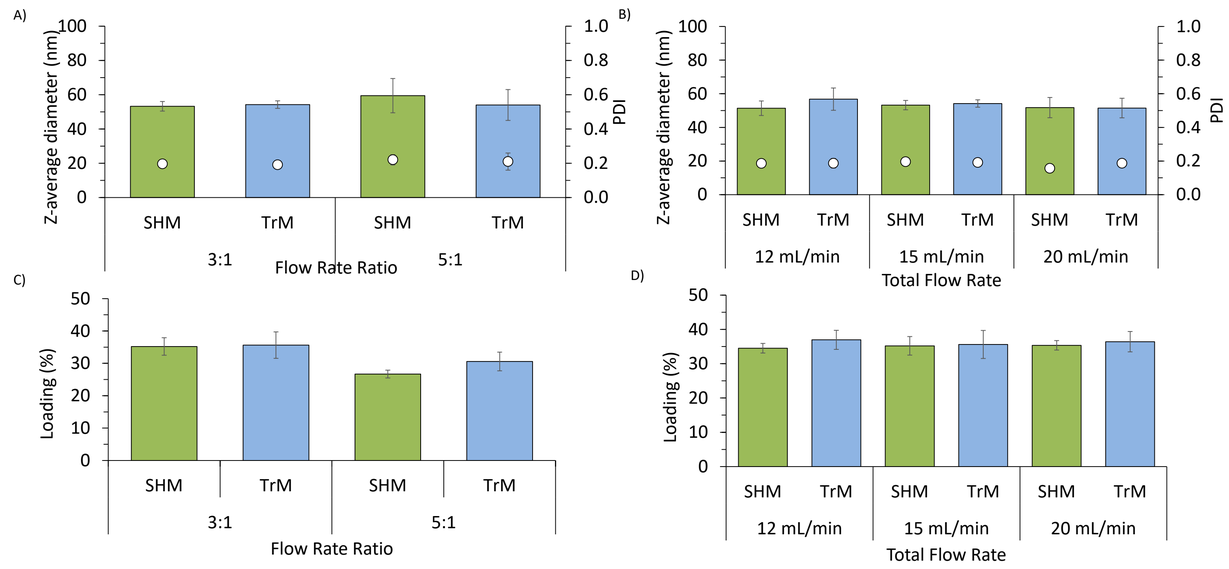


Figure 2: *Detailed study investigating FRR and TFR on each system investigated. (A-B) represents the effect of size and loading efficiencies respectively as the FRR is increased from 3:1 to 5:1 aqeuous:organic phase. (C-D) represents the effect of size and loading efficiencies respectively as the TFR is increased from 12-20 mL/min. Results represent mean ± SD, n = 3.*

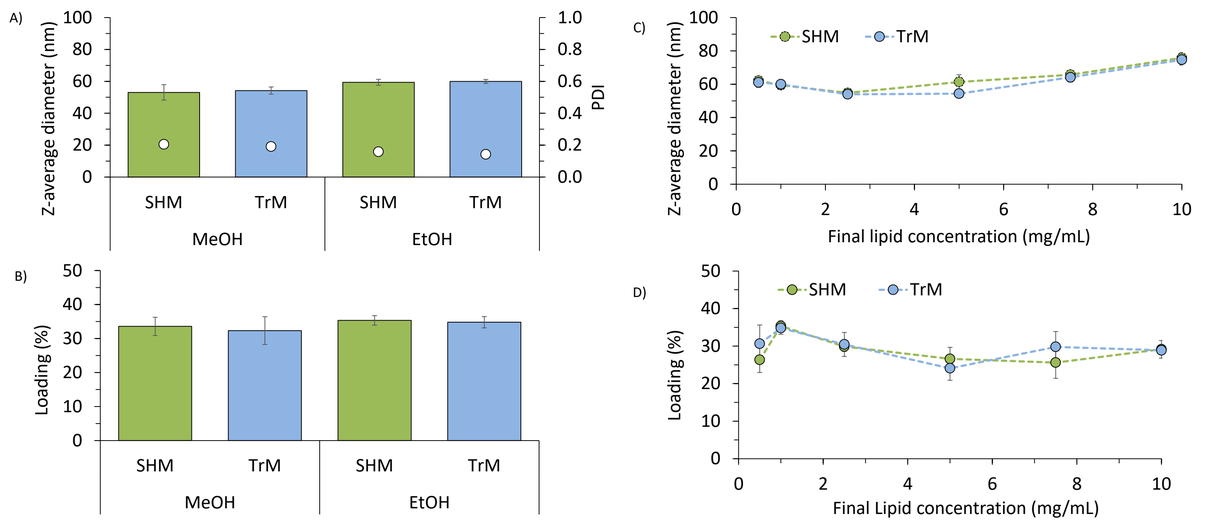


Figure 3: *Investigating the effect of solvent on liposome size and PDI (A) and the resulting entrapment efficiencies (B) using MeOH and EtOH. Liposomes were composed of DSPC:Chol (2:1 wt/wt) and produced using SHM and TrM at an initial concentration of 4 mg/mL and 0.25 mg/mL initial OVA concentration. A 3:1 FRR and 15 mL/min TFR was used and resulting sample purified using TFF. Results represent mean ± SD, n = 3*

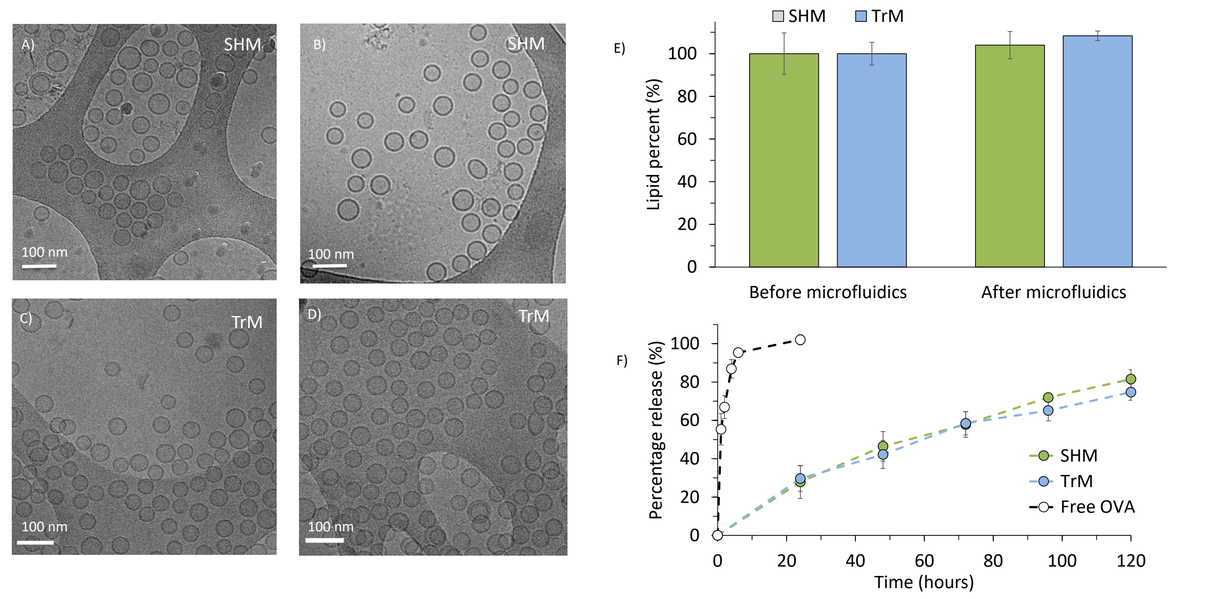


Figure 4: *CryoTEM (conducted by The University of Nottingham) representation of liposomes with entrapped OVA using the SHM (A-B) and TrM (C-D) micromixers. Phospholipid quantification assessing lipid concentration before and after microfluidics (E). Liposomes were produced in MeOH using DSPC:Chol (2:1 w/w); FRR of 3:1; TFR of 15 mL/min; initial lipid concentration 4 mg/mL with 0.25 mg/mL initial OVA (A-E). Protein release from DSPC:Chol liposomes (F). The release of ovalbumin from liposomes produced using the HSM and TRM cartridges was tracked over 120 hrs at 37 °C. Liposome formulations were produced at 3:1 FRR, 15 mL/min TFR and matched initial lipid of 16 mg/mL and initial OVA of 1 mg/mL (F). Results represent mean ± SD, n = 3.*

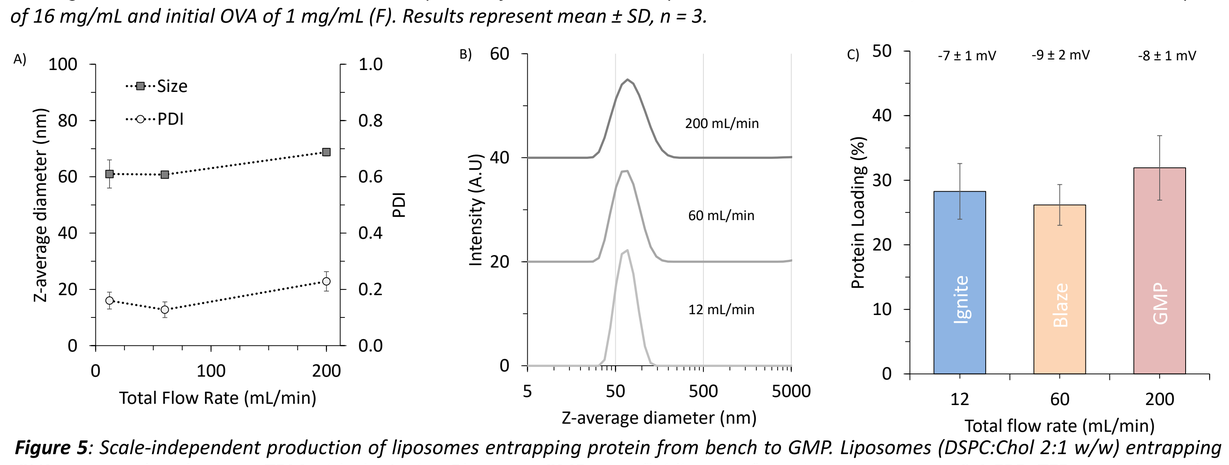


Figure 5*: Scale-independent production of liposomes entrapping protein from bench to GMP. Liposomes (DSPC:Chol 2:1 w/w) entrapping OVA were produced using a TRM with the Ignite, Blaze or a GMP microfluidic manufacturing system ran at 3:1 FRR. TFR was increased to demonstrate scale-independent production from 12 mL/min (Ignite™) to 60 mL/min (NxGen Blaze™; MF-60) or 200 mL/min (GMP; NxGen 500). The liposome z-average diameter and PDI (A), intensity-weighted size distribution (B) and protein loading quantified by micro-BCA (C) are shown. Results represent mean ± SD of three independent batches for the Ignite and NxGen Blaze systems and 1 large-scale batch on the GMP system.*

Conclusions

- ✓ Demonstrated moving between different microfluidic architectures produces comparable physiochemical characteristics in terms of size, PDI, charge and protein entrapment efficiencies
- ✓ Demonstrated manufacturing speeds can be adjusted without any impact to formulation characteristics.
- ✓ Highlighted a direct manufacturing pathway from the lab bench to GMP manufacturing using standard operating procedures

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