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Microfluidic-controlled manufacture of liposomes for the solubilisation of a poorly water soluble drug

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ABSTRACT

Besides their well-described use as delivery systems for water-soluble drugs, liposomes have the ability to act as a solubilizing agent for drugs with low aqueous solubility. However, a key limitation in exploiting liposome technology is the availability of scalable, low-cost production methods for the preparation of liposomes. Here we describe a new method, using microfluidics, to prepare liposomal solubilising systems which can incorporate low solubility drugs (in this case propofol). The setup, based on a chaotic advection micromixer, showed high drug loading (41 mol%) of propofol as well as the ability to manufacture vesicles with at prescribed sizes (between 50 and 450 nm) in a high-throughput setting. Our results demonstrate the ability of merging liposome manufacturing and drug encapsulation in a single process step, leading to an overall reduced process time. These studies emphasise the flexibility and ease of applying lab-on-a-chip microfluidics for the solubilisation of poorly water-soluble drugs.

1. Introduction

The delivery of drugs by liposomes was first described in the 1970s by Gregoriadis (Gregoriadis and Ryman, 1971) and there is now a range of clinically approved liposome-based products that improve the therapeutic outcome for patients. Whilst liposomes are commonly considered for the delivery of aqueous soluble drugs, they are also well placed to act as solubilisation agents for drugs with low aqueous solubility. This is of considerable interest given that more than 40% of all new chemical entities in discovery have low solubility and subsequent issues in bioavailability (Savjani et al., 2012; Williams et al., 2012). The encapsulation of low solubility drugs into the bilayer of liposomes allows not only for their solubilisation in an aqueous media, but furthermore can offer protection from degradation and control over the pharmacokinetic drug distribution profile and improved therapeutic efficacy.

When solubilising drug within the liposomal bilayer, drug incorporation and release rates has been shown to depend on the properties of the drug, the composition of the liposomes, the lipid choice and concentration (Ali et al., 2010, 2013; Mohammed et al., 2004). For example, the log P and molecular weight are often considered to impact on bilayer loading, and studies have shown that molecular weight may play a dominant role (Ali et al., 2013). When considering the design of liposomes, there are a range of parameters that impact on bilayer loading efficacy. For example, we have previously shown that increasing the bilayer lipophillic volume (by adopting longer alkyl chain lipids within the liposomes) increases the loading ability of liposomal systems (Mohammed et al., 2004; Ali et al., 2013). Similarly, incorporation of charged lipids within the liposomal system may also impact on bilayer loading through electrostatic repulsion of drugs with like-charged liposomal bilayers (Mohammed et al., 2004). Incorporation of cholesterol, whilst stabilising the liposomes was also shown to inhibit bilayer drug loading (Ali et al., 2010) due to the space-filling action of cholesterol in the liposomal bilayer. By increasing the orientation order of the phospholipid hydrocarbon chains, cholesterol decreases bilayer permeability. Indeed, the presence of cholesterol in liposomes solubilising propofol was shown to shift the drug release profile from zero-order (when no cholesterol was present) to first order (when 11–33 mol% of cholesterol was incorporated). This maps to the idea that without cholesterol, the bilayer can be thought of as more 'porous' in nature compared with the more condensed and less permeable cholesterol-containing liposome bilayers (Ali et al., 2010).

However, whilst a wide range of studies have looked at the effect of formulation parameters on the application of liposomes as solubilising agents, more focus is required into making liposomes a cost-effective solubilising agent. Recent advances in lab-on-a-chip based tools for process development has already lead to microfluidic-based methodologies in drug development (Dittrich and Manz, 2006; Weigl et al., 2003; Whitesides, 2006). Indeed, microfluidics-based methods (which exploit controlled mixing

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of streams in micro-sized channels) have been described for the manufacture of liposomes and lipid nanoparticles (van Swaay, 2013). Liposome formation by microfluidics primarily depends on the process of controlled alterations in polarities throughout the mixer chamber, which is followed by a nanoprecipitation reaction and the self-assembly of the lipid molecules into liposomes. Generally, two or more inlet streams (lipids in solvent and an aqueous phase) are rapidly mixed together and flow profiles in the chamber itself are of low Reynolds numbers and categorized as laminar. Using microfluidic systems, a tight control of the mixing rates and ratio between aqueous and solvent streams is achieved, with lower liquid volumes required, which facilitates process development by reducing time and development costs. The systems are designed with the option of high-throughput manufacturing and are generally considered as less harsh compared to conventional methods of liposome manufacturing that are based on mechanical disruption of large vesicles into small and unilamellar ones (Wagner and Vorauer-Uhl, 2011). Within the range of microfluidic mixing devices, we use a chaotic advection micromixer, a staggered herringbone micromixer (SHM). The fluid streams are passed through the series of herringbone structures that allow for the introduction of a chaotic flow profile, which enhances advection and diffusion. A chaotic advection micromixer, as well as flow focusing methods, were shown to allow for scalability, associated with defined vesicle sizes (Belliveau et al., 2012; Jahn et al., 2007). The method based on chaotic advection was shown to reproducibly generate small unilamellar liposomes (SUV) with tight control of the resulting liposome sizes at flow rates as high as 70 mL/min in a parallelised mixer-setup. We have previously shown that microfluidics can be used to produce cationic liposomal transfection agents (Kastner et al., 2014), where design of experiments and multivariate analysis revealed the ratio between aqueous and solvent phase having a strong relevance for the formation of size-controlled liposomes. Within this study, we have exploited microfluidics to develop a high-throughput manufacturing process to prepare liposomes solubilising drug within their bilayer (Fig. 1).

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (PC) and cholesterol were obtained from Sigma–Aldrich Company Ltd., Poole, UK. Ethanol and methanol were obtained from Fisher Scientific UK, Loughborough, UK. Tris Ultra Pure was obtained from ICN Biomedicals, Inc., Aurora, OH. Propofol (2,6-bis(isopropyl)phenol) and 5(6)-carboxyfluorescein (CF) was obtained from Sigma–Aldrich Company Ltd., Poole, UK.

2.2. Micromixer design and fabrication

The micromixer was obtained from Precision NanoSystems Inc., Vancouver, Canada. The mixer contained moulded channels which were 200 μm × 79 μm (width × height) with herringbone features of 50 × 31 μm. 1 mL disposable syringes were used for the inlet streams, with respective fluid connectors to the chip inlets. Formulations using the micromixer were performed on a NanoAssemblr™ (Precision NanoSystems Inc., Vancouver, Canada) that allowed for control of the flow rates (0.5–6 mL/min) and the flow ratios (1:1 to 1:5, ratio between solvent:aqueous) between the respective streams.

2.3. Formulation of small unilamellar vesicles using microfluidics

Lipids (16:4 molar ratio of PC and cholesterol, 8:1 w/w) were dissolved in ethanol. SUV were manufactured by injecting the lipids and aqueous buffer (Tris 10 mM, pH 7.2) into separate

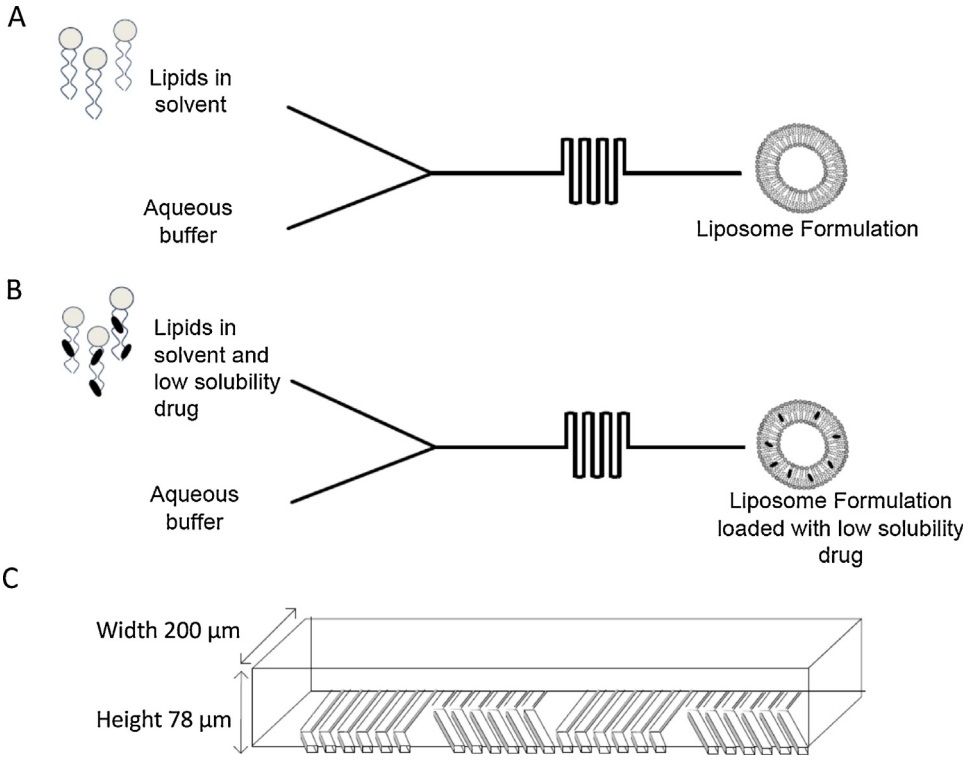

Fig. 1. Schematic depiction of the liposome formation process based on the SHM design, a chaotic advection micromixer for (A) empty liposomes, (B) drug loaded liposomes and (C) chamber layout.