ABSTRACT
This study utilizes an established microfluidic technique to synthesize small, nearly monodisperse liposomes of tunable diameters within the size limit for passive transport across dermal layers to investigate specific relationships between size and drug transport. Our findings provide more intricate results than present studies using polydisperse vesicles prepared via traditional bulk-scale methods, which result in vesicles that are too large to passively traverse dermal layers (>80 nm). Here we utilize the microfluidic technique to synthesize liposomes within a range of sizes unattainable by traditional methods (<40 nm) to demonstrate size-dependent passive uptake of liposomes into porcine dermal tissue.

KEYWORDS: Transdermal drug delivery; Liposome; nanoparticle; Bionanotechnology

INTRODUCTION
As an appealing alternative to oral delivery and a potentially promising substitute for hypodermic injection, transdermal drug delivery is anticipated to have a significant impact on drug delivery, with clinical applications ranging from pain management to dementia [1]. Numerous tactics have been studied for transdermal delivery of macromolecules, including novel chemical enhancers, electroporation, cavitation ultrasound, microneedles, thermal ablation and microdramabrasion [2]. Though these techniques have provided constructive advancements for transdermal drug delivery, all involve disrupting the stratum corneum (SC), a 10-20 µm thick protective layer of the epidermis, to reach the underlying tissue.

An alternative strategy for delivering molecules to deeper tissues of the skin is to passively traverse the SC using nanoparticles. Topical application of nanoparticles is a rapidly growing field for transdermal delivery of active reagents through the skin for systemic treatment of a variety of clinical conditions [1]. Nanoparticles with evident potential for transdermal drug delivery are of lipid, polymeric, mineral, or organic origin, demonstrating enhanced drug penetration and expanding the range of molecules which may be passively delivered by the transdermal route in a noninvasive manner [3]. Despite these advantageous qualities, safety is a prevailing concern for nanoparticulate transdermal drug delivery systems, particularly the biodegradability of inorganic nanomaterials which can be highly toxic once they have been taken up and retained by the reticuloendothelial system [3].

Liposomes, nanoscale vesicles composed of natural physiological and completely biodegradable lipids, exhibit low toxicity and excellent tolerability as well as enhanced drug permeation and chemical stability. It has been proposed that liposomes have potential as vehicles for transdermal delivery of reagents due to their ability to improve transport across the SC, which is primarily composed of a lipid/protein matrix [4]. Although liposomes possess ideal qualities for transdermal drug delivery, their maximum potential has yet to be demonstrated as preliminary studies have indicated that liposomes are unable to traverse the SC in significant quantities [5]. Various studies have examined the dermal transport of liposomes with diameters ranging from 60 nm to micrometers with little indication for transport of intact liposomes through the SC [6], however, it has been shown that 20-30 nm diameter lipid dendrimers possess the ability to traverse the SC [7]. Additionally, smaller 40 nm particles have proven the ability to transport through dermal layers while larger particles were excluded [8]. The accruing indications that nanoparticles within the range of 20-40 nm can passively cross the primary dermal barrier in addition to the benefits of liposomal drug delivery necessitate the ability to create small, nearly monodisperse populations of vesicles within this size range. Nevertheless, conventional liposome production techniques are based on bulk-scale processes, resulting in large (typically >80 nm), polydisperse populations of vesicles, limiting the full capacity of liposomes as transdermal drug carriers to be attained.

A previously demonstrated microfluidic flow-focusing technique offers an alternative method for liposome synthesis which enables production of nearly-monodisperse vesicles within a range of sizes not achievable through customary methods (40 nm and below) [9]. The microfluidic method enables the production of unilamellar lipid vesicles of tunable size within the necessary size range limitations than traditional methods, enabling studies to be performed with deconvoluted data describing size-based phenomenon.

Figure 1. Comparison of liposomes made via microfluidic flow-focusing at different flow rate ratios (FRRs) and traditional bulk homogenization following multiple filtration steps. The microfluidic method enables production of liposomes of tunable size which exhibit narrower size distributions plus lower size limitations than traditional methods, enabling studies to be performed with deconvoluted data describing size-based phenomenon.
range to passively cross dermal tissue with much lower levels of polydispersity than conventional methods (Fig. 1).

Microfluidic-directed synthesis of nanoscale liposomes is realized through hydrodynamic focusing of a stream of lipid solvated in ethanol by two adjacent sheath flows of aqueous buffer [9], resulting in populations of liposomes as small as 30-40 nm in diameter. This unique liposome synthesis method exploits the controlled diffusive mixing of chemical species at the nanoscale as a result of the laminar flow in microfluidics to facilitate precise self-assembly of lipids into nearly-monodisperse populations of liposomes whose sizes may be adjusted by altering the flow conditions within the microchannels. Here, we exploit continuous-flow microfluidic lipidosome synthesis to generate small, nearly-monodisperse lipid vesicles within the size range of interest for transdermal drug delivery to exhibit size-dependent passive uptake of liposomes into ex vivo porcine dermal tissue.

RESULTS AND DISCUSSION

Populations of liposomes near the size range of particles which have been observed to transport through porous tissues were produced via microfluidic flow-focusing (Fig. 2). Liposomes on the order of size necessary to traverse skin layers (32.4±11.3 nm) as well as larger liposomes (287.1±77.3 nm) of identical composition were produced for comparison. Typical liposome populations produced using the microfluidic technique. Liposomes with diameters both above and below (FRR 5 and FRR 50, respectively) the size range expected to passively traverse the dermal layer.

EXPERIMENTAL

Microfluidic devices made of polydimethylsiloxane (PDMS) and glass were fabricated using soft lithographic methods and bonded via oxygen plasma. Dimyristoylphosphatidylcholine (DMPC), cholesterol, and phosphoethanolamine-[methoxy(polyethylene glycol)-2000] (PEG(2000)-PE) were mixed in chloroform in molar ratio 75:25:5 then placed in a vacuum desiccator for at least 24 hours for complete solvent removal. PEG-lipid facilitates the formation of smaller liposomes, provides steric stabilization, and serves as a protective shield from the immune system for vesicles which reach the vasculature. The dried lipid mixtures were re-dissolved in anhydrous ethanol for a total lipid concentration of 40 mM. Phosphate buffered saline (PBS) was used as a hydration buffer.

Liposomes were prepared by injecting the lipid-solvent mixture between two buffer inputs into the PDMS glass microfluidic device. The flow rate ratio, or the ratio of the volumetric flow rate of buffer to the volumetric flow rate of solvent, was set to FRR 5 and FRR 50 to produce two populations of liposomes above and below the size range likely to passively traverse the dermal layer. Linear flow velocity of the total flow for all FRRs was kept constant (0.125 m/s) for a volumetric flow rate of 112 L/min. The liposome populations were characterized for size using a Malvern Zetasizer and ZSP. The vesicles produced contained Dil-C18 in their bilayer to assist in fluorescence imaging, which helped assess the depth of penetration. To remove any remaining dye not incorporated into the liposomes during the formation process, the samples were purified via size exclusion chromatography on Sephadex G-25 (PD-10) columns that were equilibrated with PBS.

A Yorkshire pig (4 weeks, 5 kg) which was being sacrificed for another study was used for these experiments. Porcine tissue was used to assess passive transdermal diffusion of the microfluidic-synthesized liposomes due to its morphological and functional similarities to human skin [14]. One ear from the pig was removed following general anesthesia and used immediately. Liposome solutions (50 L) each size population (32.4 nm and 287.1 nm) were applied to different locations on the outer side of the ear and placed in a freezer in covered petri dishes following a 15 min incubation at room temperature. The ear tissue was embedded into cryo-OCT media and placed in a freezer (-80 °C). Once frozen, the embedded tissues were mounted in a cryostat microtome (HM550 series, Richard Allen Scientific), sliced into small sections (30 µm), and placed onto gelatin-treated glass slides. The sections were imaged using an inverted epifluorescence microscope (ikon TE-2000 S). Bright field and fluorescence images at a 528 nm-553 nm range excitation wavelength (green filter) were taken and overlaid to assess and validate the depth of liposome penetration into the dermal tissues.

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samples (Fig. 3 (e) and (f)). The small, highly-monodisperse 32.4 nm liposomes are on the size scale of intracellular junctions and other sites which provide openings in the skin, which is a possible reason for their capacity to passively diffuse through the dermal layers while the larger 287.1 nm liposomes simply remain on the top layer of the skin. This is in contrast to other studies, in which larger, more polydisperse liposomes do not penetrate the SC.

CONCLUSION

These findings confirm the ability of microfluidic-synthesized liposomes to be passively incorporated into dermal tissue. The capacity to produce liposomes within a size limit that can traverse the skin enables the use of liposomes for transdermal drug delivery applications with results unprecedented by liposomes prepared through traditional methods. These promising results may be used to plan studies in which microfluidic-synthesized liposomes with small incremental size differences are investigated to optimize formulations for dermal uptake.

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REFERENCES


CONTACT
*D.L. DeVoe, tel: +1-301-405-8125, ddev@umd.edu