UNIVERSITY OF URBINO CARLO BO

Department of Pure and Applied Sciences

Doctoral Program in Research Methods in Science and Technology

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CHIM/09

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Microfluidic formation of deformable lipid vesicles for dermal and transdermal delivery

Introduction

The skin is a very promising non-invasive drug delivery route as it represents the most easily accessible human organ. Nonetheless, it is challenging to deliver drugs by topical and transdermal routes, due to the its complex structure (1,2). In order to overcome the difficulties of drug transport through the skin, improve the pharmacokinetic properties and reduce adverse effects, several organic drug delivery systems (DDS) were specially developed for skin passive delivery.

Liposomes are a lipid based vesicular DDS first studied by Alec D. Bangham in the 1960s (3). Their use in topical delivery has been reported later since the 1980s (4). These interesting vesicles are composed by a mono or multi-phospholipid bilayer resulting in a spherical structure with an aqueous core (5,6). This conformation is suitable for the incorporation of molecules with different solubilities since the hydrophilic molecules easily "accommodate" in the core of the vesicle while the hydrophobic ones bind to the lipid bilayer. The advantages of liposomes in drug delivery are well known, such as biocompatibility, biodegradability, and non-toxicity or immunogenicity (7). However, they present the disadvantage of poor ability to penetrate the skin, which is a clear limitation for topical and especially transdermal delivery (8,9). As a result, other specific types of liposomes, such as ultradeformable vesicles (UDV), have been developed to improve their physicochemical properties, successful and sustained skin drug delivery, among other properties (10–12).

One of the main challenges in the manufacturing of liposomes or UDV is to obtain a formulation with a consistently reproducible size and homogeneous distribution using the conventional methods (13,14). The microfluidic technique is an emerging technology that has been attracting attention as it allows a more accurate control of the flow rate of multiphase fluids, letting processes such as mixing and droplet generation to occur under more controlled conditions (15,16). As reported in Hood et al. work, the microfluidic synthesis of nanoscale liposomes enabled the generation of highly stable monodispersed vesicles with a smaller size that allowed the passive transdermal drug delivery (17). Despite the promising opportunities for liposome preparation, the application of the microfluidic technique to produce the UDV also requires further investigations.

Aim of the project

The main goal of the project is to acquire further knowledge on microfluidic synthesis of UDV for topical application to achieve dermal and transdermal delivery.

More specifically the project aims to:

- Identify the best device and fabrication of the microfluidic device;
- Investigate the formulation parameters (lipid mixture, edge-active substance selection, and hydration buffer) to obtain optimal mixtures;
- Produce and characterize the developed systems, on the basis of morphology, surface chemistry, and stability;
- Evaluate the cytocompatibility *in vitro*;
- Assess the interactions with cells;
- Explore the *in vitro* and *in vivo* behaviour.

Methodology and expected results

Product design and manufacture

The first phase will consist in fabricating and choosing the microfluidic devices that will be used to form small, nearly monodisperse liposomes and ultradeformable vesicles.

Formulation development: Microfluidic Liposome Synthesis and Characterization

Liposomes will be prepared by injecting the lipid-solvent mixture between two buffer inputs into the microfluidic device. The flow rate ratio and linear flow velocity will be determines. For small vesicles only buffer will be used; for deformable vesicles surfactant and/or ethanol will be solubilised in the buffer. A lipophilic model drug (solubilized in the lipid mixture) and a hydrophilic model drug (solubilized in the buffer solution) will be incorporated and encapsulated into the vesicles, respectively.

The liposome populations will be characterised by:

- Dynamic light scattering (DLS): used to determine the vesicle size and polydispersity index (PDI);
- High Performance Liquid Chromatography (HPLC): used to quantify the incorporation efficiency and to evaluate the amount of the drug during release studies;
- Transmission electron microscopy (TEM): used in the evaluation of vesicle morphology.

Cytocompatibility in vitro and interactions with cells

The biocompatibility of the systems will be assessed on different cell lines representative for the desired target, for instance keratinocytes and dermal fibroblasts.

Development of a pharmaceutical form suitable for liposome topical application

The best strategic semi-solid pharmaceutical form will be selected to perform the *in vitro* and *in vivo* assays.

***In vitro* and *in vivo* studies on diseased models**

In summary, the expected outcomes of the project involve the successful development of an innovative system that validates microfluidics as a suitable technique to produce stable, reproductible, and biocompatible UDVs. Furthermore, the produced formulation would be used to prove the biocompatibility and efficiency of the formulation.

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Feasibility of the 3-years project:

Time period	**Activities**
First Year Nov 2023 – Oct 2024	Acquisition of knowledge about: - Microfluidic device design and manufacture - Materials - Applications Starting with the development of the microfluidic devices Selection of nanocarriers composition Optimization and characterization of the developed devices Visiting period abroad
Second Year Nov 2024 - Oct 2025	Testing the microfluidic devices for the production of small nearly-monodisperse liposomes and deformable liposomes Dosage form development Visiting period abroad
Third Year Nov 2025 - Oct 2026	Final *in vitro* and *in vivo* studies Final thesis