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"Off-the-shelf" microfluidic devices for the production of liposomes for drug delivery

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ABSTRACT

An "off-the-shelf" microfluidic chip approach, utilizing lowcost, commercially available components, for liposome production, is presented. Microfluidic devices with different geometries have been conveniently designed and assembled, allowing the production of narrowly dispersed unilamellar and very reproducible liposomes. The presented results indicate that off-the-shelf microfluidic devices can hold great promises for the efficient preparation of different lipid based colloidal systems for biomedical applications.

1. Introduction

Lab-on-a-Chip (LOC) devices have been largely proposed as miniaturized bioanalytical systems for chemical/biological applications being able to perform multiple tasks associated with many laboratory procedures [1]. LOC devices offer indeed many advantages over standard (i.e. macroscopic) systems, including reduced sample and reagent consumption, faster analysis and higher levels of throughput and automation.

In spite of these advantages, the production and use of microfluidic chips still remain largely confined to academics. The costs for their development and fabrication remain indeed rather high; commercial standard chips are expensive (e.g. the price of a flow focusing chip ranges between 150 and 200 US dollars) and the price to pay for a tailored chip with particular channel geometry can dramatically increase up to thousands of dollars. In addition, the fabrication of complete chips (with ports and connecting tubing) requires specific knowledge, facilities and equipment, therefore, only marginally applicable for protocols requiring rapid evaluation of prototypes [2]. Moreover, the life span of a microfluidic chip can be dramatically short, especially if the channel width is below 100 μm, due to always-possible channel clogging.

In order to reduce the high cost of microfluidic chips, some authors have proposed the use of "off-the-shelf" devices as alternative to the generally used PDMS, COC or glass 2D chips [3–5].

The concept of "off-the-shelf" microchip was first described in 2010 by Alex Terray and Sean J. Hart that described the construction and operation of a device assembled using only standard parts, available in the market as HPLC component. The authors demonstrated that, using this device, they were able to obtain a precise focusing of particles in suspension; particularly, a flow focusing of particles was observed at the exit of the nozzle and within a connected microfluidic exit tubing [3].

Later, other authors described the use of "off-the-shelf" as droplet generator devices for the preparation of O/W or W/O emulsions [4,5]. Unfortunately, these manuscripts described the preparation of very simple emulsions, constituted of pure water, mineral oil and span 80, representing a formulation very far from "real" emulsions, suitable for food, cosmetic or pharmaceutical applications. Commercial formulations are normally indeed constituted of many excipients (i.e. especially constituting the oil phase) and at least a drug.

Following the "off-the-shelf" microfluidic concept, in the current paper the use of 2 alternative devices, constituted of readily available commercial products, was described and compared.

Both devices were tested for the production of supramolecular colloidal system (i.e. liposomes) by a mechanism described as "self-assembly" in a controlled diffusion process. Attention was paid to the production of liposomes characterized by a lipid content/composition and drug content strictly resembling commercially available medicines based on liposomal drug-delivery system [6].

As a model drug, ivermectin was selected since recent studies have demonstrated that this drug could be used for the treatment of some RNA viruses [7]. Ivermectin is the semisynthetic derivative of avermectin B1 (a natural compound belonging to the macrocyclic lactone family) that is used in domestic animals and livestock, for the control of internal and external parasites.

Ivermectin is largely used in humans; millions of people are indeed treated with ivermectin for many diseases including onchocerciasis,

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lymphatic filariasis, and scabies. Administration of ivermectin in polyparasitised poor communities around the world is increasingly recognized as a mechanism to effectively improve overall quality of life and health for everyone [8,9]. Importantly, ivermectin has recently proved to be a potent inhibitor towards both HIV-1 and dengue virus [10], therefore the drug has potential in the clinical setting as a dengue antiviral [11].

In order to apply this drug to virus treatment, it is mandatory to develop a convenient drug formulation able to improve the cellular internalization of ivermectin, reducing at the same time the unfavorable effect of the drug. In this respect, in the current paper the preparation of liposomal formulations by "off-the-shelf" microfluidic devices is described.

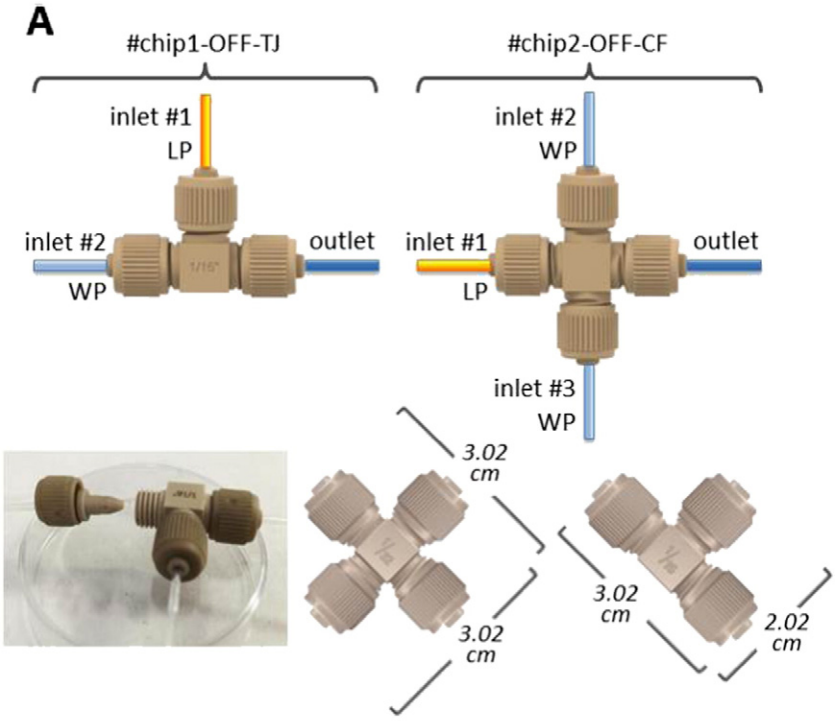
2. Materials and methods

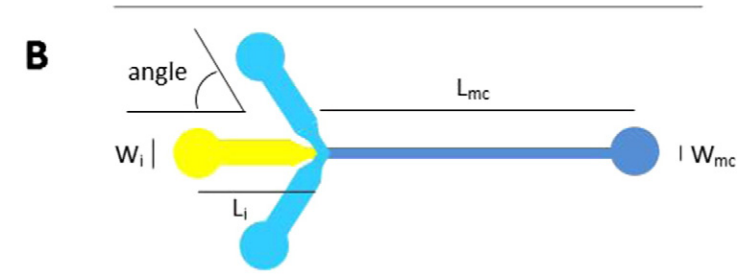
2.1. Chemicals

Highly pure phosphatidylcholine (PC) 90% from soybean (Phospolipon 90G Lipoid, Germany); cholesterol 97% (Fluka, Germany); dimethyldioactdecylammoniumbromide (DDAB). Ivermectin (Sigma, UK) has the following specifications: molecular formula: $C_{48}H_{74}O_{14}$; molecular weight: 875.09; CAS registry number; 71827-03-7; solubility in water: sparingly soluble (0.080 g/l) (25 °C); density: 1.23 ± 0.1 g/cm^3 (20 °C 760 Torr); xlogp3: 4.1; hydrogen bond donor count: 3; hydrogen bond acceptor count: 14; heavy atom count: 62; formal charge: 0; defined atom stereocenter count: 18; appearance (color): white to off-white; appearance (form): powder; solubility (color): colorless to faint yellow; solubility (turbidity): clear, 50 mg/ml, methyl ethyl ketone; purity (HPLC): ≥90%. For the determination of drug entrapment efficiency into liposomes, size exclusion chromatography was conducted with Sepharose 4B (Pharmacia, Uppsala, Sweden) and Isotonic Palitzsch buffer pH 7.44, for 100 mL of buffer solution, 50 mM sodium tetraborate (10 mL) were mixed with 200 mM boric acid (90 mL); NaCl (270 mg) was added to adjust the tonicity of the buffer to 0.9 at 37 °C [12]. All the other regents and solvents were from Sigma-Aldrich and were analytical grade.

2.2. Microfluidic chips

Two different "off-the-shelf" chips were employed (see Fig. 1), namely #chip1-OFF-TJ (characterized by a T-junction geometry and 2





chip code	material	geometry	channel dimensions (width/depth. μm); (length. mm)	channel angle
#chip3-HFF	PDMS/glass	HFF	150/100; 30	40°

Fig. 1. Scheme showing the geometrical characteristics of "off-the-shelf" (A) and conventional cross-flow (B) microfluidic devices. Two different "off-the-shelf" devices were employed, namely: #chip1-OFF-TJ, characterized by 2 inlets and a T-junction geometry and #chip2-OFF-CF characterized by 3 inlets and a cross-flow geometry.