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Department of Pure and Applied Sciences
Doctoral Program in Research Methods in Science and Technology
Chemical and Pharmaceutical curriculum
Research Project

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3D PRINTING REVOLUTION IN THE FABRICATION OF MICROFLUIDIC DEVICES

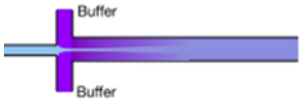
Introduction

During the last few years, microfluidic-based nanocarriers (NCs) production has been widely applied for multiple biological usages. Compared to conventional bulk methods, microfluidic-assisted NCs production shows significant advantages, such as narrower particle size distribution, higher reproducibility, improved encapsulation efficiency and enhanced scaling-up potency [1].

Microfluidics enables precise control over small volumes in devices with microscale dimensions, allowing processes such as mixing, droplet generation, or nanoprecipitation to occur with precise control. The same is not possible using conventional techniques [2].

Several microfluidic device architectures have been used for controlled NCs production; Table 1 describes the different chip geometries with their respective advantages and disadvantages [3].

Table 1 *Different chip geometries*

	Chip geometry	***Advantages***	***Disadvantages***
Microfluidic hydrodynamic flow focusing 	In this chip a narrow fluid stream flows in the same channel next to a different fluid to facilitate rapid mixing between the two fluids.	• Small size NCs (<150 nm) • Fully scalable manufacturing	• Limited use for gene therapy • Dilute sample concentration
Microfluidic staggered herringbone micromixer 	This chip involves a chaotic advection channel, meaning that transverse flows are generated that causes advection that are not in the same direction as the flow.	• Small size NCs (<100 nm) • Encapsulation efficiency >90% • Fully scalable manufacturing	• Limited solvent compatibility for device materials
Microfluidic bifurcating mixer 	This chip induces chaotic advection as the fluid travels, the channels split into two, travel a different path length, and are then merged back together inducing rapid mixing in a single-layer device by large centrifugal forces.	• Small size NCs (<100 nm) • Encapsulation efficiency >90% • Fully scalable manufacturing	• Injection molding fabrication processes not always accessible
Microfluidic baffle mixer 	This chip is an invasive NCs production device, that involves a series of perpendicular turns to rapidly mix NCs components.	• Small size NCs (<100 nm) • Fine tuning of NP size • Encapsulation efficiency >90%	• Limited use for gene therapy • Requires modified design for low production rates
T-junction mixing 	This is a method of rapid mixing operated at very high flow rates (40–60 mL/min) where two input streams are faced directly towards each other with a perpendicular output.	• Solvent compatibility • Suitable for large-scale production	• Not suitable for small volume production during discovery phase

Common microfluidic device fabrication protocols include photolithography and micromachining. Some of these techniques require much space to hold multiple equipment, intensive labor (multiple step processes to make final product), time wastage to facilitate the change in design, and limited biological materials available [4].

Recently, advancements in 3D printing in terms of resolution and speed have helped to simplify the fabrication process of microfluidic devices into a single step. 3D-printing refers to a set of additive manufacturing techniques, which can create solid three-dimensional (3D) objects layer-by-layer under precise digital control. Among these techniques, the one that is most relevant to microfluidic device fabrication is fused deposition modeling (FDM) [5].

FDM is based on the melting and extrusion of a polymer filament. The filament is fed into and melted in a heated metal cylinder ending in a nozzle. As fresh filament is supplied continuously into this component, the molten polymer is pushed out of the nozzle, forming a thread roughly the size of the nozzle diameter. To shape this thread into a plastic part, the nozzle is placed above a building plate (print bed) at a distance that depends on the desired resolution. Upon exiting the nozzle, the filament is deposited on this print bed, which can be heated to promote attachment. When the print bed and nozzle are both controllably moved in perpendicular directions, we can draw a two-dimensional figure on the print bed having the thickness of one polymer thread. This thickness is controlled by the distance between the nozzle and the print bed and the ratio between the flow rate of the filament through the nozzle and the printing speed. When the first layer is finished, the print bed is lowered by a fixed distance (the thickness of a single layer), and a second layer can be printed on top of the original one. By repeating these steps, an object is created in an additive manner [6].

This approach presents several advantages including a large selection of relatively inexpensive materials and printers, low maintenance costs, the ease of initial use and the ability to start, stop, and integrate complexity on the fly [7].

Aim of the project

The microfluidic devices will first be designed and then printed to be evaluated for the manufacturing of nanocarriers.

The project will be mainly concentrated on:

• Selecting the best materials for FDM 3D Printing;

• Investigating more functional geometries of microfluidic chips;

• Testing the printed chips for the manufacturing of nanocarriers;

• Physico-chemical characterization of the developed systems and evaluating in vitro and in vivo behavior.

Methodology

Microfluidic chip design and manufacture:

- ### 3DP-FDM approach

The first phase will focus on optimizing the production process, including the selection and study of materials to produce the microfluidic chips. These will be designed using a CAD software and then printed employing the FDM 3DP-technique.

- ### Microfluidic using syringe pumps

Precise and highly tunable control of fluidic motion is crucial in microfluidic applications. For this fluidic control, flow-rate-based control method will be employed. Flow-rate-based control is typically implemented with a syringe pump. Once a flow-rate value is set at the syringe pump, the pump pushes the plunger of the syringe at a constant speed [8].

Product Characterization

Prepared formulations will be characterized by:

- **Differential Scanning Calorimetry (DSC):** the thermal behavior of the printed device and the materials employed can be analyzed using the DSC;

- **Thermal Gravimetric Analysis (TGA):** in combination with the DSC it can be applied to obtain information, carrying out a single analysis, on all the phase transitions the materials are subjected (glass transition for polymeric materials, polymorphism);

- **Dynamic Light Scattering (DLS):** it allows to measure the size of the device;

- **Transmission Electron Microscopy (TEM):** it can be employed to visualize the structure and the morphology of the produced nanocarriers;

- **High Performance Liquid Chromatography (HPLC):** it is useful to quantify encapsulated drugs and to evaluate the amount of the drug during release studies.

References

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A provisional timetable for the project:

Time period	***Activities***
First Year Nov. 2021 - Oct. 2022	Acquisition of knowledge about: - FDM-3DP technique - Lab-on-a-chip devices - Materials - Applications Starting with the development of the microfluidic devices

	Optimization and characterization of the developed devices Visiting period abroad
Second Year Nov. 2022 – Oct. 2023	Testing the microfluidic chips for the production of NCs Visiting period abroad
Third Year Nov. 2023 – Oct. 2024	Final in vitro and in vivo studies Final thesis