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Review of the PhD Thesis by Mr. Francesco Nalin "*Novel Microfluidic Strategies for Droplet Generation and Flow Control with Applications in Biotechnology*"

Introductory comments. Introductory comments. Microfluidics has been playing a fundamental role in the recapitulation of biological niches. Specifically designed microfluidic devices serve as bioreactors for the culture of cells under conditions that closely mimic the natural environment in living organisms. They have been successfully used in producing models of various human tissue types. The applications of such systems have been typically employed in the study of fundamental biological mechanisms and in assessing the therapeutical effectiveness of drugs. In particular, given the close resemblance between cultivated tissues and their in vivo counterparts, tissues grown within microfluidic devices represent the most suitable animal experimentation alternatives.

Another relevant application of microfluidic devices is as advanced extrusion printing heads in robotic digital manufacturing. Microfluidics allows the real-time formulation of the ink through the variation of its chemical composition, including the production of heterogeneous inks (emulsions and foams). Such flexibility translates into spatiotemporal control over the composition of the matrix being deposited and the possibility of producing materials with a spatial ordered and controlled porous texture.

The current doctoral dissertation provides novel contributions to using microfluidics in the study above mentioned domains.

Outline of the thesis. The thesis is structured into three primary subjects. The first section involved the design of a microfluidic device capable of accommodating a viable cell population and delivering anticancer medicine to the cells in a predetermined dosage and at various time intervals. The significance of the developed chip lies in its ability to provide real-time monitoring of the drug's absorption by cells, hence facilitating the determination of the kinetics of this process. The chip design may be of significant interest to pharmaceutical companies engaged in developing innovative anticancer medicines.

The second subject of the thesis focuses on creating a microfluidic device that produces water-in-oil droplets. These droplets serve as a controlled environment for cultivating bacteria from the gut microbiota. The objective is to replicate the complete range of bacterial populations in a laboratory setting.

The third topic is developing and producing a microfluidic chip that can generate monodisperse emulsion through step emulsification. The enhanced benefits of the engineered chip rely on its ability to adjust the droplets' size on demand and the possibility of massive parallelization, allowing for considerable scaling up of emulsion production.

Another innovative aspect of this thesis section is the integration of the chip within a 3D printer to place in space, according to a predetermined design, emulsion inks characterized by different droplet sizes and compositions of the continuous phase. The emulsion inks are deposited within a suspending bath that supports the printed ink before the curing process triggered by light.

Summary and critical assessment. The thesis's central theme revolves around using microfluidic devices in biology and 3D printing.

The central thesis' merit relies on the innovative character of the work. All the topics are faced by developing new chip designs and novel post-fabrication surface treatment (i.e. hydrophilization of PDMS surfaces). The first two topics have relevant applicative potentials, as demonstrated by filing a patent. Of particular interest is the fabrication of a step emulsification chip endowed with a deformable surface to control the width of the junction zone. The level of in-depth investigation is not exceptionally high but is compensated by the novelty of the research involved. The discussion regarding the benefits and drawbacks of the produced technologies lacks depth. A hallmark of scientific maturity is thoroughly examining the innovative aspects introduced by new technology and its inherent limitations.

In particular, the thesis lacks an in depth analysis in the following points:

1. In the first section, the drug uptake by cells cultivated inside the microfluidic chip was live monitored by confocal microscopy, but no image analysis was conducted to obtain a curve associating a relevant parameter related to drug uptake with time. A curve like this might be analyzed to extract relevant kinetic parameters that could be used to compare the cellular absorption capability of different drugs. Has the candidate explored such a possibility to any extent?
2. In section 3, the candidate demonstrated how to prepare inks for 3D printing using step emulsification. He emphasized the benefits of extensive chip parallelization and demonstrated

the possibility of depositing inks with varied droplet sizes and continuous phase compositions. One critical piece of information is missing from the analysis of step emulsification potentials: the ability to modify the dispersed phase volume percentage to generate porous materials with varied porosities. Is the step emulsification method compatible with creating high-volume fraction emulsions?

3. There is no study of the morphology of the printed samples. The candidate demonstrated the ability to print geometrically complex items and multi-material objects, but no evidence of internal porous texture was provided. Are there any specific reasons why gradient porosity cannot be obtained? What experimental techniques can be used to obtain images of the porous texture and extract quantitative information (e.g., porosity, pore size distribution, and so on) from them?

The development of the thesis project necessitated the acquisition of several practical skills and instrumental expertise, in particular, CAD design, manufacturing of microfluidic chips by soft lithography, and digital manufacturing.
As a result, Mr Nalin has acquired solid knowledge and expertise in the diverse applications of microfluidics and digital manufacturing and data analysis through specialized software.

The thesis fulfils the legal requirements of the "Law on Higher Education and Science".
1. The doctoral dissertation demonstrates the candidate's general theoretical knowledge in microfluidics and applications related to microfluidics and the ability to conduct research work independently.
2. The subject matter of the doctoral dissertation is an original solution to specific scientific problems.

Rome, 04.01.2024 Prof. Andrea Barbetta

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