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3D printed microfluidic modules: passive mixers and cells encapsulation in alginate

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Abstract: passive mixers and droplet generation microfluidic chip modules were designed and manufactured using a commercial SLA 3D-printer. The mixing modules were designed specifically for 3D-printing and evaluated using FEM modeling. The co-flow droplet generator was used for cancer cells encapsulation and drug potency evaluation.

Keywords: 3D-printing, microfluidic, alginate, mixer, droplet

Introduction

Microfluidics is an increasingly growing technology that finds applications in many fields of life science disciplines, including cell biology and biochemistry. However, state of the art microfluidic systems have still limited availability due to the high cost and complexity of the fabrication method that is based on Silicon wafer photolithography and PDMS replicas. In recent years, 3D printing technology developed sufficient resolution to be an alternative to the traditional manufacturing methods allowing faster, cheaper and more complex structures [1]. Our goal is to develop new solutions for applications in life science based on commercial 3D printers, such as stereolithography (SLA). For this, a microfluidic droplet generator and two mixing modules were specifically designed and printed. The system was used to encapsulate breast cancer cell in alginate droplets and assess drug induced cytotoxicity at increasing concentrations. The mixer performance was evaluated via finite element modelling (FEM).

Materials and Method

All the parts were printed using Form3 SLA printer (Formlabs, US) and ClearV4 resin. The mixers were simulated and printed with a nominal 1mm diameter. Mixing efficiency was calculated using FEM with extra fine mesh in Comsol between 10 and 100 Reynolds. For the simulation the two liquids concentration was set to 1 mol/m^3 and 0 mol/m^3.
The biocompatibility of the microfluidic devices was tested by assessing cytotoxicity on MCF-7 cells after 30 min incubation inside a 1 mm diameter 3D printed channel, thus simulating the operative conditions. The control material was a non-cytotoxic dental resin 3D printed part of the same shape.
MCF-7 Cells were encapsulated in alginate using on-chip partial external gelation of water in oil droplets [2] and collecting the droplets in 1.5 wt.% $CaCl_2$ to complete gelation. Water phase included cells, 1 wt.% alginate and Ca-free culture media. Oil phase was made by dispersing 1 wt.% Ca-acetate with an ultrasonic finger (30 s-30 % amplitude) in soybean oil and adding 3 wt% Span80. After cells encapsulation, alginate beads were incubated for 48 h together with cytostaticum doxorubicin at different concentrations. Cell viability was measured using WST-8 colorimetric assay The liquid flow of mixers and droplet generator was controlled with an OB1 Elveflow system (Elvesys, F).

Results and Discussion

The resolution limits of the printer were assessed by printing circular channels of different size and inclination in a 20x20x20mm block. As summarized in Table 1, the minimum channel size is 500 µm when oriented perpendicularly to the printing platform or 0°, while it is possible to print only

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600 µm channels up to a 60° angle. With increasing inclination up to 90°, only 2000 µm channel are printable.

Table 1: printing outcome of different size channels in a 20x20x20 mm cube at various orientation. OK indicates print was always successful, POSSIBLE indicates a print success rate below 50 %, with FAIL the channels were always clogged.

	Diameter (µm)							
Angle	2000	1600	1200	800	700	600	500	200
0°	OK	OK	OK	OK	OK	POSSIBLE	POSSIBLE	FAIL
45°	OK	OK	OK	OK	OK	POSSIBLE	FAIL	FAIL
60°	OK	OK	OK	OK	OK	POSSIBLE	FAIL	FAIL
90°	OK	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL

The printing limit is explained by the increasing capillary force at narrower channel diameters that traps uncured resin. The printing of successive layers, causes the trapped resin to slowly cure because exposed to the diffused UV light that is not completely limited to the laser spot, thus affecting the minimum feature size printable. The effect is accentuated at higher printing angles above 60°.

With these printing limitations, two different mixer designs were studied with FEM analysis: Diamond and Highway, which mixing units are visible in Figure 1

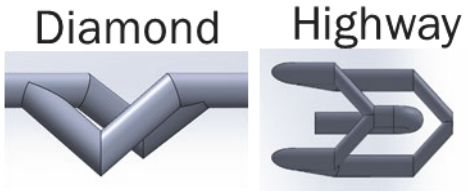

Figure 1: Diamond and Highway modules for 3D printed passive mixers. The length of both modules is 7mm.

Transparent microfluidic modules of both designs could be easily printed and connected with standard microfluidic fitting assuring a fast and secure sealing up to 2 bar. FEM analysis on the mixer composed by 5 repeating units with Ø 1 mm channels, demonstrated that the mixing performance is > 80 % already at 10 Reynolds for both Diamond and Highway. Mixing performance was defined as [3]

$$M = \left[1 - \frac{1}{N}\sum_{i=1}^{N}\sqrt{\left(\frac{\propto_i - \propto_{ref}}{\propto_{ref}}\right)^2}\right] \times 100\%$$

Where N are the simulation nodes at the exit channel surface, a_{ref} is the perfect mixing value, equal to 0.5, and a_i the mixing result at the single node.

The mixing efficiency was qualitatively verified for the Highway mixer as visible in Figure 2A where blue and yellow coloured water was introduced in the module and became completely green already after 3 mixing units due to the smaller channel diameter and the higher pressure used compared to the simulation. Highway and Diamond mixers were specifically created for 3D printing. The necessity to design new mixers was given by the impossibility to directly copy existing 2D designs due to printing limits in both resolution and angles; and to take advantage of a unique three-dimensional geometry to improve mixing through splitting, stretching, folding, and breaking processes[4].

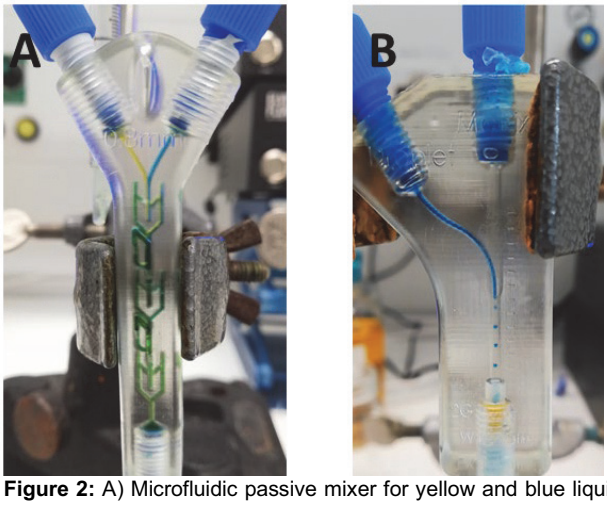

Figure 2: A) Microfluidic passive mixer for yellow and blue liquid. Channel nominal diameter 800 µm B) Co-flow alginate droplet generator. Droplet size ≈500 µm. The alginate is blue colored for better visibility

Together with the mixing modules, a 3D-printed droplet generator for cells encapsulation was developed and it is visible in Figure 1B. It is based on a co-flow design with oil in the outer channel and water in the inner one. The inner channel has a 600 µm diameter. The droplet module allows to control the production of 300-800 µm alginate droplets with the size depending on the W/O pressure ratio. The best results in terms of cells viability, beads size distribution and shape were obtained by using a cell concentration of $5 \cdot 10^6$ cells/mL and by keeping the beads diameter at 400-500 µm. Smaller beads had reduced cells viability due to the higher shear stress applied during drop tearing by the increased oil pressure.

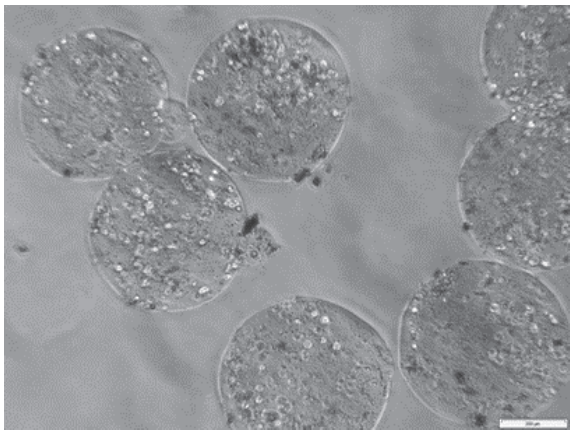

Figure 3: alginate beads with encapsulated cells. Scale bar 200µm

Despite the used material is not classified as non-cytotoxic, the short contact during the encapsulation process with the cured resin did not affect the cells viability. This outcome confirms the results from the cytotoxicity contact test performed where after 30 min, 80 ± 10 % of the cells were viable compared to the control material. It is therefore possible to use such resin for short-term contact application in biology having also other advantages compared to biocompatible resins formulations such as higher transparency, small features printability and lower cost.

The encapsulated cells, in contact with doxorubicin led, as expected, to a concentration dependent decrease in viability detected with WST-8 and with Calcein-AM staining. The measured viability, shown in Figure 4, is however higher than 2D cultures of the same cells at similar doxorubicin concentration. This result confirms the difference between 2D and 3D cultures, when assessing drug potency, and the better suitability of the latter for simulating the in-vivo environment [5].

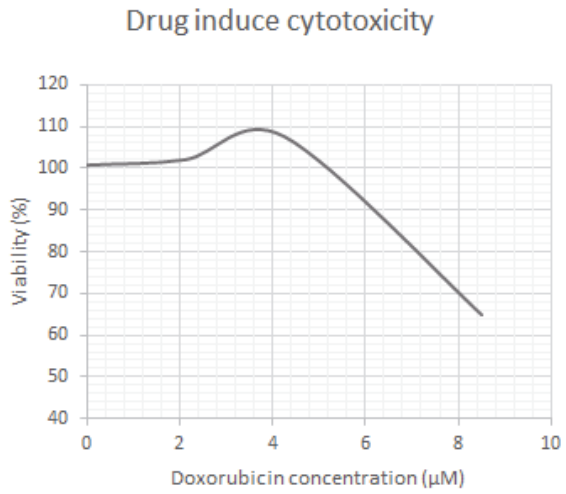

Figure 4: Cell viability after 48h treatment with Doxorubicin using WST-8 colorimetric assay

Conclusions

3D printing makes possible to fabricate microfluidic modules with novel and customizable designs at low-cost but still performant. This was demonstrated by developing 3D passive mixers with efficiency > 80% at low Reynolds. Despite the larger dimensions of the channels compared to silicon microfluidic (≈ 100 µm) a droplet generator was successfully used to encapsulate cells in alginate droplets with a size of 300-800 µm for drug screening. 3D printing benefits as well from the biocompatibility and transparency of the printed material, making it suitable for many applications in life-science and in general, an attractive alternative to 2D state-of-the-art microfabrication for specific applications. The main limit of this manufacturing method resides in the printability of small channels below 500 µm that are not yet achievable with todays commercial printers and resins unless custom setups are used.

Author Statement

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