Centrifugal microfluidic platform for optical monitoring and treatment of biofilms

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It is known that bacteria can adhere to surfaces and human tissue forming biofilms [1], which have shown an increasing tolerance to antibiotics and disinfectant chemicals [2]. Traditionally in hospitals, the evaluation of antibiotic resistance is performed using disc diffusion on solid agar, without taking into account the bacterial natural growth environment and setting. In contrast, studying bacterial biofilms in a continues flow environment has demonstrated *in vivo* like conditions. Nowadays in research laboratories, a bacterial perfusion culture is performed in a miniaturized flow system, where fresh culture medium is pumped through the culture chamber with the help of a rather complex and bulky system of pumps, tubes and valves [3]. Centrifugal, lab-on-a-disc (LoD), microfluidic systems require a simple liquid actuation module, a spinning motor, for pumping and liquid handling. The LoD devices are compact, with integrated liquid routing on the disc which greatly decrease the degree of complexity of the fluidic system and improve usability [4]. The lack of tubes and valves in addition results in low sample volumes, low death volumes and in case of cell culture could decrease the possibility of contamination.

We aimed to create a LoD device which enables working at low (few 100 nl/min) flow rates, optimum for a perfusion culture. The microfluidic system is fabricated from polycarbonate (PC) and therefore is compatible with standard sterilization methods, such as autoclaving. The disc is composed of two PC layers (Fig 1A), one containing the inlet, outlet reservoirs, cell culture chamber, microchannel, loading and venting holes and another one used as lid with the corresponding loading and venting holes (Fig 1B). The LoD device was fabricated with micro milling and bonded with thermal bonding (10 kN, 140°C, for 10 minutes). The flow rates were measured optically and calculated using an imagine analysis and a Matlab® code. Flow rates were evaluated between 1,125 and 0,375 Hz as it is shown in Table 1. With the lowest flow rate of 500 nL/min achieved at a 0,375 Hz rotation frequency the cell culture system is able to operate for up to 6 days, without the need to change or add cell culture medium.

The LoD device was placed on a spin stand (Fig 2), and was used for culturing *Pseudomonas aeruginosa* at 30ºC at a flow rate of 400 nL/min. We also studied the effect of shear stress and centrifugal forces on the bacterial culture using finite element analysis, and found that the used rotation speed and flow rate will not have any effects on the cells [5]. The bacterial culture was monitored daily for up to 3 days using confocal microscopy. An image from the third day of culture is presented on Fig 2. After additional optimization of the perfusion culture, we aim to study the effect of antibiotics on bacterial growth and biofilm development in the LoD system.

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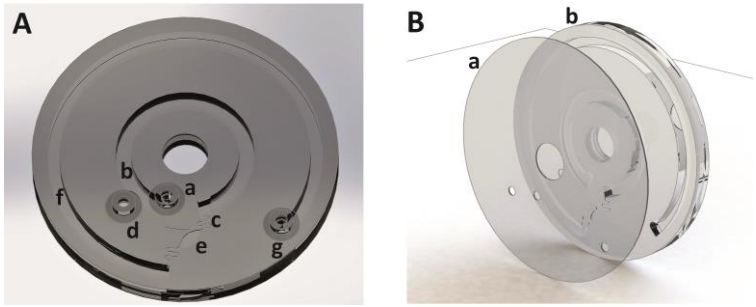

Figure 1A. LoD device for optical monitoring of biofilm formation, which contains luer connectors for filters (a,d and g); inlet reservoirs (b); microchannel (c); cell culture chamber (e) and outlet reservoir (f). B. Design of the two PC layers, the lid layer (a) and the layer containing the features (b).

Table 1. Flow rates evaluated between 1,125 and 0,375 Hz

Frequency (Hz)	Flow rate (µl/min)
1,125	14,3 ± 0,53
1	4,3 ± 0,29
0,9375	3,8 ± 0,35
0,875	3,13 ± 0,53
0,75	1,35 ± 0,28
0,625	0,83 ± 0,04
0,5	0,7 ± 0,08
0,375	0,5 ± 0,07

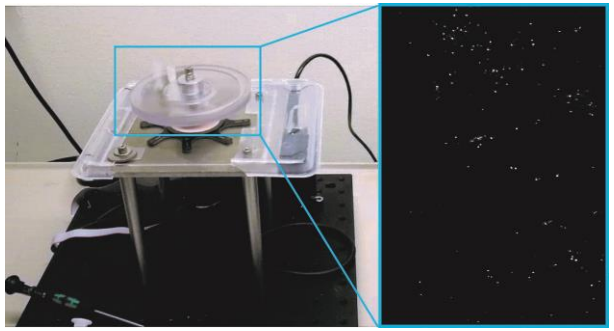

Figure 2. Experimental setup composed of a spin stand with the LoD device in incubation room, with a close up of *Pseudomonas aeruginosa* bacterial culture (white spots), on the third day of culture.