Establishing bacterial cell cultures on a centrifugal platform for monitoring of antibiotic resistance and biofilm development

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Establishing bacterial cell cultures on a centrifugal platform for monitoring of antibiotic resistance and biofilm development

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Bacteria can adhere to various surfaces and human tissue forming complex communities, biofilms, which have a high resistance to antimicrobial agents.1 Conventionally, biofilms are studied in miniaturized flow systems, where fresh medium is continuously perfused.1 Lab-on-a-disc (LoD) devices, proved to be a good alternative to conventional pressure driven fluidic system, enabling the fabrication of compact analysis units, requiring low amount of reagents.4

We present the development of a LoD system aimed to be used for perfusion cell culture and optical cell based assays. The device is composed of two PMMA discs, one disc with chambers (inlet, outlet reservoirs and cell culture chamber), microchannel, loading and venting holes and another one used as lid (Fig.1A,B), fabricated using micro milling. The discs were bonded together with thermal bonding. We aimed to achieve low flow rate in range of hundreds of nL/min in order to ensure cell perfusion without exposing the cells to high shear stress and adversary centrifugal forces.

The optimum flow rate was achieved at 1,25 Hz rotation frequency and calculated, by measuring the progression of a coloured liquid from the inlet to the waste chamber (Fig.2A,B). The calculation was carried out using imagine analysis and a Matlab® code. A finite element analysis was used to calculate the shear stress in the culture chamber (Table 1), and found to be below the level where it has a negative effect on bacterial cells. In addition, the effect of centrifugal forces was evaluated and it was found that the centrifugal forces at the defined rotation speed will not have adversary effect on the cells5. As a next step we are aiming to introduce bacterial cell cultures and study the effect of antibiotics on cell growth and biofilm development.

Figure 1A) LoD system for perfusion cell culture and optical cell based assay with a close up of the perfusion cell chamber. B) Design and illustration of the PMMA layers.

Figure 2A) Illustration of the experimental conditions during flow rate monitoring. During spinning the fluid reached the waste chamber where the liquid movement is optically recorded. B) Flow rate was measured every
30 minutes for around 5 hours. The average of the flow rate is 0.18 µl/min.

Table 1: Table showing the shear stress at specific frequencies and flow rates.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Flow rate (µl/min)</th>
<th>Shear stress (Pa)</th>
</tr>
</thead>
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<tr>
<td>1.25</td>
<td>0.5</td>
<td>4.11 × 10⁻⁵</td>
</tr>
<tr>
<td>1.35</td>
<td>1.5</td>
<td>1.68 × 10⁻⁴</td>
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<tr>
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<td>0.00123</td>
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References