A MICROFLUIDIC APPROACH TO STUDY THE INTERACTIONS OF CYSTIC FIBROSIS PATHOGENS

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Two microfluidic devices have been designed and fabricated to study the interaction between the bacterial species Staphylococcus aureus and Pseudomonas aeruginosa, responsible for lung infections in cystic fibrosis patients.
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1. Introduction

Microfluidic devices have proven to be excellent tools for biomedical investigation, and nanofabricated chips have been used to study bacterial functionality, based on an accurate spatial and temporal analysis [1]. In the present work, two microfluidic chips have been designed and fabricated, to investigate the behaviour of bacterial pathogens causing serious infections in patients with cystic fibrosis (CF), which is a progressive genetic disease. The two most common bacteria that causes persistent lung infections in CF patients are *Staphylococcus aureus* and *Pseudomonas aeruginosa*; it is known that early-infecting *P. aeruginosa* strains produce anti-staphylococcal compounds and inhibit *S. aureus* growth [2]. To investigate the mechanisms at the basis of the interaction between these two bacterial pathogens, two different microfluidic devices have been fabricated.

2. Materials and Methods

Both microfluidic devices consist of a cover glass and a micromolded silicon elastomer (*i.e.*, polydimethylsiloxane, PDMS) that are covalently bonded. Each chip is composed of two *reservoirs* (compartments) for bacterial loading, and a set of micrometric channels, that can be colonized by bacteria. The microchannels layout is obtained using electron beam lithography and the macrostructures (*i.e.*, bacterial compartments) using photolithography. The structures have been imprinted on PDMS, using soft lithography.
P. aeruginosa PAO1(pMMR) expressing red fluorescent protein (mCherry) and *S. aureus* RN4220(pCN36) expressing green fluorescent protein (GFP) have been monitored in the microfluidic channels at different time intervals. The two bacterial strains were grown in Mueller Hinton II broth at 37°C for 16 h. After incubation, bacterial cultures were diluted to reach the OD_{600}= 1 value, and 50 µl of the two bacterial suspensions were inoculated into microfluidic *reservoirs*.
The interactions of fluorescent bacteria have been analyzed using a laser-scanning confocal microscope Leica SP5.

3. Results

Two different microfluidic structures have been fabricated. The first chip is composed by two compartments connected by a set of microchannels (Fig. 1A). The two bacteria species do not enter in contact because a cell trap is inserted at the center of each microchannel [3]. The other chip presents an additional central compartment filled with 1% agarose, that acts as a physical barrier, separating the two bacterial strains (Fig. 1B). Both devices allow an ordered growth of bacterial cell within each microchannel. Therefore, it is possible to statistically monitor the bacteria grown and analyse the effects of bacterial interaction in terms of replication rates.

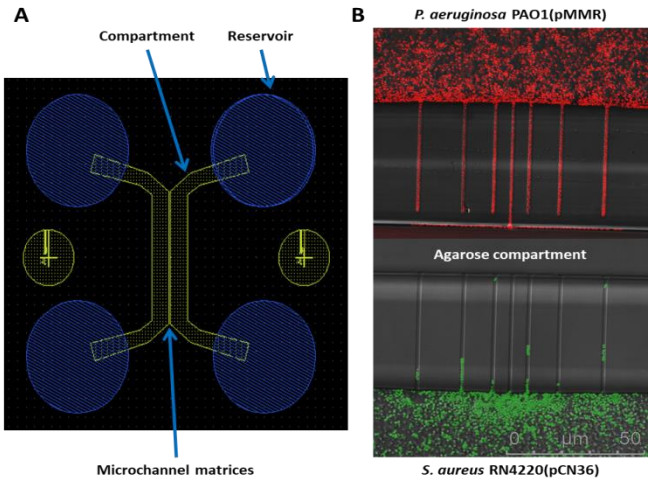

Fig. 1 A) Microfluidic device layout using cell traps. B) Detail of the microfluidic device using agarose, showing the colonization of microchannels by *P. aeruginosa* (red cells) and *S. aureus* (green cells).

References

1. Rafeeq M. M., Murad H. *J. Transl. Med.* **15,** 84 (2017).
2. Briaud P. et al. *Sci Rep.* **9**, 16564 (2019).
3. Baltekin O. et al. *Proc. Natl. Acad. Sci.* **114**, 9170-9175 (2017).