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Envirobot RTD 2010

FNSNF

Development of microfluidic devices to measure the bacterial chemotaxis response

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ENVIROBOT PROJECT

The aim of Envirobot project is the development of a self-navigating robot that samples and measures a set of relevant water quality parameters through a variety of incorporated sensors. This project requires biosensors with a rapid response in order to provide quasi real-time measurements, so that the robot modifies its direction and finds the source of the contamination.

Common whole cell bacterial biosensors are based on reporter protein expression, such as gfp or luciferase. The response usually takes a couple of hours before it can be detected with sufficient confidence, which is too long for the robot guidance. An interesting behavior of bacteria, exploitable to develop biosensors with quick response, is chemotaxis. In presence of a gradient of attractant, bacteria biais their swimming direction towards the highest concentration of attractant. The aim of our study in particular was to design systems, which may permit to quantitatively and rapidly measure chemotaxis.

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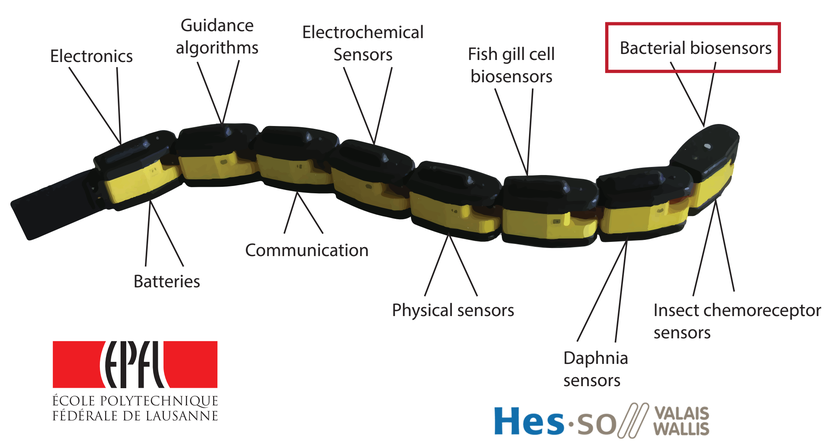


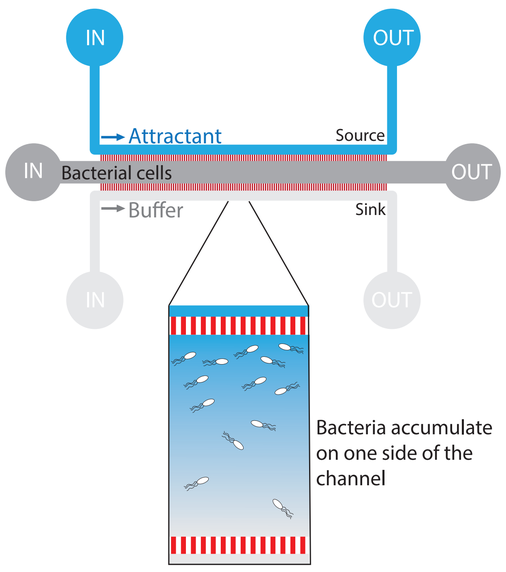
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

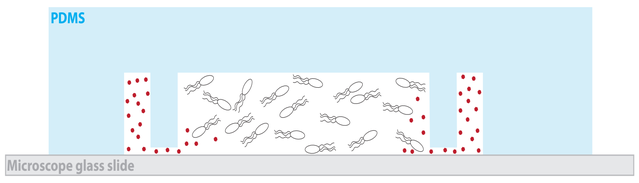
Hes·so VALAIS WALLIS

PDMS CHIPS TO MEASURE CHEMOTAXIS

PDMS (Polydimethylsiloxane) is a transparent and oxygen-permeable polymer allowing developing of microfluidic chips. The flexibility and the miniaturization of the microstructures would be a real advantage to study chemotaxis.

We designed microfluidic chips in which to generate a gradient of attractant and measure bacterial chemotaxis.

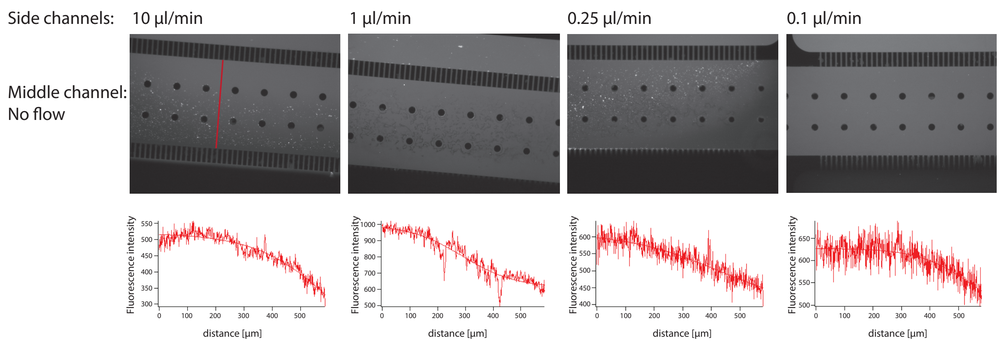




The principle is based on filters composed of channels that measure only 700 nm height, which allow the diffusion of small chemical molecules but prevent the passage of cells.

GRADIENT FORMATION

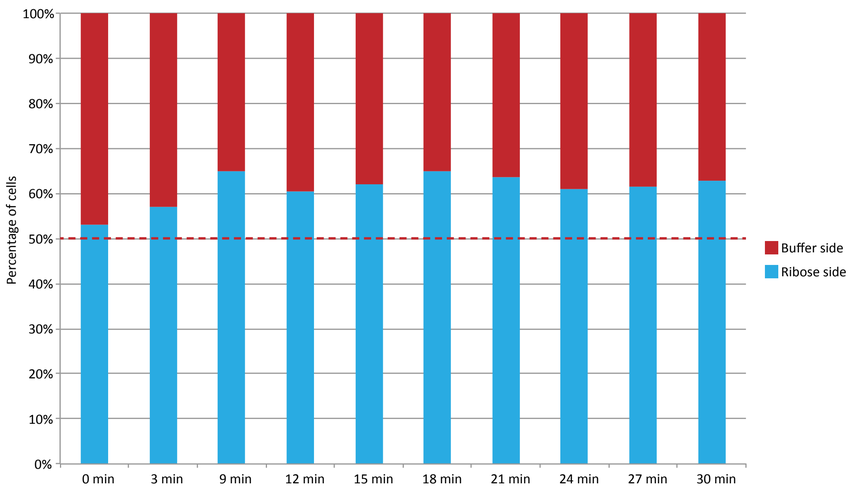
In order to create a stable gradient perpendicularly to the middle channel, solutions of attractant and buffer are flowed in the side channels and the molecules diffuse from source to the sink. Cells are introduced in the middle channel and swim towards the attractant, thus accumulating on one side of the channel.



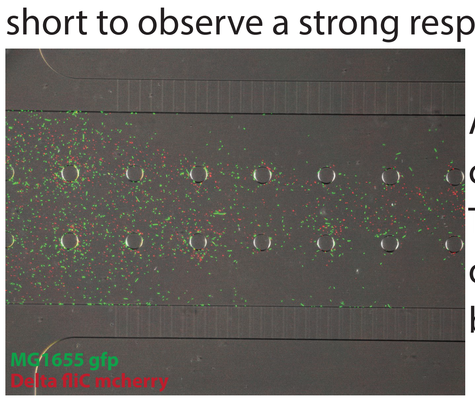
Gradient formation was tested with a fluorescent dye, which showed that the gradient steepness depends on the flow rates in the side channels. Low flow did not produce a gradient since the flow is not sufficent to wash away the dye in the sink channel.

CHEMOTAXIS ASSAY

Escherichia coli MG1655 constitutively expressing mcherry was challenged with a solution of 10 mM ribose as attractant under a flow regime of 0.25 µl/min. Snapshot pictures were taken every 3 min during half an hour in a zone near the beginning of the channel. The cells distribution across the channel was determined by image analysis.



We observed a small tendency of the cells to migrate more to the ribose side of the channel. But since the pictures were taken in the beginning of the channel, the time for the cells to react is probably too short to observe a strong response and attraction to ribose.



An important issue with this design is the fact that the flow from the side channels enters into the filter and pertubs the flow in the middle channel. This showed that the fluidic resistance of the filter is not high enough to prevent the liquid flowing through, instead of allowing only diffusion of chemicals. The consequence is that the cells are pushed towards the center of the channel and their directional bias towards the attractant cannot be very well detected.

CONCLUSION AND OUTLOOK

We show that chemical gradients can be produced in a microfluidic design consisting of three parallel channels. The current design is not optimal because of interference of flow between the side channels and the middle channel, which needs to be improved by the use of longer or shallower filters, or by using valves. Cells introduced in the middle channel seem to experience chemo-attraction, but this needs to be verified by using chemoreceptor or chemotaxis-negative mutants.

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