MICROFLUIDIC MAGNETIC FLUIDIZED BED FOR BACTERIA EXTRACTION, GROWTH AND DETECTION

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ABSTRACT

We present a two-step microfluidic assay for the rapid detection of bacteria in liquid samples based on a magnetic fluidized-bed recently developed by our group. First, an immunoextraction and preconcentration step of *Salmonella typhimurium* is performed with evaluated capture rates of 90%. Immediately thereafter the captured bacteria are cultured *in situ* with a constant flow of nutritionally rich medium. Their growth results in an expansion of the fluidized-bed enabling the direct detection of salmonella by visual monitoring. Moreover, the expansion times directly depend on the sample concentration, allowing precise quantifications of the initial bacteria coupled with a very high sensitivity.

KEYWORDS: Fluidized bed, magnetic beads, salmonella, on-chip culture

INTRODUCTION

The detection and identification of bacteria is crucial in many fields of science and society, but very particularly in the food industry. The standard method, cultured colony counting with different growth media, is a rather laborious and time consuming procedure with results obtained after 24 to 48 hours.

The field of microfluidics can offer a platform for faster and more automated detection systems. In this sense, a variety of separation methods can be found in the literature, such as size sorting [1], electrophoresis [2] or antibody capture [3]. For bacteria identification these methods are often associated to a PCR detection or immuno-recognition thus increasing the complexity of the system.

Our team recently developed a pre-concentration module taking inspiration from fluidized-bed reactors. By making use of an external magnetic field and a constant flow, beads inside a cone-shaped microfluidic chamber are in equilibrium due to opposing magnetic and drag forces, leading to a high bed porosity and recirculation [4]. A first proof of concept on protein extraction has been presented. We demonstrate here for the first time the capture of living entities with this system and we further take advantage of the intrinsic size self-regulation of the fluidized-bed to obtain a label-free detection mechanism, integrating the capture and detection steps in the same microfluidic chamber.

EXPERIMENTAL

Disposable PDMS chips were used, consisting of three inlets (for buffer, bacteria sample and LB-broth), one outlet and an internal 1 cm long cone-shaped chamber (Fig 1). The height of all the chip channels and chamber was 50 µm. Pressure control and flow-rate regulation were achieved with an MFCS® and Flow-unit® (Fluigent) respectively. 50µg of Dynabeads® anti-salmonella (Life Technologies) were introduced in the chip chamber for each experiment. *Salmonella typhimurium* and *Escherichia coli* (*E. Coli*) were the bacteria strains chosen for positive and negative experiments respectively.

The experimental protocol was as follows: after the introduction of the beads, 50 µL of bacteria sample were made pass through the fluidized bed at 1 µL/min for its specific capture. This was followed by a washing step with 40 µL of buffer (PBS + 1% BSA) at 1.5 µL/min. For detection experiments a final step was introduced, in which nutritionally rich medium (LB-broth) was made pass through the chip at 0.150 µL/min and the temperature was set at 37°C by placing the chip on a heating plate. To record the evolution of the bed expansion, images were taken every minute with a Dino-Lite® camera.

RESULTS AND DISCUSSION

The efficiency of the immunocapture step was first estimated by direct culture plating of the magnetic beads after removal from the chip. A mean value of 90% was found, confirming the high efficiency of the protocol. Further, the immunocapture selectivity was investigated by the use of *E. Coli* as a negative control, resulting in no capture of bacteria.

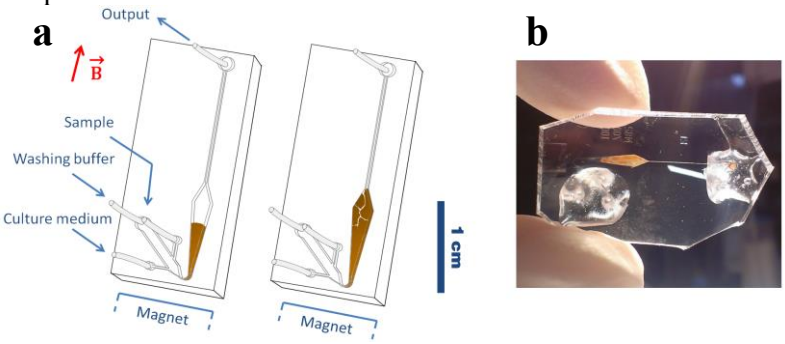

Fig.1: (a) Drawings of the PDMS chip used: a normal bed length is shown on the left and a bed expansion (right) after the growth period indicates the positive capture of bacteria. (b) Picture of chip at the end of experiment after bed expansion (chip is 2.5 cm in length).

Once the bacteria are captured on the beads, their detection requires a further incubation step with growth medium to grow the Salmonella *in situ*. Once a certain amount of bacteria is reached in the chip in a constant flow-rate configuration the bed starts expanding until it completely fills the conic chamber. This allows the easy detection of the presence of bacteria since the chamber is big enough for a direct visual inspection (Fig. 1b).

A more detailed view of the expansion of the front of the fluidized bed can be seen in Fig. 2. As observed the front stays homogeneous and the process takes place relatively quickly.

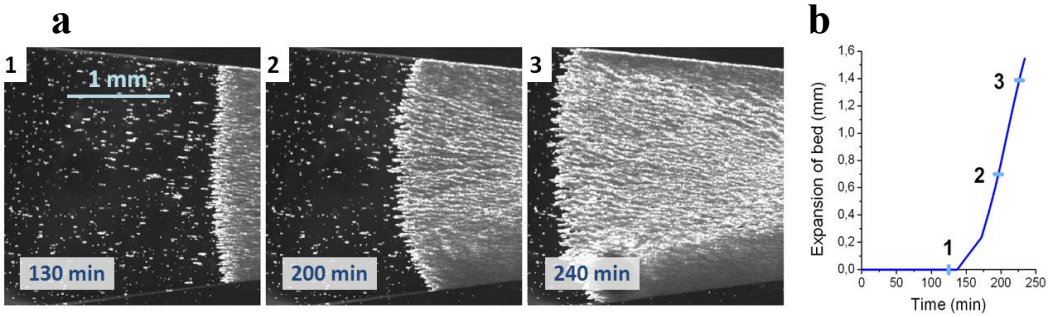

Fig. 2 : (a) Images of fluidized bed front at different times during expansion and (b) corresponding curve obtained by following the front position as a function of time (number of initial bacteria was 7000).

To further evaluate the quantification achievable with this technique a series of experiments at different initial concentrations of Salmonella were performed (Fig. 3). As seen, the time of expansion is directly proportional to the initial concentration of bacteria. Hence, and in analogy to other amplification methods such as qPCR, the initial concentration can be inferred from the appearance of a measurable bed growth. Furthermore, the detection of as little as 4 bacteria has been confirmed, proving the high sensitivity of the system. To confirm its specificity, cultures of up to 48 hours starting with highly concentrated *E. Coli* samples were performed, resulting in zero expansion of the bed.

Finally, to verify the growth of bacteria inside the fluidized bed and establish its growth rate fluorescent GFP-salmonella were used and their fluorescence measured during the culture step (Fig. 4). As shown the growth of salmonella is indeed exponential and bacteria tend to initially grow in colonies (Fig. 4b), presumably around the position of an initial single bacterium. However, and as seen in the last image, the distribution of bacteria ends up homogenizing due to the recirculation of the beads.

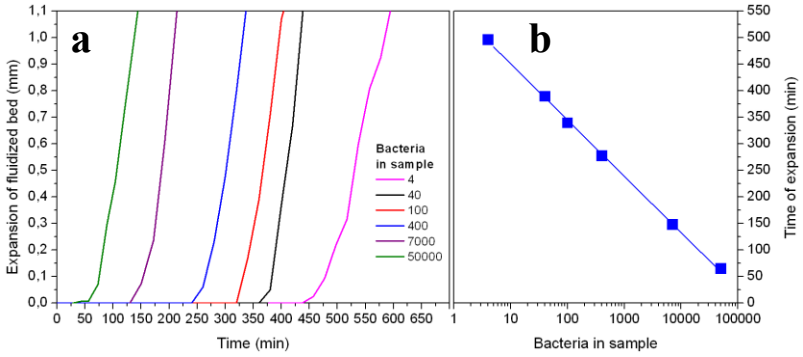

Fig. 3 : (a) Quantification of bed expansion as a function of time during bacteria growth for different initial concentrations of captured bacteria (zero represents the initial position of the front, and the expansion indicates any further increase in the total length of the bed) ; (b) Calibration curve for times after an expansion of 0.2 mm.

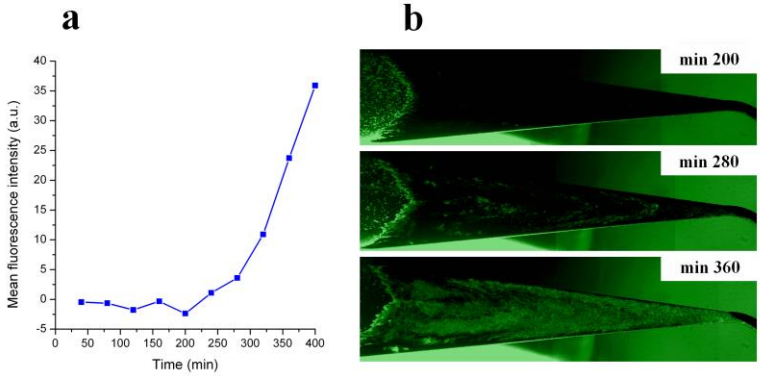

Fig. 4: The fluidized bed was closed every 40 minutes during incubation and fluorescence from GFP-expressing salmonella was measured: (a) mean intensity of fluidized bed (first measurement is the reference and the ROI was the whole bed) and (b) FITC images of the bed from three points in time (total width of images is 4 mm)

CONCLUSION

We have developed a pre-concentration and enrichment system able to detect the specific presence of *Salmonella typhimurium* from a complex sample, after a few hours versus 2 days in conventional cell culture methods. This automated system provides an estimation of the initial concentration in the sample by monitoring the fluidized bed expansion with a low-cost camera. This new concept can bring major breakthroughs in various areas of bacteria detection, e.g. in the environment, food industry and medicine with a working period significantly shorter than current standard methods.

ACKNOWLEDGMENTS

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