Dielectrophoretic separation of blood pathogens ou Bacterial extraction from biological samples using DEP forces

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ABSTRACT
The abstract should be a single paragraph which summarises the content of the article. Any references in the abstract should be written out in full *e.g.* [Surname *et al., Journal Title*, 2000, **35**, 3523].

1 Introduction

Rapid detection and identification of pathogens from biological matrices is a major issue in microbiological diagnosis. Reference techniques are time-consuming methods based on culture, increasing time significantly between the collection and the analysis of pathogens. For diagnosis of sepsis, traditional techniques detection provide a measuring result after 48h to 7 days, because pre-enrichment of the low micro-organism concentration in blood (below 100 cfu/mL) is usually required to acknowledge its presence. Faster sample preparation would provide great benefits, and would mainly alow medical staff to prescribe broad spectrum antibiotics and thus would reduce the risk of new multi-resistance pathogens emergence. In addition, more accurate therapies could be set up for patient wellness.

Dramatic advances in microfluidic and microtechnology research provide numerous emerging methods to cope with the lack of simple sample preparation methods.

- surface-based approaches (antibody-antigene interaction,...) limited by diffusion
- bulk based approaches (MACS, FACS,...) rapid expansion because of micro-system emergence
- focus on DEP
- DEP applications in other fields than cell sorting (high throughput drug assay[1]/ concentration of biothreat agents from food matrices[2])

essor de la DEP depuis la maitrise des micro-technologies

citation : trapping based method = target cells suspended in the sample stream are selectively trapped and concentrated onto the designated area inside a microfluidic channel while other cells are continuously drawn out by the flow[3]

We report here a method based on DEP separation to concentrate pathogens out of a biological sample by combining positive and negative DEP to separate pathogens from the sample matrix. In this approach, we take advantage from the large tolerance of micro-organisms towards osmotic shock to perform dielectrophoretic separation in a low electric conductivity medium. This condition enables to collect micro-organisms by positive DEP, while lysed blood cells are repelled from the electrodes by negative DEP.
Our microfluidic device is designed based on numerical simulation.
This approach is validated on a large range of micro-organisms, since it has been tested with different species of bacteria (*S. epidermidis* (Gram +) and *E. coli* (Gram -)) and yeasts (*C. albicans*).

2 Methods and materials

2.1 Device design

The device used for the DEP sorting experiments is made up of an electrode array topped with a microfluidic chamber. The electrode array is made of 5 pairs of interdigited electrodes : the electrode width and gap are 90µm and 10µm, respectively. An overview of the device is presented in figure 1. (faire aussi un schema des electrodes ?)

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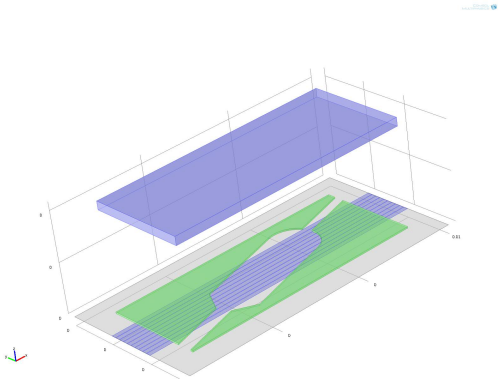

Fig. 1 Schematic of the microfluidic device for cell separation using DEP

2.2 Device fabrication

The device was fabricated using standard photolithography techniques.
Si + SiO_2 + Al + SiO_2
+ de details sur la fabrication : *mesure d'epaisseur par ellipsometrie la semaine du 19/03*
The electrodes are covered with a SiO_2 passivation layer to minimize electrochemical reactions with the processed sample.

double-sided tape over the electrodes (Notto Denko MC-2033) to design the sorting chamber with an accurate height of 30µm. Then the chamber is closed with a coverglass to enable microscopic observation.

2.3 Theory and electrid field simulation

2.3.1 DEP theory. When a dielectric particule is suspended in a conductive medium and an electric field is applied, the particule will become polarised. The interaction between the induced dipole and the applied non-uniform field will generate a force. When the applied electric field is non-uniform, the strength of the electric field on one side of the dipole is greater than on the other side. Thus a force is induced, called dielectrophoretic force (DEP force) because this force will set the particule in motion[4].

The time average DEP force in the dipole approximation for a spherical cell is given by equation 1.

$$\vec{F}_{DEP} = 2\pi\varepsilon_m r^3 Re[f_{CM}]\vec{\nabla}|E|^2 \qquad (1)$$

where r is the particule radius, ε_m is the permittivity of the suspending medium, $Re[f_{CM}]$ is the real part of Clausius-Mossoti factor and E is the root-mean-square electric field.

The Clausis-Mossoti factor, given by equation 2 for an homogenous particule, stands for the relative polarizability of the particule in the suspending medium. It depends on the applied field frequency and can take values from -0.5 to 1.

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

where $\varepsilon_p^* = \varepsilon_p - j\sigma_p/\omega$ and $\varepsilon_m^* = \varepsilon_m - j\sigma_m/\omega$ are the complex permittivities of the particule and the medium, respectively. ε_p, ε_m and σ_p, σ_m are the permittivities and the conductivities of the particule and the medium, respectively.

Depending on the sign of $Re[f_{CM}]$, particules will move in opposite direction : if the particule is more polarisable than the suspending medium ($Re[f_{CM}] > 0$), particule motion will be induced towards the local minima of the applied electric field (positive dielectrophoresis or DEP+), and conversly if the particle is less polarisable than the medium ($Re[f_{CM}] < 0$), particule motion will be induced towards the local maxima of the applied electric field (negative dielectrophoresis or DEP-). tri en fonction de l'amplitude de la force ou de la direction

Thus depending on the frequency of the applied electric field, DEP can be used to separate two different populations of cells by binary sorting (ref).

2.3.2 Electric field simulation. We solved numerically the electric field produced by interdigited electrodes by using finite element analysis software (Comsol Multiphysics 4.1).
It enables to create the geometry and the mesh and then to solve the associated differential equations. The software solved the electric potential using the Laplace equation (or Electrostatic Poisson Equation ?). Then the computed electric field ($\vec{E} = -\vec{\nabla}V$) is used to determine $\vec{F}_{dep}$, which is proportional to $\vec{\nabla}|E|$.
We defined half of a pair of electrodes because of symetry.
Simulation conditions / boundary conditions should be specified ?

To assess the effect of the passivation layer on the electric field in the biological medium, we can model this layer as a capacitor and the biological medium as a parallel combination of a capacitor and a resistor. The resolting model for this overlay (faire un schema ?) can be described as a high-pass filter with a cut-off frequency f_c and a gain G_0 defined by equation 3.

$$f_c = \frac{\sigma_m}{2\pi\varepsilon_m}\frac{2}{2+\alpha} \quad \text{and} \quad G_o = \frac{\alpha}{2+\alpha} \qquad (3)$$

$$\text{with} \quad \alpha = \frac{l_{gap}\varepsilon_{passiv}}{\varepsilon_m h_{passiv}}$$

where l_{gap} is the width of the electrode gap and h_{passiv} and ε_{passiv} the thickness ant the permittivity of the passivation layer, respectively.

faire l'application numerique pour la freq de coupure avec la vraie valeur de conductivite du milieu ! ! !

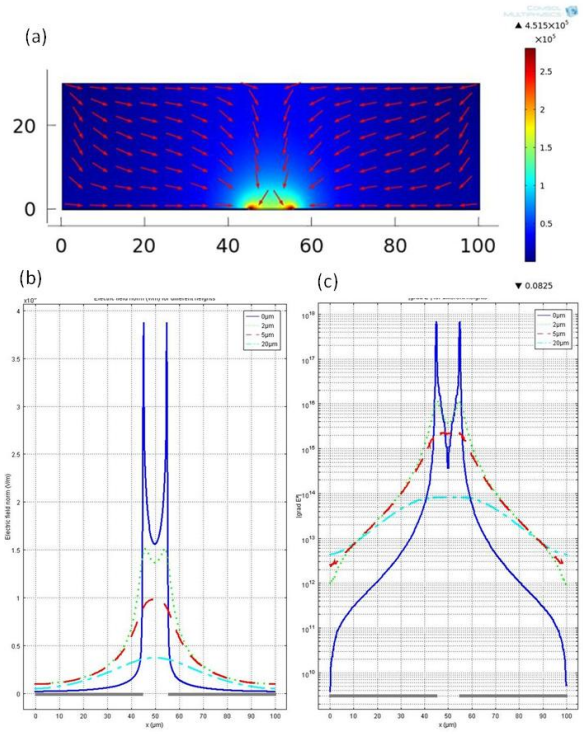

Fig. 2 (a) surface : $|E|$ The red color represents regions with strong electroc field, where particules with $Re[f_{CM}] > 0$ are attracted. The blue color represents regions with weak electric field, where particules with $Re[f_{CM}] < 0$ are taken away ; arrows : direction of $\vec{\nabla}|E|$ Particules with $Re[f_{CM}] > 0$ will move following these gradient lines.
Estimation of $|E|$ (b) and $|\vec{\nabla}|E|^2|$ (c) for different heights away from the electrode plane

Taking into account that DEP force decreases very quickly with the distance from the electrodes as illustrated by figure 2, the microsystem heigh is limited to 30 µm for DEP sorting : this height was set as a compromise between the sorting efficency and the volume of the sorting chamber.

2.4 Sample preparation

2.4.1 Bacteria. *Escherichia coli* (strain ATCC 35421) were grown overnight in 3mL Tryptic Soy Broth medium at 30˚C and subcultured at a 1 :50 dilution for 2h at 37˚C. Then the bacteria are washed twice by centrifugation at 10 000tr/min during 5 min and resuspended in deionised water at a cell concentration of 5.10^8 cfu/mL.

ref for bacteria viability in deionised water ?

2.4.2 Yeasts. *Candida Albicans* (strain ATCC 18804) were grown overnight in 10mL Sabouraud medium at 30˚C and subcultured at a 1 :50 dilution for 4h at 30˚C.

Then the yeasts are washed twice by centrifugation at 10 000tr/min during 5 min and resuspended in deionised water at a cell concentration of 10^7 cfu/mL.

2.4.3 Red blood cells. Human blood was colleted from an anonymous healthy donor in EDTA vaccum tubes provided by the French Blood Service. A 1mL sample was centrifuged at 600g in order to separate red blood cells from the plasma and the buffy coat. 10 µL of red blood cells were then collected.

2.4.4 Mix. The previous micro-organisms and red blood cells suspensions are then mixed with the following ratio : 25 µL of micro-organisms suspension, 1 µL of red blood cells suspension and 25 µL of deionised water. The red blood cells are immediatly lysed due to the osmotic choc generated by the deionised water. The final concentration of bacteria and blood cell ghosts in the sample were $2,5.10^8$ cfu/mL and 2.10^8 cells/mL, respectively.
The dilution of the sample with deionised water enables us to work in low conductivity medium, so both DEP+ and DEP- were conceivable. On the other hand it was identified as an indisputable way to lyse blood cells without resort to chemical agents that would also have a potential affect on micro-organisms viability.

Mix conductivity : manip semaine du 19/03

1.5µL of the solution was finally injected by capillarity in the micro-system. To avoid evaporation during the expermients, two oil drops were applied at both ends of the chamber.

2.5 Experimental setup

A 10 $V_{peak-peak}$ sinusoidal signal was supplied by a function generator (AFG32,Tektronic). The frequency can take value from 0 to 80MHz. Particule motion was observed with an optical microscope (Zeiss) and a x20 objective. Images were captured with a CDD camera ( ) with a AxB pixel^2 resolution.

one image every 300ms
card-edge connector to connect the device to the generator.

schema

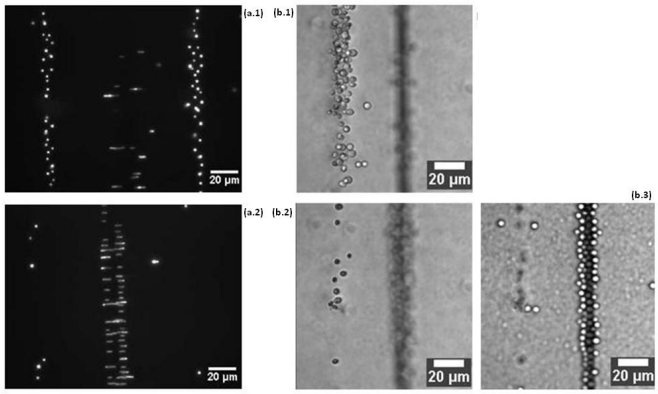

Fig. 3 illusration of negative and positive dielectrophoresis for E coli (a) and C albicans (b)

3 Results and discussion

3.1 micro-organisms separation in a simple buffer

3.1.1 ***E coli*** **separation in** H_2O**.** DEP+ or DEP- depending on the frequency (figure 3(a))
Either in positive or negative DEP regime, we observed that E Coli bacteria ligned up with the field line.
consistent with other papers (ref)
Its own motility seems to prevent it from unspecific adsorption. However it is not the case for other species sush as Staphyloccocus epidermidis : the experiments carried out with the strain ATCC 14990 revealed that once adsorbed on the surface of our micro-system, any force from electric field could move the cells away.

3.1.2 ***C albicans*** **separation in** H_2O**.** cross-over frequencies different from those with E coli
(figure 3(b))

3.2 pathogens separation from blood cells in a low conductivity medium

As illustrated in figure 4, cells can have differents equilibrium positions. Pictures for different frequencies of the electric signal :

- 50kHz : every cell goes under DEP-
- 1MHz : every cell goes under DEP+
- 20 MHz : DEP- for RBC and DEP+ for E. coli

comparison with other articles with experiments with the same conductivity ?

3.2.1 DEP repulsion and reversibility. In these experiments, we perform binary sorting between two cell

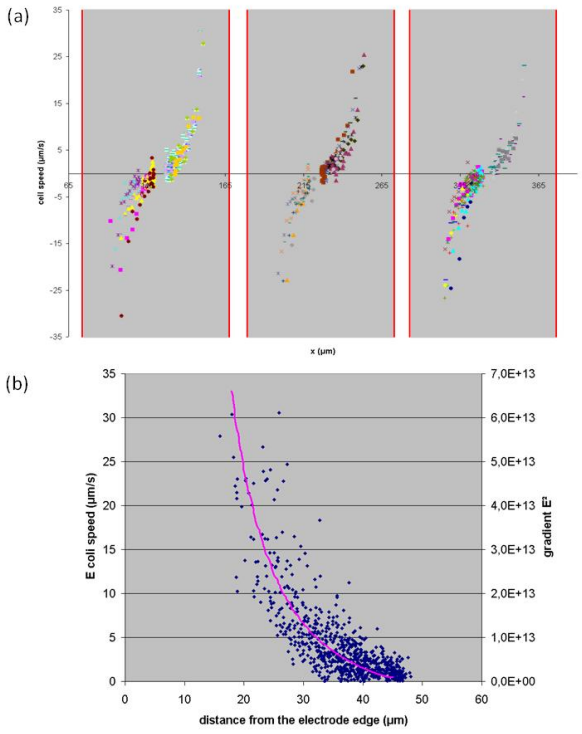

Fig. 5 (a) *E coli* speed evolution with the distance from the electrode edges (red lines)
(b) *E coli* speed comparison with $\nabla|E|^2$ computed for h=2µm from the electrodes

populations (micro-organisms and red cell ghosts). In a real contaminated sample, the micro-organisms are far less abondant than blood cells (1 cfu versus 10^9 cells) : thus during the sorting, it's of great importance that blood cells are repelled from the surface of the electrodes in DEP-, therefore it prevents from saturation near the electrodes (pas clair...).

The displacement of the cells, and especially micro-organisms, is reversible : during the experiments bacteria can be attracted or reppeled from the electrodes depending on the frequency as often as required. Thus micro-organisms can be recollected in a buffer after the elimination of the lysed cells to allow further analysis.

3.2.2 average cell velocity. The image interpretation of DEP experiments was performed using the image processing software ImageJ with the particule tracker plugin.
The positions of 40 *E coli* cells were tracking during the DEP-stage (working frequency = 20MHz) : the computed speed of *E coli* cells is in good agreement with the evolution of the simulated electric field.

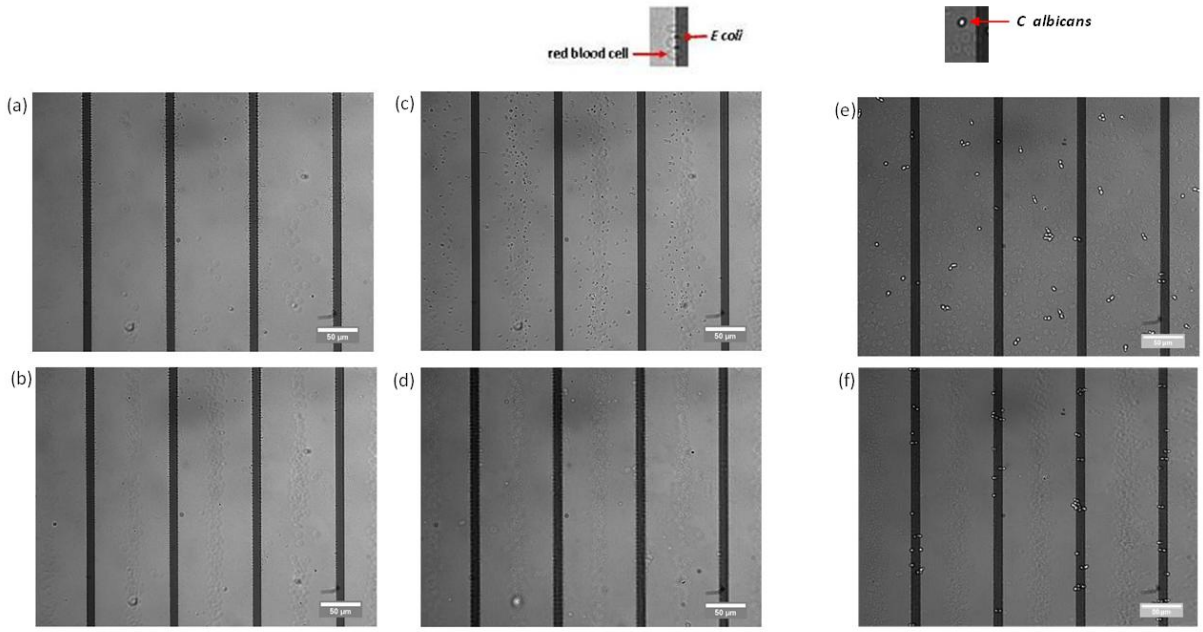

Fig. 4 illusration of negative and positive dielectrophoresis for E coli and red blood cells separation in low conductivity medium

3.2.3 ***C albicans*** **and blood cells separation.** common frequency found for E coli and C albicans (compulsory for your application because we don't know what king of micro-organisms we are looking for)

3.2.4 blood cell populations. In this study we only take into account red blood cells for it is the most representative population cell in a blood sample (roughly 10^9cells/mL). Yet the other cell populations (white blood cells and platelets) have to be considered in more detail because their concentrations will still be far more important than the concentration of the pathogen in the sample. However we can reasonably assume that once lysed, the other blood cells will have the same behaviour than red cell ghosts.
manip de lyse des globules blancs lundi 19/03

3.2.5 other discussions ?.
Rendement ?

Viability (cell lysis ?)

En statique : only 1.5µL, so it's necessary to perform this sort with a stream

Indispensable de se placer dans un milieu avec une conductivite plus faible que celle des milieux biologiques : ici c'est la dilution qui permet de faire baisser cette conductivite (de 1,5 S/m vers environ 30 mS/m). Si on souhaite supprimer ou tout du moins diminuer la conductivite, il faudra trouver un autre moyen de faire baisser la conductivite avant l'etape de tri.

4 Conclusions

In this study, we developed a microfluidic device for separation and concentration of pathogens from a blood sample. This method took advantages of the large tolerance of micro-organisms towards osmotic shock to perform dielectrophoretic separation in a low electric conductivity medium. A well known eletrodes configuration was used to create a highly non-uniform electric field and thus to perform dielectrophoretic sorting. The performance of the device was validated on different pathogen species : indeed the versatility of the method is a key issue, as we don't know the nature of the pathogen prior to its isolation. We believe our device can fill in the critical gap in sample preparation between the collection of potentially contaminated samples and the analysis of pathogens. In future work, the sorting will be processed with a fluidic interface, so that we can concentrate the micro-organisms outside the microsystem once separated by DEP. Next step will be to determine the maximum flow rate for which the separation is still significant in our microfluidic device to ensure that conventional methods can be challenged.

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