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A generic and label free method based on dielectrophoresis for the continuous separation of microorganism from whole blood samples

Emilie Bisceglia[a,b,c], Myriam Cubizolles[a], Claudia Irene Trainito[b], Jean Berthier[a], Catherine Pudda[a], Olivier Français[b], Frédéric Mallard[c], Bruno Le Piouffle[b,*]

[a] Department of Microtechnology for Biology and Health, CEA LETI – Minatec, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France
[b] SATIE, CNRS, ENS Cachan, 61 avenue du Président Wilson, 94235 Cachan Cedex, France
[c] bioMerieux, Technology Research Department, 5 rue des Berges, 38000 Grenoble, France

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ABSTRACT

Current methods for the in vitro diagnostics of bloodstream infections are based on blood culture, followed by phenotypic characterization of the pathogen. Nevertheless, such methods are too long in the case where a fast and appropriate medical treatment is needed. There is thus a very strong interest in blood sample decomplexification methods to be coupled to the existing analytical means.

In this paper, we report the continuous flow capture and concentration of microorganisms spiked in a blood sample, flowing within a dedicated microfluidic device. The separation is achieved with dielectrophoresis (DEP) forces, using hypotonic conditions for the buffer. We demonstrate efficient and real-time accumulation of target pathogens from blood in our microfluidic device, thus providing a decomplexified sample suitable for further characterization. In particular, up to 97% capture rate could be observed with *Escherichia coli* micro-organisms. Simultaneous separation of different micro-organisms (Gram+, Gram− and yeast) could also be achieved, showing the versatility of the method and device.

1. Introduction

Bloodstream infection by micro-organisms is a severe cause of mortality [1,2]. The success of a therapeutic strategy strongly depends on the administration of the appropriate antibiotic therapy as soon as possible, ideally within hours after the sepsis suspicion has been confirmed. Methods based on blood culture [3] have shown their interest in the past decades for the sensitive detection and phenotypic characterization of blood pathogens. However, these methods require a long growth time – days – and do not fit with the need for faster information delivery.

Some alternative analytical methods allow for the rapid identification of bacteria and yeast starting from much less bacterial biomass than the above-mentioned phenotypic methods. Molecular biology was already shown to detect and characterize very low amounts of pathogens in whole blood in hours [4]. However, this approach is strongly dependent on the quality of the sample preparation and the good elimination of PCR inhibitors [5]. Fluorescence or Raman spectroscopy has been reported to provide phenotype-related information even at the single bacterium level [6]. However, due to the complexity of blood, such methods could not be applied to raw samples.

In all cases mentioned above, extraction of microorganisms from the complex blood environment prior to further analytical steps appears to be the main limiting step for really fast microbiological testing. As the phenotypic characterization of pathogens remains necessary, bacterial extraction from blood should preserve the viability of micro-organisms while eliminating blood compounds, mainly white and red blood cells. Moreover, since a wide range of micro-organisms is being susceptible to cause septicaemia [7], the separation method has to be effective for a large variety of pathogens.

Several trapping and sorting methods have been developed and optimized during the previous decade. A number of them rely on the capture of micro-organisms by affinity molecules (antibodies, phage proteins, aptamers, etc.), fixed on a planar [8] or colloidal substrate [9–11]. More recently, other methods have emerged, that can separate cells according to differences in their physical parameters: hydrodynamic sorting [12], lateral displacement filtration [13], magnetophoresis [14], electrophoresis [15], acoustophoresis [16] or optical forces [17], AC electrokinetic techniques [18]. In particular, the use of dielectrophoresis forces, electrorotation, and travelling wave dielectrophoresis showed to be quite efficient for cell trapping [19–21], cell separation [22–24]and cell

* Corresponding author. Tel.: +33 147407736.
E-mail address: bruno.le-piouffle@ens-cachan.fr (B. Le Piouffle).

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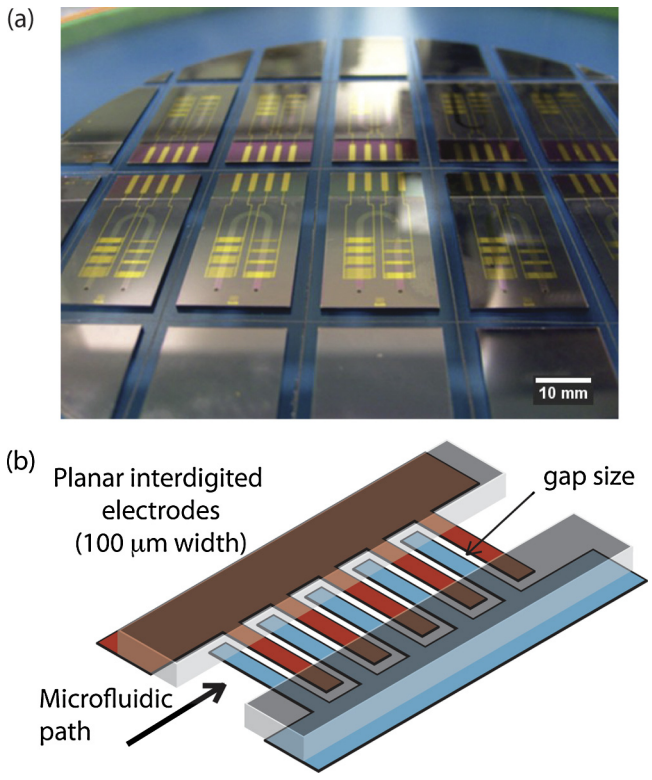

Fig. 1. Photography of the microfluidic devices on a 4 in. silicon wafer (a) and scheme of the architecture involving interdigitated electrodes within the flowing microchannel (b).

characterization [25,26]. These label-free and non-contact techniques offer the advantage of being minimally invasive and less sensitive to the inherent pitfalls of surface-mediated capture.

Within this context, we demonstrate in this paper the feasibility of a continuous flow separation and concentration of pathogens from a blood sample, within a micro-system. Our method combines the application of dielectrophoresis forces (Eq. (1)) with an osmotic shock to capture and extract with high efficiency pathogens from the blood and their subsequent dynamic separation inside a fluidic device with an interdigitated electrodes array (Fig. 1), that generates a highly non-uniform stationary electric field.

The DEP binary sorting has previously been achieved between pathogens and blood cells under static conditions [27]. The buffer containing the biological sample was optimized in order to perform binary DEP sorting: pathogens were trapped by positive DEP forces while the blood cells were repealed by negative DEP forces. The method requires a buffer exchange to set the Clausius–Mossoti factor at the required value leading to DEP forces presenting proper intensity and direction.

In the present work the trapping of micro-organisms is performed under dynamic flow conditions, and the repealed blood cells are flowed downstream. Moreover, we tuned the configuration and experimental parameters to optimize the trapping rate while not altering the survival rate of microorganisms. Using these conditions we could successfully trap different pathogens (yeast, Gram positive and Gram negative bacteria) from a raw blood sample, with high efficiency (up to 97% in the case of *Escherichia coli*).

2. Materials and methods

When immersed in an electric field, the blood components (blood cells and pathogens) experience adielectrophoretic force [28], which time averaged expression is:

$$\langle \vec{F}_{DEP} \rangle = 2\pi\varepsilon_m r^3 Re[f_{CM}]\vec{\nabla}|E_{rms}|^2 + 4\pi\varepsilon_m r^3 Im[f_{CM}]\sum_{xyz} E^2_{rms,i}\vec{\nabla}\Phi_i \quad (1)$$

where r is the biocomponent radius (spherical case), ϵ_m is the permittivity of the suspension medium, f_{CM} is the Clausius–Mossoti (CM) factor, E_{rms} is the root-mean-square electric field, and Φ the phase of the electric field.

When the amplitude of the electric field is non-uniform ($\vec{\nabla}E \neq 0$), then the first term of the Eq. (1) is prevailing. The force direction is determined by the sign of the real part of the CM factor. We used such conditions to perform the trapping of micro-organisms from the whole blood flowing through the device, using therefore interdigitated electrodes to generate the non-uniform field.

When the phase of the electric field is non-uniform ($\vec{\nabla}\Phi \neq 0$), then the second term of the Eq. (1) prevails and is used to induce electrorotation (ROT), or travelling wave dielectrophoresis (twDEP). Electrorotation experiments were used to determine the dielectric parameters of the different cell types used in our bacterial extraction experiments (see Section 2.5).

The electrorotation torque (expressed in Eq. (2)) opposes to the friction resistant torque (Eq. (3), [29]).

$$\vec{\Gamma}_{ROT} = -4\pi r^3 \varepsilon_m Im[f_{CM}] E^2_{rms}\vec{z} \quad (2)$$

$$\vec{\Gamma}_f = 8\pi\eta r^3 \varepsilon_m \Omega \vec{z} \quad (3)$$

The rotational velocity of the cell then equilibrates to:

$$\Omega = -\frac{\varepsilon_m}{2\eta} E^2_{rms} Im[f_{CM}] \quad (4)$$

where η represents the dynamic viscosity of the medium ($\eta = 0.001$ Pa s), and $\vec{z}$ is the unity vector, normal to the plane of electrodes.

2.1. Electric field simulation and DEP force calculation

We estimated the electric field produced by our interdigitated electrodes network using numerical simulation, using a finite element analysis software (Comsol Multiphysics 4.3). The potential V is calculated by solving in each element the Laplace equation ($\Delta V = 0$, no volumic charge distribution), and is then used to determine the electric field ($\vec{E} = -\vec{\nabla}V$) and the dielectrophoresis force $\vec{F}_{dep}$ which is proportional to $\vec{\nabla}|E|^2$.

The screening effect of the nitride layer (thickness $e_{nit} = 200$ nm) is considered in the simulations, even if it is small at the electrical frequency used for our experiments ($f = 10$ MHz). Indeed, above the characteristic frequency $f_c = (1/2\pi)(\sigma_m/\varepsilon_m)(2\varepsilon_m e_{nit}/(l_{gap}\varepsilon_{nit} + 2e_{nit}\varepsilon_m))$ the actual electric field in the medium is attenuated by the factor $G_o = (l_{gap}\varepsilon_{nit})/(2\varepsilon_m e_{nit} + l_{gap}\varepsilon_{nit})$, due to the passivation layer [27]. Here σ_m represents the conductivity of the medium, l_{gap} the distance between electrodes and ε_{nit} the relative permittivity of the nitride ($l_{gap} = 10$ μm and $\varepsilon_{nit} = 7.5$ gives $G_o = 0.7$ for the simulation). The attenuated voltage is imposed as a boundary condition in the simulation.

2.2. Simulation of the trajectories of cells and micro-organisms

In the microfluidic device, cells and micro-organisms mainly experience the buoyancy force, the fluid drag force and the dielectrophoretic force. Through the action of the DEP force, it is possible to concentrate and separate the pathogen cells from blood cells. To explain this differential behaviour, we simulated the cells trajectories inside the separation channel according to the sum of forces