Pragmatic setup for bioparticle responses by dielectrophoresis for resource limited environment application

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Abstract. Various dielectrophoretic responses of bioparticles, including cell-chain, spinning, rotation and clustering, are of high interest in the field due to their benefit into application for biomedical and clinical implementation potential. Numerous attempts using sophisticated equipment setup have been studied to perform those dielectrophoretic responses, however, for development into resource limited environment application, such as portable, sustainable and environmental friendly diagnostic tools, establishment of pragmatic setup using standard, non-sophisticated and low-cost equipment is of important task. Here we show the advantages in the judicious design optimization of tip microelectrode, also with selection of suspending medium and optimization of electric signal configuration in establishing setup that can promote the aforementioned dielectrophoretic responses within standard equipments, i.e. pragmatic setup.

1. Introduction

Development of diagnosis tools for resource-limited environment such as war-torn and natural disaster situation are among major studies implementing dielectrophoresis research. While the development is in extensive progress with various sophisticated instrumentation and setup, the need for a pragmatic setup, i.e. portable, sustainable, environmental friendly is often overlooked. Dielectrophoretic response of particles relies on their dielectric constant as well as of the suspending medium, and electric signal [1]. While the particles properties are of the major subject of the studies in this field and non-changeable parameter, the suspending medium and electric signal configurations are customizable.

Researchers can choose parameter for suspending medium, by selecting such as culture medium [2], sugar alcohol [3] and salty solution such as phosphate buffered saline (PBS) [4], or deionized water (DIW). However, for a portable, sustainable and environmental friendly selection for a point-of-care (POC) or war-torn resource limited and quick diagnostic tool, DIW is of the best choice. As the process of changing the suspending medium typically is centrifuge based process, which is simple, low cost and portable, the use of DIW as suspending medium is not labour-intensive, i.e practical option. In fact, DIW suspended bioparticles remain viable, at least for a period up to an hour as demonstrated in this work, which is sufficient enough for a quick diagnostic test.

Selection of electric signal parameters, i.e. the voltage, and frequency should be based on a standard function generator configuration so that the setup is non-sophisticated, portable and low cost. A standard function generator can produce a signal of voltage up to around tens V_{pp} and frequency range up to several MHz. There are a number of attempts to use sophisticated configuration such as GHz
frequencies range, however this configuration might cost a larger space, less portability and higher expenses \cite{5,6}. We showed that frequency range up until several MHz, which producible by a standard function generator, is sufficient enough to induce various responses of bioparticle, i.e. repel, attract, spin, rotate, and chaining. One should note that at frequencies less than 1 kHz, electro-osmosis and electrolysis could disrupt the cellular motional responses. Therefore, the ideal frequency ranges for bioparticles to exhibit dielectrophoretic responses in DIW is between tens of kHz to several MHz, as demonstrated in this work.

However, on top of mentioned parameters, the design of of the microelectrode design is the topmost important parameter. The best design is the one which can construct the maximum difference of the electric field gradient difference within the suspension region. In this work, we demonstrated a judicious design of microelectrode where microtip configuration is being employed, as it constructs non-continuous high electric field spots which can focus the bioparticle at positive dielectrophoretic response region (described in next section), instead of continuous high electric field region which reduce the contrast response of bioparticle between opposite responses (positive DEP and negative DEP).

We performed this work using widely used model organism, \textit{Saccharomyces cerevisiae}, a type of yeast cell, which is a well-known and widely used in proof-of-concept studies, prior to expansion to study of various cells of interest. Well-customized parameters in this work serves as a pragmatic setup to achieve bioparticle responses especially for the development of quick diagnostic tools for resource-limited environment, while at the same time open for future work using other targeted cells.

2. Dielectrophoresis

Dielectrophoresis is a motional phenomenon of polarizable dielectric materials, including bioparticles when suspended in a non-uniform electric field (Figure 1). Bioparticles are polarized, establishing dipoles within their membrane. Unequal net Coulombic forces are generated at opposite sides, pushing the bioparticles either towards or away from regions of electric field with high gradients. The acting force is known as dielectrophoretic (DEP) force, \( F_{\text{DEP}} \), which is governed by \cite{7,8}

\[
F_{\text{DEP}} = 2\pi a^2 \varepsilon_m \text{Re}[f_{\text{CM}}] |E|^2
\]

where \( a \) is the radius of suspended particle or bioparticle, \( \varepsilon_m \) is the absolute permittivity of the suspending medium, \( f_{\text{CM}} \) is the Clausius–Mossotti factor and \( E \) is the electric field. The \( f_{\text{CM}} \) indicates the correlation between dielectric constants of two different media. For a spherical bioparticle, \( f_{\text{CM}} \) is given by

\[
f_{\text{CM}} = \frac{\varepsilon_p^* \varepsilon_m^*}{\varepsilon_p^* + 2 \varepsilon_m^*}
\]

where \( \varepsilon_p^* \) and \( \varepsilon_m^* \) are the complex permittivities of the suspended bioparticle and the suspending medium, respectively. The complex permittivity is determined by

\[
\varepsilon^* = \varepsilon_0 \varepsilon_j \omega
\]

where, \( \varepsilon_0 \) is vacuum permittivity (8.854 \( \times 10^{-12} \) F/m), \( \varepsilon \) is permittivity while \( \sigma \) is conductivity, \( j = \sqrt{-1} \), and \( \omega \) is angular frequency of supply voltage. The polarity of the DEP force depends on the sign of the real part of CM factor, \( \text{Re}[f_{\text{CM}}] \). Positive \( \text{Re}[f_{\text{CM}}] \) indicates positive DEP (pDEP) force, pushing the bioparticles towards regions of high electric field gradients, while negative \( \text{Re}[f_{\text{CM}}] \) indicates negative DEP (nDEP) force, pushing bioparticles in opposite direction. The pDEP and nDEP responses of bioparticles alternate vice versa at a frequency known as crossover frequency, \( f_{\text{crossover}} \), where the polarizabilities of the bioparticles and the suspending medium are equal.
3. Experimental

3.1. Computations and design.

Real part of the Clausius-Mossoti factor, Re[\(f_{CM}\)] is computed using Octave 4.0 for a frequency range between 1 kHz to 10 MHz. Electric field distributions \(E\) is simulated using Agros2D.

3.2. Device fabrication.

DEP platform consists of a glass microscope slide substrate with patterned DEP microtip electrodes, fabricated using standard photolithography and wet etching. The electrodes is structured by a 50 nm chromium (Cr) underlayer and 150 nm gold (Au) overlayer. A thick PDMS (DowCorning Corp, Sylgard 184) superstrate, formed using standard soft-lithography, consists of an inlet port, microchannel (150 \(\mu\)m \(\times\) 50 \(\mu\)m) and outlet stage, and is bonded by corona plasma bonding to the top of the glass substrate.

3.3. Sample preparation.

Sample composed of yeast suspended in DIW with a volumetric ratio of 0.12% w/v, is capillary force driven into into the microchannel. The yeast suspension was sonicated in an ultrasonic bath at 37 °C for 30 min. The sample suspension is introduced into the microfluidic channel followed by a buffer period of 30 min prior to DEP response observation.

3.4. DEP response observation.

The DEP platform is mounted on the stage of an optical microscope (Motic) and the image is monitored by a monitor. Electric signal is applied to the electrodes by a function generator (Topward, 8120) while the signal configuration, i.e. voltage and frequency are monitored with an oscilloscope (Tektronix, TDS1012B). The voltage and frequency amplitudes are set at 7 \(V_{rms}\) (20 \(V_{pp}\)) and the frequency is varied between 20 kHz and 2 MHz.

4. Results and discussions

4.1. Simulation result

Simulation result of Re[\(f_{CM}\)] indicates that yeast cells suspended in DIW experience nDEP force at low AC frequencies, while pDEP force is experienced at higher frequencies, and cross over frequency takes place at \(f_{0}=90\) kHz (Figure 2).
Figure 2. Simulation of Re[f_{CM}] for yeast cells (multi-shell model) suspended in DIW. Crossover frequency, f_{xo} = 90 kHz. Negative DEP (nDEP) is experienced at f < f_{xo}, while positive DEP (pDEP) occurs at f > f_{xo}.

Simulated electric field distribution (Figure 3) shows that highest electric field regions (230 kV/m) exist at the edge of microelectrode tips, i.e. 6 points (x = ±100 and 0 μm of y = ±20 μm), while lowest electric field regions exist at vicinity of the microelectrode base, i.e. along y = ±100 μm that without microtip.

Figure 3. Simulation of electric field distribution, E.

4.2. Bioparticle responses
Observation of the yeast solution under bright field microscopy prior to the experiment confirms that the cells were in equilibrium and well dispersed before the application of the DEP force (Figure 4a).
Under this pragmatic setup, cells can demonstrate the forming of chain structures with high frequencies (2 MHz) application. The cells at first move towards the microtip electrode at which the field intensity $E$ is the maximum due to pDEP forces, and subsequently align into chain-like formation. The chains are formed throughout the microchannel while moving to finally localized at the centerline between the microelectrode pairs with cells fill in the gap to complete the bridging (Figure 4b). The chains spans a wide range of electric field intensities, from 230 kV/m as maximum located at the microtips edge to 110 kV/m as minimum located at the midpoint between the opposing microtip electrodes pairs. Establishment of the cells chain forms a stable contact between cells, promising for future application into studies of intercellular communication, bioelectricity and electrofusion.

Spinning of cells and rotation of cell clusters can be generated in addition to cells attract-clustering at 200 kHz frequency, not yet crossing the crossover $f_{xo}$ (90 kHz). This frequency attracts the cells to accumulate at the microtip vicinity by pDEP force while at the same time the cells exhibiting spinning and rotational motion. The cells congregate close around the microtip edge with the electric field intensity ranges between 230 to 150 kV/m (Figure 4c). The characteristic of cells spinning and clusters rotational motion is cells dependent, hence promising for application into development for diagnostic tools and cells properties database.

In contrast to demonstration of cell-chaining, spinning, rotation and attract-clustering, the assembly of cells by repel-clustering can be formed at frequencies lower than the crossover frequency $f_{xo}$, which demonstrated at 20 kHz in this work. The cells are pulled away from regions of high field intensity in the vicinity of the microtip electrodes due to nDEP force to regions of low field intensity between 10 and 50 kV/m close to the microchannel side wall or the base regions (Figure 4d). Unlike the attract-clustering demonstrated in the pDEP response, cells in these nDEP repel-clustering exhibit a stable contact between the cells, i.e. do not spin and rotate, which expected to have potential application into

**Figure 4.** Bioparticle contact configurations under DEP force; a) prior application of AC signal, b) pDEP response at 2 MHz, c) pDEP response at 200 kHz, d) nDEP response at 20 kHz.
studies based on as cell-chain formation such as cellular communication, however, with advantage in multi-directional configuration instead of linear configuration by cell-chain format.

5. Conclusions
Various bioparticles responses to DEP mechanisms are of high interest into application to development of biomedical and clinical studies. The different type of responses produced under pragmatic setup consist of customization of microtip electrode design, suspending medium selection and different standard electrical frequencies for resource limited environment have been discussed. Lower frequencies below the crossover frequency provides cell repel-clustering in low electric field intensity regions. Whereas attract-clustering can be obtained at higher frequency just above cross over frequency, with additional specific response in the form of cell spinning and cluster rotation. The construction of cell-chain structures can be generated at higher frequencies, i.e around MHz frequencies.

This pragmatic setup to induce various bioparticles responses is of highly potential implementation into studies in cellular communication and bioelectricity. As cell contact can be established at certain frequencies, how cell to cell communicate can be done by forming cell contact and later perform the desired analysis. As for cell spinning and cluster rotation, the characteristics such as relation of spinning speed and frequency is cell type dependent. Thus, characterization of various cell type especially disease related cells such as cancerous cell can be performed and later database could be established for disease diagnosis protocol in future. The development of a quick diagnostic tools for resource limited environment should get most benefit from this pragmatic setup optimization.

Acknowledgements
We thank the Ministry of Higher Education of Malaysia (MOHE) for support under Fundamental Research Grant Scheme FRGS/1/2015/TK04/MMU/02/9 and the Ministry of Education of Malaysia (MOE) for support under the Higher Institution Centre of Excellence (HiCOE) Grant, AKU-95.

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