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How medium osmolarity influences dielectrophoretically assisted on-chip electrofusion

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ABSTRACT

Cells submitted to an electric field gradient experience dielectrophoresis. Such a force is useful for pairing cells prior to electrofusion. The latter event is induced by the application of electric field pulses leading to membrane fusion while cells are in physical contact. Nevertheless, the efficiency of dielectrophoretic pairing and electrofusion of cells are highly dependent on medium properties (osmolarity and conductivity). In this paper, we examine the effect of medium osmolarity on volume, viability and electrical properties of cells. Then we characterize in real time the impact of electropermeabilization of cells on their dielectrophoretic response. To do so, a microfluidic device, inducing particular field topologies is used. These real time observations are correlated to numerical analysis of the Clausius–Mossotti factor. Taking into account the identified changes, an electrofusion protocol adequate to the optimal medium (100 mOsm, 0.03 S/m) is defined. Up to 75% simultaneous binuclear rapid electrofusions were achieved and monitored with average membrane fusion duration lower than 12 s.

1. Introduction

The application of micro and millisecond electric field pulses has well-known effects on cells. High amplitude electric field solicitations can cause cell death, while using moderate intensities induces reversible permeation of the membrane offering an easier access to cytoplasm to introduce some desired molecules [1–3]. During the last decades, this method, called electropermeabilization, became an important tool in cell/molecular biology [4], food industry and medicine [5,6]. Indeed, electropulsation was very quickly introduced in clinical applications for drug, antibody and plasmid delivery [7,8]. Since the last few years, interactions of ultra-short pulses (nanosecond scale) with cell membranes and cytoplasmic components have also been studied [9–11].

Using the appropriate conditions, it is also possible to create hybrids by fusing two contacting cells subjected to electric pulses [12–14] avoiding chemical (polyethylene glycol [15]) or biological (virus [16,17]) contaminants. Besides, the former method was reported to be more efficient than the last ones [18–20]. Hybridoma generation has been studied for antibody production [21], reprogramming of somatic cells [22] and cancer immunotherapy [23]. The technique using electric pulses (electrofusion) is simple. However, due to the lack of knowledge concerning electropermeabilization mechanisms [24], it is difficult to optimize the conditions for cell electrofusion. These reasons can explain why this method is not already the universal fusion tool. Different parameters can improve cell electrofusion. We can summarize some of them as follows:

- Increasing the electric field amplitude improves the fusion yield. However, it also reduces cell viability [25]. Thus, a compromise should be made.
- Increasing the conductivity (especially using Mg ions [26]) enhances cell permeabilization [27–30] but, combined to electric field, produces Joule heating that must be controlled. Moreover, electroporation in lower conductivity media preserves cell viability [31].
- A brief osmotic shock improves electrofusion yield [32–35]. Hypotonic media are considered as "optimized buffers".
- The application of pulses through different directions can improve bulk cell electrofusion [36].
- The use of miniaturized microfluidic structures improves electrofusion yield (using electric field constrictions for example [37–40]). It can also maximize one-to-one cell fusions and reduce polynucleated hybridoma [38,41].

As said before, the modification of medium parameters, especially osmolarity, can drastically improve fusion efficiency. In Usaj et al. work [34], the effect on fusion yield was deeply studied using a modified adherence method for cell contacts. In the present paper, cell pairing being achieved in a different way, using dielectrophoretic forces (DEP) [33,42–46], we consider the effect of an osmotic swelling on DEP force efficiency prior, during and after fusion. We used several biodevices, that

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we previously characterized in [39] and [41], to investigate the influence of buffer tonicity on each step involved in our electrofusion process (DEP during: i) pairing, ii) fusion initiation and iii) post-pulse). In particular, the "optimized" hypotonic fusion buffer [33,34] was considered.

Firstly, the effect of medium osmolarity on DEP force; necessary for the pairing protocol is examined. To do so, the cytosol conductivity was evaluated in the different media. In parallel, the viability in such hypotonic buffers was determined.

Secondly, the electrodeformation of cells, maintained into contact by DEP during the application of electrofusion electric pulses, was investigated. The usability of spherical shape model (generally used for DEP simulations) was confirmed. Besides, observed membrane shape modifications (as blebbing) were linked to fusion efficiency.

Finally, as DEP is maintained after fusion, the repercussion of electric pulses on cells characteristics (slight swelling, cytosol conductivity modification) and electrical behavior (DEP repulsion/attraction) were considered. These last questions are important when choosing the fusion procedure (pulse first/contact first protocol).

2. Materials and methods

2.1. Chemicals and cell culture media

Dulbecco's Modified Eagle Medium (Gibco ® GlutaMAX™ DMEM), Roswell Park Memorial Institute medium (Gibco ® RPMI), Penicillin–Streptomycin antibiotics (Gibco® PS), Fetal Bovine Serum (Gibco® FBS), Bovine Serum Albumin (BSA) and trypsin were purchased from Invitrogen (Life Technologies SAS, Saint-Aubin, France). D-sorbitol, Tris, Magnesium Chloride ($MgCl_2$) and Calcium Acetate ($C_4H_6CaO_4$) were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Germany).

2.2. Cell culture

Two cell lines were used during the different experiments: murine melanoma cells (B16F10) and human malignant epithelial cells (HeLa). Cultures were maintained in a 5% CO_2 incubator at 37 °C and passed every 2–3 days. Cells were cultured in 75 cm^2 culture flasks using DMEM supplemented with 10% FBS and 1% PS antibiotics until they reached 80–90% confluence.

2.3. Buffer

Three main buffers were used: iso-, semi-hypo and hypotonic buffers. The composition of these media is shown in Table 1. The *pH* was adjusted to 7.5 using Hydrochloric acid (HCl) and conductivity measured with the CyberScan Series 600 conductimeter (Eutech Instruments Pte Ltd).

Table 1
Composition of the main media used in the study. The medium conductivity σ_m/(S/m) is measured cell free at a temperature $T = 20$ °C. The bovine serum albumin protein is noted as BSA.

Ingredient/unit	Isotonic	Semi-hypotonic	Hypotonic
[Sorbitol]/mM	300	200	100
[Tris]/mM	1	1	1
[$MgCl_2$]/mM	0.7	0.7	0.7
[Ca(CH3COO)2]/mM	0.1	0.1	0.1
[BSA]/(mg/mL)	1	1	1
Osmolarity/mOsm	300	200	100
pH	7.5	7.5	7.5
σ_m/(S/m)	0.03	0.04	0.03

For dielectrophoretic experiments, a low conductivity medium is needed. To obtain conductivities of $\sigma_m = 0.003$ S/m, the original medium ($\sigma_m = 0.03$ S/m) is diluted then osmolarity adjusted using sorbitol.

2.4. Cell preparation

Before the experiment, adhered cells were trypsinized during 2–3 min and the adequate amount of cells suspended in culture medium. After centrifugation, buffer was removed and the cell pellet was rinsed twice with the experimental medium before suspension. Preparations of 1 to 4 million cells per mL were used.

2.5. Cell viability

Viability of osmotically shocked B16F10 cells was assessed by trypan blue exclusion method and cell count performed on Malassez plates. Each data point represents the mean value of 9 Malassez compartments; including triplicates (method variability) of 3 independent experiments (biological variability). Cells are kept at ambient temperature during the experiment. Measures on B16F10 cells suspended or adhered in culture medium represent, respectively, the first and second control.

2.6. Cell radius measurement

After centrifugation, rinsing and suspension in the experimental medium, a droplet of cells was placed on a cover slide and observed under a reflection microscope (Axio Scope.A1, Carl Zeiss SAS) with 20× objective. Bright field images were acquired with a calibrated CCD video camera (Moticam Pro 252B, Motic, Spain) and PC software Motic Images plus 2.0. All measurements were performed on fresh droplets from cell suspension. Each data point represents the $D(t)$ mean value of 9 cells from 3 independent biological samples. Time values include rinsing time in the corresponding medium. Measures in culture medium (diameter $D_0 = 15$ µm and volume V_0 for both cell types) are considered as reference. The median diameter deviation ($DV(t) = 100 * (D(t) - D_0)/D_0$) and volume ratio ($V/V_0$) were then compared at different osmolarities.

2.7. Microfluidic devices fabrication

Only biocompatible materials are used for the fabrication of microfluidic devices:

- Thin sputtered (150 nm) or thick electroplated (3–5 µm) gold electrodes,
- SU8 channels (a biocompatible negative thick photoresist-MicroChem©),
- Glass or PDMS (Polydimethylsiloxane polymer-Sylgard® 184, Dow Corning©) covers.

The device microfabrication is based on standard photolithography processes. The optimized protocol was detailed in previous work [39,41].

2.8. Electric field and dielectrophoretic force numerical calculation

Immersed in a non-uniform electric field E/(V/m), cells are subjected to dielectrophoretic (DEP) force due to the interaction between the external field and the induced dipole. The direction and intensity of DEP force depend on cells and buffer's dielectric characteristics. For a spherical particle with a radius R_{Cell}, the DEP force is given by:

$$\vec{F}_{DEP} = 2\pi R_{Cell}^3 \varepsilon_m \mathrm{Re}[f_{CM}] \vec{\nabla} \left| E^2 \right|, \quad (1)$$