Sensing and Bio-Sensing Research 7 (2016) 162–167

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Analysis of the role of elution buffers on the separation capabilities of dielectrophoretic devices

Rossana Di Martino [c,*], Massimo Camarda [d], Michele Cascio [a], Michele Gallo [a], Antonino Magliano [a,*], Salvatore Baldo [a,e], Alessandra Romano [b], Luigi Minafra [c], Giusi I. Forte [c], Giorgio Russo [c], Maria Carla Gilardi [c], Francesco Di Raimondo [b], Silvia Scalese [a], Antonino La Magna [a]

[a] CNR-IMM Section of Catania, Z.I. VIII Strada 5, I-95121 Catania, Italy
[b] Division of Hematology, AOUP Vittorio Emanuele, via S.Citelli, Catania, Italy
[c] IBFM-CNR, Cefalù, PA Sicilia, Italy
[d] Laboratory for Micro- and Nanotechnology Paul Scherrer Institute, CH-5232 Villigen-PSI, Switzerland
[e] Department of Physics and Astronomy, University of Catania, via S. Sofia 64, 95123 Catania, Italy

A R T I C L E I N F O

Article history:
Received 31 August 2015
Received in revised form 2 December 2015
Accepted 4 December 2015

Keywords:
Dielectrophoresis
Elution buffer
Circulating tumor cells
Cell motility

A B S T R A C T

Field flow fractionation dielectrophoretic (FFF-DEP) devices are currently used, among the others, for the separation of tumor cells from healthy blood cells. To this end specific suspension/elution buffers (EBs), with reduced conductivity (with respect to that of the cell cytoplasm) are generally used. In this paper we investigate the long-term alterations of the cells and elution buffers. We find that the EB conductivity is critically modified within few minutes after cells suspension. In turn, this modification results in a change the ideal separation frequency of the FFF-DEP device. On the other hand we prove that DEP manipulation is preserved for more than three hours for cells suspended in the considered EBs.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Dielectrophoresis (DEP) is currently used to determine the electrical characteristics of the cells, which are then analyzed [1] and exploited for the manipulation [2,3] and the selection of target cells from a mixture in suspension [4]. Metastatic diseases, a major cause of poor prognosis, are caused by the detachment and dissemination, through the blood stream, of cancer cells from the primary tumor mass. Circulating Tumor Cells (CTC) are able to take root and continue to grow into new tissue districts very distant from the primary site, leading to a, sometimes more aggressive, new tumor development [5–7]. Moreover, the identification of the CTC and their counts in cancer patients cannot only be considered as a prognostic factor, but it can follow the trend of a certain treatment indicating possible changes and/or improvements from a simple analysis of the blood avoiding invasive biopsies [8].

The dielectrophoretic forces are generated in a non-homogeneous electric field, respectively, as positive-DEP (+DEP) (with respect to the gradient of the electric field) or negative-DEP (−DEP) cell movement under the action of this electric field is used to sort cells [9–11].

The direction of cells' movement for a given frequency of the electric field depends on the polarizability of the cells compared with that of the extracellular medium in which they are suspended. This parameter also depends on the specific dielectric constant of the cell membrane (in turn dependent on the radius of the cells), a factor known as Clausius–Mossotti factor [12].

The exposure to a high electric field, however, induces a condition of high stress for the cells, which results in a change of the inner biochemical and biophysical properties, leading in the extreme cases either to cell death via cell lysis or apoptosis [13]. For these reasons, the cells can be manipulated for DEP only in buffer with low conductivity.

The search for an optimal buffer for the manipulation of cells on a dielectrophoretic device was the purpose of our research. We analyzed the physical (in terms of conductivity, pH) and chemical (in terms of composition of the buffer and dissolved salts) properties to characterize the best elution buffer (EB). In particular, we have turned our attention to the relationship between the buffer type and the capacity of DEP induced movement of the cells when the device is in action. Moreover, we have correlated these evidences with the capability of survival of the cells in the specific buffers and the variation of the physical–chemical

* Corresponding authors.
E-mail addresses: rossanadim@gmail.com (R. Di Martino), antonino.lamagna@imm.cnr.it (A. Magliano).

http://dx.doi.org/10.1016/j.sbsr.2015.12.001
2214-1804/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

parameters of the buffer, following the time variation of different measurable quantities.

These preliminary analyses were conducted on different tumor cell lines, from solid and liquid tumors, to evaluate the existence of any specificity of the cell type with buffer type.

2. Materials and methods

MDA-MB-231(breast cancer triple negative) and K562 (human immortalized myelogenous leukemia) were obtained from American Type Culture Collection (ATCC; Manass, VA, USA), and were cultured under condition recommended by ATCC.

Both cell lines were grown in DMEM supplemented with 10% heat-inactivated FBS (Heat-Inactivated Fetal Bovine Serum, from Gibco) and antibiotics (100 U/ml penicillin and 100 U/ml streptomycin; Sigma-Aldrich). All cells were maintained at 37% with 95%/5% air/$CO_{2.}$ and are grown to 70% confluence up to 48 h before being detached with trypsin (Trypsin-EDTA, Sigma-Aldrich) for MDA-MB-231 or to be re-suspended (K562). After neutralizing the trypsin with the complete medium, such type of cells was centrifuged for 5 min at 1200 rpm at 4 °C, re-suspended in specific extracellular medium to give a final concentration of 500,000 cells/ml and finally centrifuged again at the same condition.

Media whit the same conductivity, osmolarity and pH were prepared mainly by varying the type of salts. Our DEP-buffer consisted of an aqueous solution of sugar with the following percentage: 9.5% sucrose (S7903, Sigma-Aldrich), 0.1 mg/ml dextrose (D9559, Sigma-Aldrich), 0,1% pluronic F68 (Pluronic F68 non-ionic surfactant 100×, Gibco).

This buffer has a conductivity equal to 5 μS/cm and represents our elution buffer, which is then brought to the desired conductivity with solution 1 M of KCl (Sigma-Aldrich) or DMEM (11965, High glucose, GIBCO). The composition of the buffers used in comparison to the total composition of the culture medium is shown in Table 1.

The conductivity and pH of the media was calibrated using a conductivity/pHmeters (SG23 Seven Duo pH/conductivity, Metter Toledo).

2.1. Design, fabrication on the devices DEP

The electrode design used to study the kinetics of the cells is based on a polynomial schema: the electrodes' shapes can be described by the following parametric system:

$$\begin{aligned} &D \le x \le L \\ &y = \pm\sqrt{x^2 + D^2} \end{aligned} \quad (1)$$

for each electrode, rotated of 90° one with respect to the other. In Eq. (1), D represents half the distance of opposing electrodes whereas L is related to the electrode width. The polynomial electrode design is known to produce well defined 3D non-uniform electric fields and it is used for the study of negative and positive DEP. This electrodes schema has been fabricated by deposition of a very thin multilayer of 20 nm of Chrome (to improve adhesion), 100 nm of Gold on a standard microscope glass. The electrodes were delineated by lithographic methods followed by wet etching.

2.2. Experimental set-up for experiments DEP

80 μl of each type of suspension was put over the devices DEP, at the intersection between the 4 electrodes. To generate the out-of phase AC voltage for the DEP operation on the devices, a waveform generator (Agilent 33500B Series) was employed. A sinusoidal signal at fixed frequencies was applied to adjacent electrodes while two opposed electrodes were maintained at the same electrical potential. Electrical input signals were checked using a digital oscilloscope (Agilent Infini Vision DSO-X 2014A). The final distribution was observed with a standard contrast inverted microscope (Zeiss Axiovert 40 C). Movies and images were captured and recorded with a CANON camera connected to a microscopy. Data analysis was conducted with Excel, through the analysis of the t-test and ANOVA test, and program MATLAB.

3. Results and discussion

3.1. Effect of different cell types on the chemical–physical characteristics of the elution buffer and effect on the mobility of the cells on the device DEP

Once prepared the colloidal solution, we monitored in time the trend of the conductivity of the medium with the various cell types in suspension. Two different types of behavior have been recovered, related to the two types of cells, despite that they are re-suspended in the same type of buffer (EB-KCl 300 μS/cm) (Fig. 1).

This variation can be attributed to the different chemical, physical and biological properties, due to the different origin of the two tumor cell lines. Moreover, it also depends on the ability of cells to adapt in different ways to changes in the external environment: i.e. expressing different classes of receptor or ionic channels and pumps, express adhesion complex [13], deregulating the PI3K-AKT-mTOR pathway trough modulation of PTEN a p53 expression [14], fitting the area of the cell membrane to changes in metabolic activity [15,16].

In addition, there is another factor that should not be underestimated. Indeed, it is well known that the electrical properties of cell membranes that are in suspension depend on the morphology that they have before being detached and removed from their normal site of growth.

Microscopy investigations show that the change in the size of cells suspended in a medium derived from the loss of cytoplasm.

Table 1
Composition of different buffers used in our experiments.

	Elution buffer	Buffer KCl (low cond.)	Buffer DMEM (low cond.)	Buffer KCl (high cond.)	Buffer DMEM (high cond.)	DMEM (mg/L)
Sucrose	9.5%	9.5%	9.5%	9.5%	9.5%	–
Dextrose	01 mg/ml	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml -	–
KCL	–	0.02%	–	0.04%	–	400
$Fe(NO_3)_3.9H_2O$	–	–	–	–	–	0.1
$MgSO_4$ (anhyd)	–	–	–	–	–	97.67
NaCl	–	–	–	–	–	6400
NaH_2PO_4	–	–	–	–	–	125
Sodium pyruvate	–	–	–	–	–	110
$CaCl_2$	–	–	–	–	–	200
Amino acids	–	–	–	–	–	100% as per catalog
Vitamins	–	–	–	–	–	100% as per catalog
DMEM	–	–	15%	–	30%	
Conductivity	5 μS/cm	300 μS/cm	300 μS/cm	600 μS/cm	600 μS/cm	1856 mS/m
pH	6.5	6.8	7.20	6.8	7.40	

Download English Version:

https://daneshyari.com/en/article/807380

Download Persian Version:

https://daneshyari.com/article/807380

Daneshyari.com