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Applications to Cancer Research of "Lab-on-a-chip" Devices Based on Dielectrophoresis (DEP)

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The recent development of advanced analytical and bioseparation methodologies based on microarrays and biosensors is one of the strategic objectives of the so-called post-genomic. In this field, the development of microfabricated devices could bring new opportunities in several application fields, such as predictive oncology, diagnostics and anti-tumor drug research. The so called "Laboratory-on-a-chip technology", involving miniaturisation of analytical procedures, is expected to enable highly complex laboratory testing to move from the central laboratory into non-laboratory settings. The main advantages of Lab-on-a-chip devices are integration of multiple steps of different analytical procedures, large variety of applications, sub-microliter consumption of reagents and samples, and portability. One of the requirement for new generation Lab-on-a-chip devices is the possibility to be independent from additional preparative/analytical instruments. Ideally, Lab-on-a-chip devices should be able to perform with high efficiency and reproducibility both actuating and sensing procedures. In this review, we discuss applications of dielectrophoretic(DEP)-based Lab-on-a-chip devices to cancer research. The theory of dielectrophoresis as well as the description of several devices, based on spiral-shaped, parallel and arrayed electrodes are here presented. In addition, in this review we describe manipulation of cancer cells using advanced DEP-based Lab-on-a-chip devices in the absence of fluid flow and with the integration of both actuating and sensing procedures.

Key words: Dielectrophoresis; Lab-on-a-chip; cell manipulation.

Introduction

The "Lab-on-a-chip technology" (1-11), involving miniaturisation of complex analytical procedures, is expected to enable laboratory testing employing several equipment to move from the central laboratory into non-laboratory settings (12-19). An example of techniques of great interest in applied medical sciences is the isolation of homogeneous cell cohorts (even if present in low amounts) from heterogeneous cell populations. A second example is the handling of individual cells, a very important technology in cell engineering, greatly facilitating gene introduction/transfer, drug injection and clone technology. Furthermore, Lab-on-a-chip devices are also expected to perform molecular biology characterization of isolated cells, employing cell lysis, entrapment and analysis of target molecules.

In this respect, dielectrophoresis (DEP) (20-24) has been reported as a very valuable approach in projects aimed at the design and production of Lab-on-a-chip devices (25-28). This technique allows the development of Lab-on-a-chip

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Abbreviations: DEP, dielectrophoresis; PCB, printed circuit board; CMOS, complementary metal oxide semiconductor; RBC, red blood cells.

devices based on microelectronic technologies and exhibiting great flexibility and multiple applications in several biomedical fields, including molecular and cellular oncology. We have to underline that new advances in microelectronic technologies allow integrating in the substrate of the biochip circuits that could carry out functions of both actuating and sensing type (29-34). This approach takes clue from the development of bioseparation methods based on microchip manufactured on an active substrate, produced with microelectronic technologies compatible with the fabrication flow of CMOS integrated circuits (31). This class of microsystems has some innovative characteristics. In particular, thanks to the use of the technology of integrated circuits, the generated DEP fields immobilize and allow controlling single biological objects, like cells, liposomes or microspheres immersed in a liquid overhanging the same chip and in contact with it. Examples of particles investigated by DEP have been virus (such as Herpes simplex type 1) (35-38), bacteria (*Escherichia coli, Bacillus subtilis, Micrococcus luteus*) (39-41), protozoa (*Cryptosporidium parvum*) (42), yeast (*Saccaromices cerevisiae*) (43-45), plant and mammalian cells (including erythrocytes, cancer cells, stem cells) (39, 46-50), liposomes (51) and artificial nanoparticles (52-54).

Theory: What is Dielectrophoresis (DEP)?

The physical effect called dielectrophoresis (23, 24) is the movement of particles in non uniform electric fields (22, 23, 27, 29, 30, 55-62). Charges in the particle itself are not necessary for the effect to occur. This is due to the fact that when an electric field is applied to system consisting of particles suspended in a liquid, a dipole moment is induced, due to the electrical polarizations at the interface between the particle and the suspending liquid. If the field is non-uniform, the particles experience a translational force (DEP force) of magnitude and polarity, depending not only on the electrical properties of the particles and the medium, but also on the magnitude and frequency of the applied electric field. This means that for a given particle type and suspending medium, the particle can experience, at a certain frequency of the electrode applied voltages, a translational force directed towards regions of high electric field strength (this phenomenon is called pDEP). Or, by simply changing the frequency, they may experience a force that will direct it away from high electric field strength regions (this phenomenon is called nDEP).

In the case of cells, the DEP properties largely depend on several biological parameters, including membrane capacitance (determined by the membrane dielectric permittivity, thickness and area) and conductance. Interestingly, permittivity (62) strongly depends on membrane composition (55, 56). It should be noted that most of the parameters influencing DEP properties are the basis for dielectric differences between different cell types and dramatically change during cell differentiation (55-57) as well as neoplastic transformation (58), suggesting that DEP-based approaches could lead to the development of devices for cell discrimination and separation, as reported in several studies (55-58).

Compared with devices that use other electrokinetic approaches to move particles, such as electrophoresis or electroosmosis, DEP systems operate using a low AC instead of high DC voltage and can be easily combined with electronic detection technologies (e.g. resistive and/or capacitive sensing), to give a real fully-electronic Lab-on-a-chip (18).

However, devices based on the various DEP kinds do not permit a precise and deterministic control of the particle positioning, therefore exhibiting lack of accuracy and flexibility whenever rare cells or microorganisms must be selected, counted or manipulated (22-24). Moreover, these devices still require microfabrication techniques unsuited for a low cost industrial production.

For spherical geometries, a first order approximation of the DEP force can be expressed as:

$$\langle \vec{F(t)} \rangle = 2\pi\varepsilon_0\varepsilon_m r^3 \{\mathrm{Re}[f_{CM}(\omega)]\vec{\nabla}(E_{RMS})^2 + \mathrm{Im}[f_{CM}(\omega)](E_{x0}^2\vec{\nabla}\varphi_x + E_{y0}^2\vec{\nabla}\varphi_y + E_{z0}^2\vec{\nabla}\varphi_z)\}$$

where ε_0 is the vacuum dielectric constant, r is the particle radius, $E_{\alpha 0}$ and φ_α (α = x, y, z) are the magnitude and phase of each component in a Cartesian coordinate frame, E_{RMS} is the root mean square value of the electric field, $\mathrm{Re}(f_{CM})$ and $\mathrm{Im}(f_{CM})$ are the real (in-phase) and imaginary (out-of-phase) components of the Clausius-Mossotti factor,

$$f_{CM} = (\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* + 2\varepsilon_m^*) \quad [1]$$

which is a function of the complex permittivities of the particle ε_p and the medium ε_m, defined as $\varepsilon = \varepsilon + \sigma/j\omega$, being ε the dielectric permittivity and σ the conductivity. For nDEP, it should be $\mathrm{Re}(f_{CM})<0$. At low frequency ($\omega << \sigma/\varepsilon$) equation [1] can be approximated by:

$$f_{CM} = (\sigma_p - \sigma_m)/(\sigma_p + 2\sigma_m) \quad [2]$$

while at high frequency ($\omega >> \sigma/\varepsilon$):

$$f_{CM} = (\varepsilon_p - \varepsilon_m)/(\varepsilon_p + 2\varepsilon_m) \quad [3]$$

Thus particle levitation by nDEP is possible at low frequency if $\sigma_p < \sigma_m$, and at high frequency if $\varepsilon_p < \varepsilon_m$.

A more complete analysis of the theory of DEP can be found in the articles by Wang *et al*. (59), Jones and Washizu (60)

and Clague *et al*. (61), explaining in detail all the features of DEP and how the DEP force is analysed theoretically. This information is crucial, in order to properly design electrodes for specific biotechnological and biomedical applications.

Computer-aided Simulation of Driving of Cages

Figure 1 shows a numerical simulation of the effects of the general working principle that is the basis of the architecture of most proposed biochips and prototypes of interest in the development of final versions of Lab-on-a-chip devices. By applying suitable potentials to the electrodes, it is possible to generate time-dependent electric fields in the liquid (Figure 1). These fields can then generate DEP fields acting on the particles in the fluid. A DEP force is then generated thanks to the differences in the dielectric permittivities of the different materials. An important point of this approach is that the overall system can be designed to force the DEP fields to create closed cages that can trap inside particles in a stable way. These cages are created when suitable electric potentials are applied to the electrodes. By looking at the left side of Figure 1, it is possible to see a local minimum of electric fields associated with the presence of a DEP-cage (arrowed). Since these electric potentials can be applied under software control, it is possible to change how particles are moved modifying the settings on a computer. In addition, it is possible to change in time the location of these closed DEP cages. After changing the potentials applied to the electrodes, the location of the cage changes (Figure 1, right side of the panel, step 1-3) but remains closed, allowing to maintain the control of enclosed particle(s).

A Short Review on Recent Applications of DEP in Cancer Research

DEP-based manipulation of tumor cells has been recently described by several laboratories (26, 45, 48). Figure 2 outlines some features of published devices such as those containing spiral electrodes (Figure 2A) (45), parallel electrodes (Figure 2B) (32-34, 46), low-density electrode arrays (Figure 2C) (47, 64) and high-density electrode arrays (Figure 2D) (31). Figure 2 shows also what is possible to obtain using these devices, in terms of motion of cells, possibly allowing separation and sorting (right side of each panel). Only for few of these devices both actuating and sensing operations can be conducted. In any case, most of these devices were found to be useful to manipulate tumor cells. For instance, Huang *et al*. (63) reported manipulation of human leukemic HL-60 cells using dielectrophoretic field-flow fractionation and devices containing parallel electrode arrays. By combining application of electric fields and liquid flow, cell fractionation was obtained. In the reported studies, as well as in similar experimental conditions, cells growing in suspension are good candidate for manipulations. However, separation

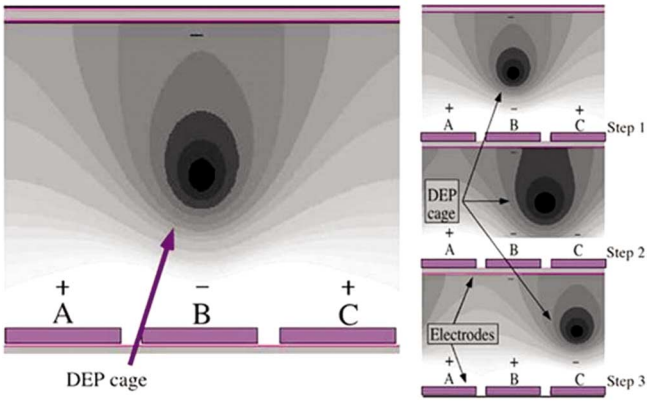

Figure 1: Shape of the DEP field as a result of a numerical simulation (left side of the panel). A local minimum of the DEP potential is associated with the presence of a cage (arrowed). After changing the potentials applieds to the electrodes (right side of the panel), the location of the cage changes (Step 1 - Step 3), but the cage remains closed. The Software used was the DESSIS Software, a multi-dimensional electro-thermal mixed-mode device and circuit simulator for one, two and three-dimensional semiconductor devices (ISE, Integrated Systems Engineering AG, Switzerland).

and/or concentration were also performed using adherent cells growing in monolayer, such as the breast cancer cell line MDA-MB231 (45), using a device with concentric spiral electrodes. In this case, cells were treated with a solution containing 0.25% trypsin and 0.02% EDTA before manipulation. These authors reported cell entrapment, levitation and motion. The cell concentration approach which is possible with these particular DEP-based devices is reported in Figure 2A (right side of the panel).

Table I shows a partial list of research reports focusing on manipulation of tumor cells using DEP-based devices (45, 63-65). In general, these approaches were proposed for cell concentration and cell isolation and detection. However, DEP-mediated cell separation methods could be associated with gene expression analysis, as recently described by Huang *et al*. (64) using a 5 × 5 electrode array. These authors used this DEP-device for isolation of different types of cells and analysis of their gene expression profiling by RT-PCR analysis using primers amplifying IL-1, TNF-alpha and TGF-beta cDNAs.

We like to underline that the DEP-based devices so far described in the literature exhibit a high degree of interplay. Many of them are flow-based systems and appear to be of great interest in performing isolation of low numbers of tumor cells from hundred of millions of normal cells; while handling of single cells is possible using flow-based Lab-on-a-chip devices (66), the simultaneous, but independent handling of hundreds of single cells appears not to be the first choice of these systems (32-34). In this respect, devices, which do not need flow and are appealing for software-controlled levitation and motion of single particles, have been recently described by Medoro *et al*. (32-34) and by Manaresi

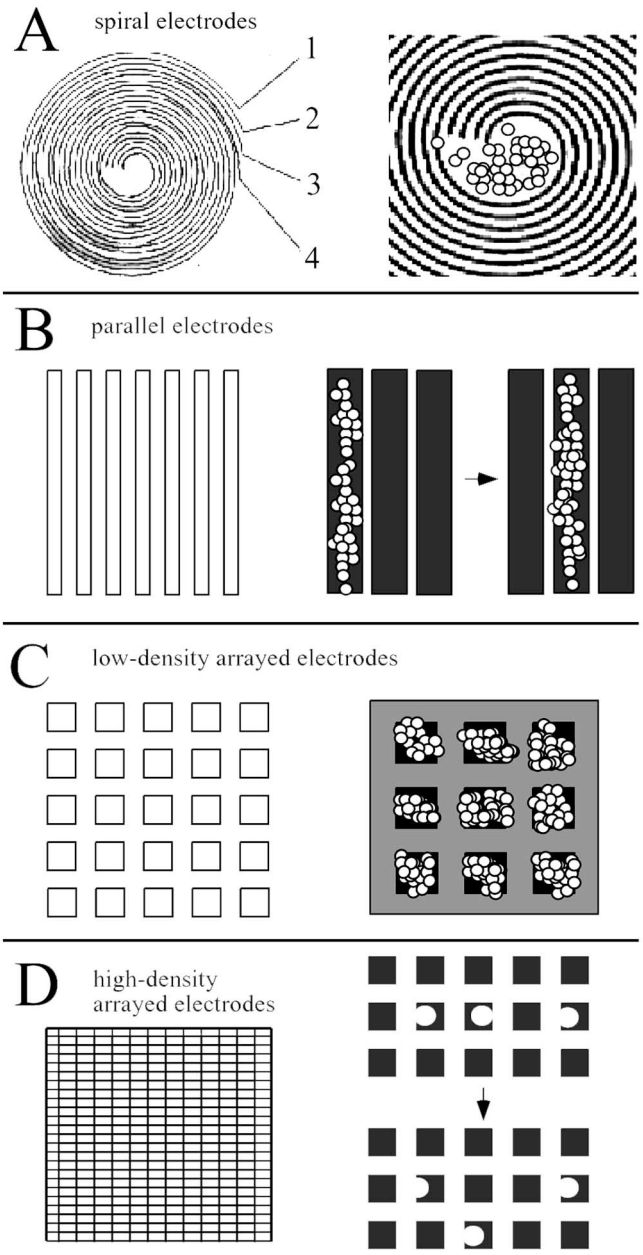

Figure 2: Schematic representations (left) and performance (right) of DEP-based chip devices carrying spiral electrodes (A) (45), paralled electrodes (B) (46, 65), arrayed electrodes (C, D) (31, 47, 64). Cell concentration (A, C) and cell motion (B, D) functions have been proposed as potential features of these devices (31, 45-47, 64).

et al. (31). In the first case, the printed circuit board (PCB) based chip is able to generate cylinder-shaped cages; in the second case a DEP-array is proposed for generation of sphere-shaped cages and simultaneous manipulation of high numbers of single cells (see schemes in Figure 2B and 2D).

The combined use of flow-based devices and arrayed devices could be of great interest to move from tumor cell isolation to functional characterization and study of the effects of drug treatment.

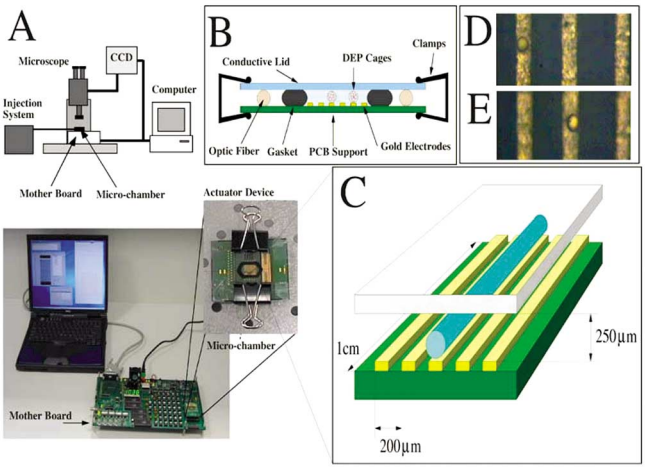

Figure 3: Schematic view of the PCB-based chip prototype device proposed by Medoro *et al*. (32). A: System setup: a mother-board is used to generate and apply the stimuli to each electrode in the device. Both the actuation and the sensig phases are executed under software control by means of a computer. B,C: Sketches of a cross section of the PCB-based chip device: two optic fibers are used as spacer and a gasket is used to delimit the micro-chamber on the sides, while a conductive lid and a PCB support close the micro-chamber respectively on the top and on the bottom. In (C) is sketched a cylinder-shaped DEP cage in correspondance of the central electrode. D, E: Software controlled motion of 50 µm microbeads. Data are modified from Medoro *et al*. (32).

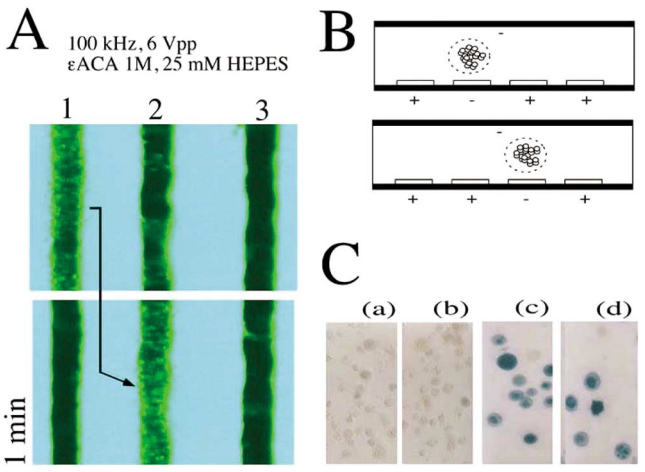

Figure 4: A: Levitation and movement of fluorescein-labelled human leukemic K562 cells from electrode n.1 to electrode n.2, using the PCB-based chip device described by Medoro *et al*. (32) and a buffer containing 1.5 M ε-ammino-caproic acid, 2.5 mM Hepes. In panel B the motion of a cylinder-shaped cage is sketched. C: Effects of the DEP treatment on erythroid differentiation. K562 cells were DEP treated (b, d) in the biochip for 1 min, recovered and cultured in the absence (b) and in the presence (d) of 1 µM cytosine-arabinoside (ara-C). After 7 days, the proportion of benzidine-positive, erythroid differentiated cells was determined. Control K562 cells were cultured in parallel in absence (a) and in presence (c) of ara-C. As expected in the case DEP treatment does not cause alteration of the biological properties of K562 cells, uninduced DEP-treated cells are negative to the benzidine stain (b), while ara-C induced DEP-treated cells are benzidine-postitive (d). Results presented in this Figure are modified from Altomare *et al*. (65).

Table I
Manipulations of Tumor Cells by Dielectrophoretic-based Devices

Cell/cell line	Feature of the device	Manipulation	References
MDA-MB231 breast cancer cells	concentric spiral electrodes	cell entrapment levitation motion	Wang et al. (1997) (46)
HL-60 human leukemic	parallel electrodes	levitation separation from RBC	Huang et al. (1997) (63)
U937 human monocytic HTB glioma cells SH-SY5Y neuroblastoma cells	5 x 5 electrode array	cell separation	Huang et al. (2002) (64)
HeLa cervical carcinoma cells	5 x 5 electrode array	cell isolation	Cheng et al. (2002) (49)
K562 human CML cells	39 parallel electrodes	levitation separation from RBC	Altomare et al. (2002) (65)

PCB-based Chip: Cylinder-shaped Cages

A cross section of the device proposed by Medoro *et al*. (32) is represented in Figure 3 (A-C): a microchamber is delimited on the top by a conductive and transparent lid (which is itself an electrode and is electrically connected to the PCB device by means of a conductive glue) and on the bottom by a PCB support (Figure 3, B and C). A spacer (realised by two optic fibers) determines the chamber height, while a silicon elastomer gasket delimits and seals the microchamber on the sides (Figure 3B). This device has 39 parallel electrodes by which it is possible to realise from 0 to 19 cylinder-shaped DEP cages. A mother-board is used to generate and distribute to each electrode in the device the proper phases needed to create and move the DEP cages and to perform the sensing operations, while a software tool allows to control the actuation and sensing operations flows (Figure 3A). By changing the electrode programming, each DEP cage can be independently moved from electrode to electrode along the whole microchamber, dragging with it trapped particles, such as microspheres and living cells. As far as injection and recovery of cells, the system, as described in Figure 3A, is integrated with a micro-fluidic set-up composed of a microsyringe and two capillaries for sample injection in to the micro-chamber. A third capillary is used to eject materials from the micro-chamber. Figure 3C sketches a cylinder-shaped DEP cage in the center of the device. To the best of our knowledge, this is the first device to integrate this possibility in a fully-electronic system without the need for fluid flow control, which tend to be bulky and expensive in a system perspective. This system is very useful for levitation, movement and separation of a large number of cells exhibiting identical DEP properties.

Arrayed Chips: Lab-on-a-chip Devices Generating Sphere-shaped Cages

A sketch of the DEP array recently described by Manaresi *et al*. (31) is presented in Figure 2D: a microchamber of about 4 μl is defined by the chip surface and a conductive-glass lid spaced 100 μm apart. The chip surface implements a two-dimensional array of micro-sites, each consisting of a superficial electrode, embedded sensor and logic. The DEP-array consists of 320 × 320 electrodes, allowing an implemented generation of DEP-cages able to accommodate single cells. By changing the pattern of voltages applied to the electrodes in a software-controlled way, the DEP cages can be independently moved around the device surface, thus dragging with them the trapped particles. This system is very useful for levitation and motion of single cells.

Applications of PCB-based Chip to Levitation and Movement of Tumor Cell Populations

Both DEP-based chips containing parallel and arrayed electrodes can be used for levitation and motion of several particles of great biological interest, such as viruses (37, 38), bacteria (41), yeast (44, 45), plant (28) and mammalian (26, 29, 30, 48) cells. In addition liposomes and microspheres were demonstrated to be suitable for manipulation using DEP-based devices (51-54). For instance, Figure 3 (panels D and E) shows the levitation and motions of microbeads on the PCB-based chip equipped with parallel electrodes. As it is readily observed, microbeads can be moved from one electrode to other electrodes without any liquid flow. PCB-based chip and DEP-array were used to manipulate also yeast, bacteria and mammalian cells (not shown). While the use of PCB-based chips allows also analysis of limited numbers of cells, it is optimal for separations of large numbers of cells ($0.5\text{-}3x10^6$). Altomare *et al*. (65) reported that a PCB-based chip device manufactured by the research and development team at Silicon Biosystems (Bologna, Italy) enable to levitate and move several tumor cell lines, including B-lymphoid Raji, T-lymphoid Jurkat, erythroleukemic HEL and K562, murine FLC, melanoma Colo38. Each cell line exhibited DEP features different from the other cell lines. As reported by Altomare *et al*., separation of red-blood cells from erythroleukemic cells is obtained using the PCB device generating cylinder-shaped cages (65). We like to underline that levitation and movement of human cells was obtained without the help of liquid flow and simply using software-controlled inputs. Figure 4A (top panel) shows

the initial state of fluorescein-labelled human leukemic K562 cells on an electrode. K562 cells were then moved to the right under software control (Figure 4A, bottom panel). The movement of the cylinder-shaped cage is shown in Figure 4B. Altomare *et al*. found that the optimal conditions for software-controlled movement of K562 cells are 100 kHz, 6 Vpp (65). In these conditions, K562 cells were forced to move as requested within seconds in the presence of a buffer containing 280 mM mannitol (65). This is a just an example of DEP-based separation of different types of cells, as reported in other excellent papers by several research groups (see Table 1 and references 26, 27, 30, 46-50, 64).

Are Cells Damaged After DEP Manipulation?

The possibility that manipulated cells can be damaged following exposure to the experimental conditions required by DEP analysis should be taken in great consideration. After electric field exposure, cells can be seriously damaged (67). For instance, electroporation of the plasma membrane by high fields and toxic reactions of cells with electrochemical species produced at electrodes have been reported (67). In this respect, it should be underlined that the conductivity of suspending buffers used in DEP-based manipulations is much below that of normal physiological medium. Therefore, at least in theory, DEP might be toxic to the cells, altering their membrane and other stress-related biological functions. In this respect, the available information is not conclusive. However, it has been reported that cells undergone to DEP forces were not damaged in the case of erythrocytes (48, 68), yeast cells (44), CD34+ cells (69). Accordingly, many research reports on applications of DEP-based devices were aimed at the separation of viable and non-viable cells in a given cell population. The demonstration that DEP-treatment does not cause major damage to cells was reported by different laboratories. For instance Stephens *et al*. (69) studied the dielectrophoresis enrichment of CD34+ cells from peripheral blood stem cell harvests. These authors isolated CD34+ cells from peripheral blood stem cell harvest samples containing an untreated natural mixed cell population. The separation was achieved by exploiting differences in the inherent DEP properties of the various cell types. In order to demonstrate that the cells remain viable after the separation process, cells were plated in colony assay cultures (stimulating GM-CFU and BFU-E) demonstrating that the cells remain normal, viable and capable of colony formation when cultured for 2 weeks. The number of colonies formed correlated with the percentage of CD34+ cells in each fraction (69). On the other hand, DEP-induced cell damage occurs using some buffers. For instance, Wang *et al*. (67) carefully studied cell damage using particular DEP conditions. Accordingly, in a recent report, Altomare *et al*. (65) determined the effects of experimental DEP conditions used for levitation and motion of tumor cells on cell growth kinetics and ability of the cells to undergo differentiation. In the case described by Altomare *et al*. (65), human leukemic K562 cells were suitable for these experiments, since this cell line can be induced to erythroid differentiation after cell culture in the presence of 1 □M cytosine-arabinoside (Ara-C) (70-72). The obtained results clearly demonstrated that the effects of DEP treatment depend on the used buffer. While is some experimental conditions inhibitory effects on cell growth were observed, in other conditions DEP treatment did not cause alterations of cell growth and erythroid differentiation (Figure 4C). The data available, therefore, suggest that careful preliminary experiments should be undertaken in order to avoid major changes of cellular biological features following DEP analysis.

Accordingly, in a recent paper Huang *et al*. analysed the expression of the stress-related gene c-fos, demonstrating that dielectrophoretic forces have little effect on cell survival and stress (64). However, minor changes in gene expression and biological functions are not excluded, and further experiments analysing the gene expression profile and function of DEP-treated cells should be carefully performed. Minor alterations of manipulated cells could alter the phenotyping characterization (introducing possible problems in using DEP-based Lab-on-a-chip in diagnostics) but also ability of the DEP-treated cells to respond to exogenously added stimuli (introducing unwanted alterations of biological parameters in pharmacological studies performed on DEP-isolated cell populations).

Future Perspectives of DEP-based Lab-on-a-chip Devices in Cancer Research

Several approaches could be feasible using PCB-based chips or DEP-arrays in different fields of molecular and cellular oncology, including separation of tumor cells from normal counterpart without using monoclonal antibodies, phenotyping characterization of tumor cells, isolation of cells present in very low concentration within heterogeneous cell populations.

Separation of Tumor Cells from Normal Counterpart Without Using Monoclonal Antibodies

This approach has been followed by several research groups, with objectives of great interest in applied oncology. For instance, Huang *et al*. demonstrated the removal of human breast cancer cells from hematopoietic CD34+ stem cells by dielectrophoretic field-flow-fractionation (DEP-FFF) (26). The array used was constituted of interdigitated microelectrodes lining the bottom surface of a thin chamber and able to generate dielectrophoretic forces that levitated the cell mixture in a fluid flow profile. CD34+ stem cells were levitated higher, were carried faster by the fluid flow, and exited the separation chamber earlier than the cancer cells. The authors suggested that the method of DEP-FFF is potentially applicable to many biomedical cell separation problems, including microflu-

idic-scale diagnosis and preparative-scale purification of cell subpopulations. It should be underlined that efficient cell separation is a key step in autologous bone-marrow transplantation in advanced cancers in which it is necessary to remove metastatic cells from the marrow of the patient. In addition, human tumor HL-60, HeLa, K562 cells subjected to a parallel electrode array could be separated from peripheral blood mononuclear cells (49, 63, 65). On the other hand, the isolation of cells present in very low concentration within heterogeneous cell populations is a crucial step in the case of the purification of stem cells to be used for in vitro expansion in cellular therapy of several human diseases. For this application, it should be demonstrated that the exposure to DEP forces does not damage cells and alter their biological properties. PCB-based chips are the most suitable for this application.

Proteomics and Gene Expression Profiling

The post-genomic era allows to perform gene expression analysis at the level of the so-called transcriptome. In this approach the purity of the cell population is an absolute requirement. Interestingly, RT-PCR and hybridization are now possible starting from a limited number of cells. Therefore, Lab-on-a-chip technology associated with microarray methods is expected to be deeply employed in the next future. In a recent paper, Huang *et al*., characterised the distinct DEP property differences between individual cell types (U937 monocytic, HTB glioma, SH-SY5Y neuroblastoma), obtaining efficient cell separations by dielectrophoresis on a 5 × 5 array (64). These authors demonstrated that the purity of dielectrophoretically-separated cells was greater than 95%. Expression profiles of IL-1, TNF-alpha, and TGF-beta genes for U937 cells mixed with PBMC before and after the separation were determined supporting the conclusion that microelectronic chip arrays for both cell separation and gene expression profiling provides a great potential for accurate genetic analysis of specific cell subpopulations in heterogeneous samples.

Phenotyping Characterization of Tumor Cells

As reported by several groups, microspheres can be moved using DEP-based devices. This has been demonstrated by Manaresi *et al*. (31) using the DEP-array for single polystyrene beads in water. This opens the possibility of characterizing single tumor cell populations with beads exposing monoclonal antibodies. DEP-arrays are required for this specific project. The possibility to bring in contact different cages is the basis for the screening of hundreds of single cells with differently located microspheres exposing different monoclonal antibodies.

Drug Discovery

At least in theory, it could be possible to screen large numbers of microspheres coated with unknown molecules either with tumor cells or with microspheres coated with molecules to be targeted for pharmaceutical applications. This strategy could allow the quantitative delivery of antitumor drugs to target cancer cells. In addition, DEP-based Lab-on-a-chip devices could bring informations on the effects of treatment on single tumor cells. According to recently published reports, functional or gene expression changes could alter the DEP properties of tumor cells. For instance, Ratanachoo *et al*. (73) studied time and dose responses of human leukemia HL-60 cells exposed to paraquat, styrene oxide (SO), N-nitroso-N-methylurea (NMU) and puromycin in respect to DEP properties. These toxicants were chosen because of their different predominant mechanisms of action, namely membrane free radical attack, simultaneous membrane and nucleic acid attack, nucleic acid alkylation, and protein synthesis inhibition, respectively. Alteration of DEP properties correlated sensitively with alterations in cell surface morphology, suggesting the feasibility of producing small instruments for toxicity detection and screening based on cellular dielectric responses. In another interesting paper, Wang *et al*. demonstrated that DEP approaches allow the detection of apoptosis more sensitively than other detection approaches, such as externalisation of phosphatidylserine and studies of DNA fragmentation using ethidium bromide (74). This is of great interest, as several anti-tumor drugs act on target neoplastic cells by activating apoptosis (74). In this study, human HL-60 cells were treated with genistein and early DEP-mediated analysis of membrane changes (capacitance and conductivity) indicated induced apoptosis. Another example showing that changes of gene expression might lead to changes in DEP properties was published by Cristofanilli *et al*. (75), who determined variations in dielectric features in MCF7 breast cancer cell lines overexpressing p185(neu). These differences may be related to the morphological alterations determined by HER-2/neu overexpression. These data introduce the exciting possibility that DEP-based Lab-on-a-chip devices could detect even little changes in biological functions without the need of complex morphological, biochemical and pharmacological techniques.

Conclusions

The development of Lab-on-a-chip technology based on approaches involving singular or combined use of flow-based DEP devices and of systems controlling motion of DEP-cages in the absence of fluid flow will enable several experiments in applied oncology research, including:

a) Separation and characterization of tumor cells from normal counterpart without using monoclonal antibodies;

b) Studies of proteomics and gene expression profiling;

c) Drug discovery and delivery.

For the methodological point of view, we should consider that:

a) In several DEP-based devices no optics is needed to follow moving cages entrapping biological objects (such as cells), since differences in DEP response are exploited;

b) In most of the proposed DEP-devices, the location and movement of cells are software-controlled;

c) DEP cages and fluid-flow allow particle concentration and recovery;

d) The majority of the DEP-based approaches allows quantitation of separated cells without the need for external instruments or additional laboratory steps;

Altogether, these features are key functions for developing Lab-on-a-chip devices performing complex laboratory approaches of great interest in tumor diagnosis, oncology research and treatment of neoplastic diseases.

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