Project: Cell / Particle Electrophoresis using Pyroelectricity

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Abstract:

Cell Electrophoresis is the study of motion of cells in a fluid under the influence of a spatially electric field. The electro-kinetic phenomenon was observe for the first time in 1807 by Ferdinand Frederic Reuss (Moscow State University), who noticed that the application of a constant electric field caused clay particles dispersed in water to migrate. Cell electrophoresis is a field driven technique, which serves two purposes: 1) to study the surface properties of cells, 2) to separate uniform cell subpopulation from cell mixtures. This process is for investigating molecules of interest, cancer cells including proteins and nucleic acids. [1][2][3] Currently, there are different types of electrophoresis process such as, Affinity electrophoresis, capillary electrophoresis, Dielectrophoresis, DNA electrophoresis, Electro blotting, Electro focusing, gel electrophoresis and many more. Electrophoresis have gained a lot of interest in the cell biology, figure below shows the application oriented electrophoresis process in the biological department.

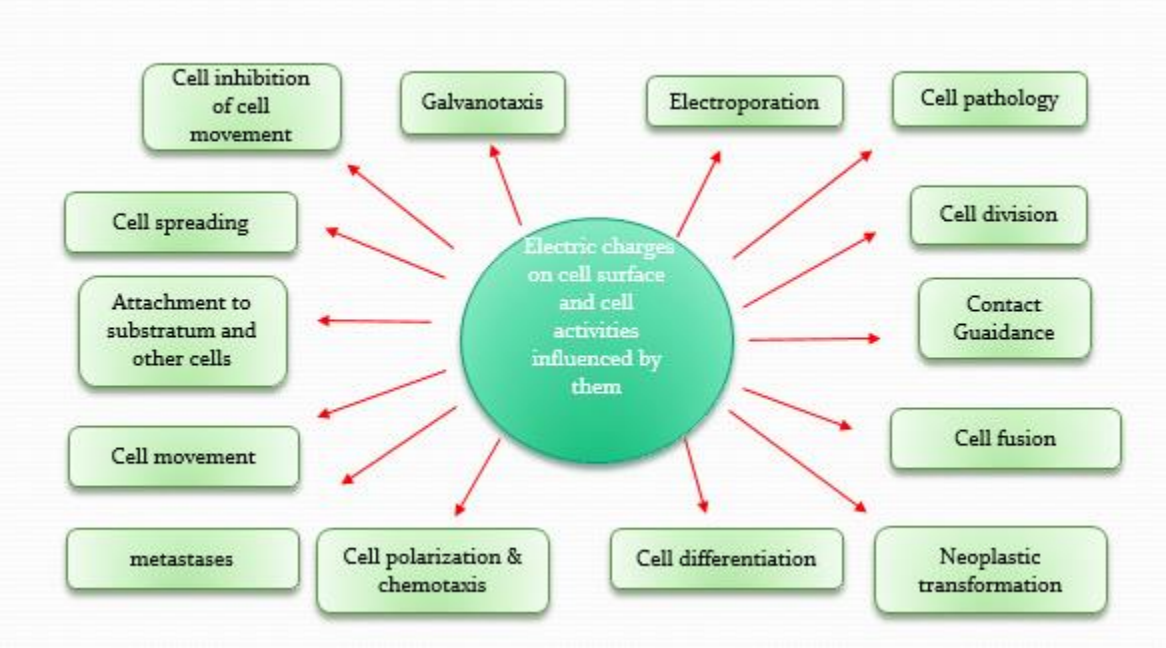

Fig1: Application of electrophoresis in biological field.

Cell electrophoresis for cell surface properties:

Microscopic methods in which single cell electrophoresis in a stationary layer of solution is directly measure under a microscope have proved very effective in the study of cell surface properties. Measurements is carried out on cells remaining in the layer of solution which does not flow when an electric field is applied to system i.e. the electro-endo-osmotic flow is zero. Significant results obtained with this methods includes in the paper related red blood cells

[1], cancer cells [3, 4], bacteria modifications of cell surface evokes in cell behaviour and function and more.

Cell Electrophoresis for separating cells from mixed cell population according to the physical properties:

Using cell electrophoresis the separation of cancer cells from normal cells also apoptotic cells were achieve, cells altered in a variety of pathological processes and cells with modified surface properties.

Capillary cell electrophoresis:

Permits for fast analysis of heterogonous population of cells, this occurring in a strong electric field due to the easy dispersion of joules heat from narrow capillaries.

Micro channel electrophoresis:

A new stream of study, which uses the micro channels, fabricated using PDMS and studying the different cell surface such as DNA, proteins and more.

Dielectrophoresis

Recently Dielectrophoresis have gained a vast interest due to the better control using electric field in a lab-on-chip. Dielectrophoresis is revived due to its potential to manipulate micro particles, nanoparticle and cells. The process occurs when a polarized is suspended in a non-uniform electric field. The electric field polarizes the particle and the poles then experience a force along the field lines, which can be either attractive or repulsive according to the orientation on the dipole. Since the field is non-uniform, the pole experiencing the greatest electric field will dominate over the other, and the particle will move. Dielectrophoresis can be use to manipulate, transport, separate and sort different types of particles. Since biological cells have dielectric properties. Dielectrophoresis has many medical applications. Prototypes that separate cancer cells from healthy cells have been made. Platelets have been separated from whole blood with a DEP-activated cell sorter. Dielectrophoresis can be used to manipulate, transport, separate and sort different types of particles. DEP is being applied in fields such as medical diagnostics, Drug discovery, Cell therapeutics, and Particle filtration.
Dielectrophoresis are implemented using electrodes where an electric field lines are created using electrodes. Generally the electrodes are active using voltage or optically using LASER.

Theory for Dielectrophoresis:

The simplest theoretical model is that of a homogeneous sphere surrounded by a conducting dielectric medium. For a homogeneous sphere of radius r and complex permittivity ε^*_p in a medium with complex permittivity ε^*_m the (time-averaged) DEP force is given by:

$$\langle F_{\mathrm{DEP}} \rangle = 2\pi r^3 \varepsilon_m \mathrm{Re}\left\{ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right\} \nabla \left| \vec{E}_{rms} \right|^2$$

The factor in curly brackets is known as the complex Clausius Mossotti function and contains all the frequency dependence of the DEP force. Where the particle consists of nested spheres the most common example of which is the approximation of a spherical cell composed of an

inner part (the cytoplasm) surrounded by an outer layer (the cell membrane) - then this can be represented by nested expressions for the shells and the way in which they interact, allowing the properties to be elucidated where there are sufficient parameters related to the number of unknowns being sought. For a more general field-aligned ellipsoid of radius r and length l with complex dielectric constant ε^*_p in a medium with complex dielectric constant ε^*_m the time-dependent Dielectrophoretic force is given by:

$$F_{\mathrm{DEP}} = \frac{\pi r^2 l}{3} \varepsilon_m \mathrm{Re}\left\{\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_m^*}\right\} \nabla \left|\vec{E}\right|^2$$

A COMSOL simulated model has been presented in the report for focusing of dielectric particle in an electrode design. Where, spherical latex particles of diameter of 6(um) and has a density of 1050(kg/m^3) is pushed through the inlet of a chip with a velocity of 1(mm/s).

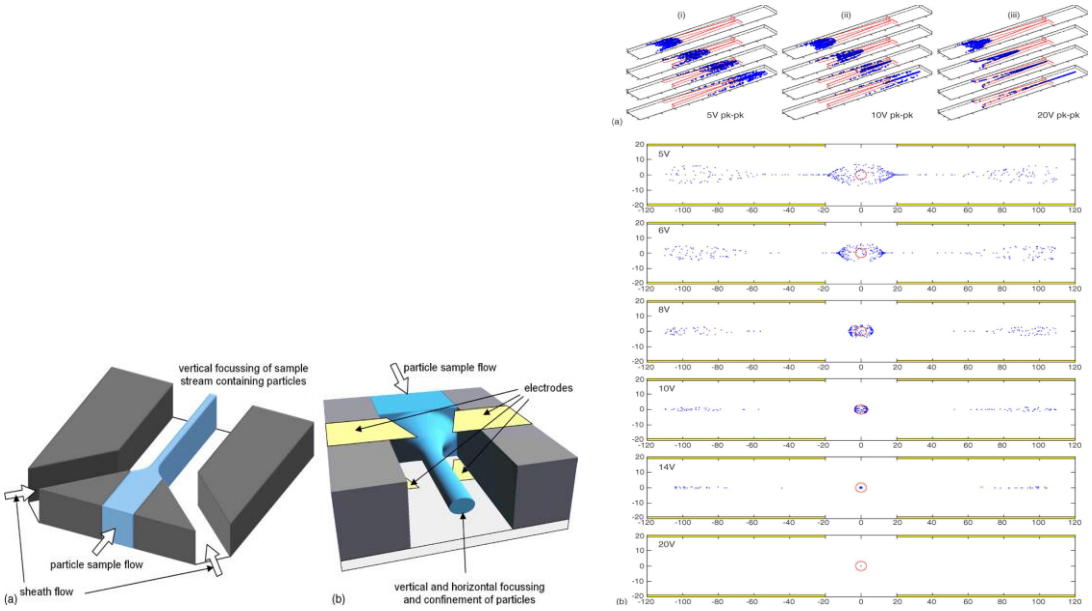

Fig 2: a) Chip design, b) particle focusing at different voltages.

Where, the relative permittivity of the fluid (water) 78, latex particle 2.55, conductive of fluid 1e-3(S/m), conductive of latex particle 8e-4(S/m), complex permittivity of particle obtained 2.55-1.438i & the complex permittivity of particle obtained was 78-1.7975i. Clausius mosotti factor calculated was -0.47547-0.012826i.

Few of the goals among other to discover of the current studies of cell electrophoresis should be concentrating on:

1) Effective way for studying cell surface, isolation from subpopulation and cell sorting process.
2) Simplification of the cell electrophoresis method to avoid the high cost, specialized and complex circuitry process.
3) Demonstration of new technique to achieve the old obtained result.
4) Opening the new window for micro channel electrophoresis since it requires a very low dilution of particle/cell.

The cell electrophoresis is less commonly applies, mainly because it requires complex, expensive and specialized equipment. In spite of this, cell electrophoresis has the considerable capacity for analytical and preparative applications in cell biology.

In the path towards the development step for a cell electrophoresis process, we need a spatially electric field (> 50-100 V/mm), a lab on chip process, fluid dynamics and to make it more cost effective. In a paper [5], it was mentioned that using a Pyroelectric crystal (Lithium Niobate) it was possible to generate electric field (typical 10kV/mm) under an electrode less configuration by only using a thermal source. It is possible to include micro fluidic chip, the electric field generated by pyroelectric process and detection technique for the cost effective and simple equipment for a cell electrophoresis.

PhD project:

To develop a lab-on-chip biosensor with an optical detection technique for a cell electrophoresis, using the facility available at IMM-CNR and University of Federico 2nd Naples, Italy.

The basic idea is to exploit the micro channel electrophoresis i.e. to design a microchip with a micro channel (PDMS microchip) and on the other side to fabricate an electrode-based design on the pyroelectric crystal for the generation of electric field and bonding them together. The advantage of having an electrode on a chip with a microfluidic channel is to help in detecting the particle not only using laser detection technique but also with microscope. The micro channel is a well-known microfluidic process where you can dilute the analyte of interest will a small dilution rate and can detect using micro-channel electrophoresis process.

An optical detection technique will be develop for the detection of the particle/ cells in the micro fluidic channel in the flow, using the optical fibre technique and laser in IMM-CNR. This will help us for better understanding the cell surface in 3D with or without fluid flow.

This process will be further continue with the thin film of pyroelectric material, this will help us to fabricate the complete device on a simple glass slide and to use it in further available applications.

PhD work carrying out over a period of 3 years:

Using the skills gained in past at IMM-CNR to Develop, designing and simulating the microchip process and obtaining an optimum recipe for the microchip device. Simultaneously working on the Pyroelectric thin films and understanding the characteristic property of the thin film on glass and silicon

Testing the chip on various cells & particles with /without microfluidic for the electrophoresis process. Simultaneously working on the optical detection technique and optimising it. Also, not forgetting the thin films where simulations and designs need to done for microchip.

Finally working, joining the parts and testing the complete system.

Reference:

1. Gascoyne, P.R.C., et al., Dielectrophoretic separation of cancer cells from blood. Ieee Transactions on Industry Applications, 1997. 33(3): p. 670-678
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4. Kaler, K.V., Jones, T.B. (1990) Biophysical Journal 57, 173-18
5. (gennari, et al., 2013)

Study plan 2014/ 2015

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Cycle XXIX

	Credits year 1							
		1	2	3	4	5	6	
	Estimated	bimonth	bimonth	bimonth	bimonth	bimonth	bimonth	Summary
Modules	18	**	**	**	**	3	1	**4**
Seminars	13	**	1	2	1	3	**	**7**
Research	34	7	7	7	7	9	7	**44**
	65	7	8	9	8	15	8	**55**

Activity list

Year	Lecture/Activity	Type	Credits	Certification	Notes	
1	Euro Progettazione	Ad hoc module	3	O		
1	**An introduction to the physics of nanostructures: phenomenology, applications and theoretical aspects**	Ad hoc module	4*	OX		
1	**Introduction to Photonics**	Ms Course	9*	OX		
1	**Three Core issues for the internet: things, security and sconimics**	AdHoc	2	O		
1	Seminar Title	Seminar	3	x	Hours	Date
	A lung-on-chip to measure oxygen affinity of single red blood cells	Seminar			4	11-Jun-14
1	**Computer generated Holograms**	Seminar			2	22-Jul-14
1	**Photochromic Materials; beyond the change of colour**	Seminar			2	23-Jul-14
1	**COMSOL: Structural Mechanics and Thermo fluid dynamics**	Workshp			8	28-Oct-14
1	**Luce e Futuro: giornata di studio**	seminar			8	01-Dec-14

1) Literature review and Using the skills gained in past at IMM-CNR to Develop, designing and simulating the microchip process and obtaining an optimum recipe for the microchip device.
2) Simultaneously working on the Pyroelectric thin films and understanding the characteristic property of the thin film on glass and silicon.