

Mini review

CELL ELECTROPHORESIS – A METHOD FOR CELL SEPARATION AND RESEARCH INTO CELL SURFACE PROPERTIES

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Abstract: In this paper, we discuss the application of various methods of cell electrophoresis in research into cell surface properties (analytical methods), and the separation of uniform cell subpopulations from cell mixtures (preparative methods). The emphasis is on the prospects of the development of simplified and versatile methodologies, i.e. microcapillary cell electrophoresis and horizontal cell electrophoresis under near-isopycnic conditions. New perspectives are considered on the use of analytical and preparative cell electrophoresis in research on cell differentiation, neoplastic transformation, cell-cell interactions and the biology of stem cells.

Key words: Cell electrophoresis, Cell separation, Cell surface

INTRODUCTION

Cell electrophoresis is a field-driven technique which serves two purposes: (i) to study the surface properties of cells and (ii) to separate uniform cell subpopulations from cell mixtures. A variety of electrophoretic methods are commonly used in biochemical laboratories for analytical and preparative investigations of the molecules of interest, including proteins and nucleic acids

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Abbreviations used: FACS – fluorescent activated cell sorter; FFE – free flow electrophoresis

[1-8]. Cell electrophoresis is less commonly applied, mainly because cell electrophoresis used to require complex, expensive and specialized equipment. In addition, the available methods were time-consuming and involved skillful manual operations [9]. In spite of the difficulties, thousands of papers show that cell electrophoresis has a considerable capacity as a method for analytical and preparative applications in cell biology. Extensive reviews of the literature on cell electrophoresis have been published [10, 11]. In this mini-review, we focus on the prospects for cell electrophoresis to become more commonly applied in research into contemporary problems of cell differentiation, neoplastic transformation, and the biology of stem cells. Attention is drawn to the need to improve and develop simpler cell electrophoresis methods than those available at present. The references given are examples, not an exhaustive list.

CELL ELECTROPHORESIS – A METHOD FOR RESEARCH INTO CELL SURFACE PROPERTIES

Microscopic methods in which single cell electrophoresis in a stationary layer of solution is directly measured under a microscope have proved very effective in the study of cell surface properties. Such research started in the 1930s and continues to this day [10-12]. These investigations contributed voluminous basic data and stimulated the contemporary study of cell surface properties. However, the methods are very time-consuming and require specialized equipment. In such methods, the electrophoresis of single cells is measured under a microscope. Measurements are carried out on cells remaining in the layer of solution which does not flow when an electrical field is applied to the system [13-15], i.e. those layers in which the electroendosmotic flow equals zero. This layer is called a stationary layer. Appropriate equations permit the localization of the position of the stationary layers in cylindrical, rectangular or two-channel electrophoresis chambers [15, 16]. The most significant results obtained with these methods include:

- i) a description of the differences in the electrokinetic potential between cells (for example red blood cells) from different animal species [3, 17, 18];
- ii) the discovery of differences in cell surface electric charges and electrophoretic mobility between various cell types, including cells of the immune system [11, 19];
- iii) the finding of differences between normal and pathological cells, including cancer cells [10, 19-28];
- iv) the identification of the changes in cell properties which accompany cell differentiation [29-32];
- v) a description of the modifications to the cell surface caused by a variety of factors which evoke changes in cell behaviour and functions [33-36];
- vi) the determination of cell function-related cell-specific changes in electrokinetic potential and electrophoretic mobility [37-41].

For example, it was shown that red blood cells from different animal species are characterized by species-specific electrophoretic mobilities [3, 11, 15, 42]. Different blood cells are characterized by distinct electrophoretic mobilities [18, 38, 43]. Cell neoplastic transformation is accompanied by changes in cell surface properties, detectable with cell electrophoresis, and these changes are additionally correlated with tumour malignancy [20, 25, 28, 44, 45]. Various pathological processes result in changes in surface properties and cell electrophoretic mobility. Additionally, cell differentiation leads to the modification of cell surface electrochemical properties [32]. Cell surface properties can be experimentally modified causing measurable changes in the electrokinetic potential at their surface, and changes in their electrophoretic mobilities.

Various strategies have been used to disclose and enhance the differences in the surface properties of observed cells. Treatment with neuraminidase showed the participation of N-acetyl neuramic acid carboxyl groups in electric charges on the surface of blood cells, and such treatment may enhance differences between regenerating liver cells and hepatoma cells [33, 34, 36]. Cell surface adsorption of polyelectrolytes (L-polylysine), lectins, or proteins and viruses was shown to specifically change cell electrophoretic mobility [37, 38, 46-50]. Changes in the medium pH or counter ion composition and concentration modified the electrophoretic mobility of various cells, increasing the differences in surface properties between these cells depending on the ionogenic groups on their surfaces [16, 18, 36, 50]. The application of microscopic methods in cell electrophoresis revealed the differences in the surfaces of bacteria species and strains that vary in their virulency [35, 39].

Thousands of papers have been published detailing the results of research carried out with analytical microscopic cell electrophoresis of bacterial, plant and animal cells [for review cf. 10, 11, 19, 26, 41, 42, 51, 52]. Numerous specialized symposia on the application of microscopic cell electrophoresis in biology and medicine have been organized and their proceedings published [26, 53]. Recently, analytical cell electrophoresis that had been carried out for years under the microscope had its potential expanded by the application of capillary cell electrophoresis [54-59]. This method was used to demonstrate the differences in the electrophoretic mobility of human red blood cells isolated from the blood of donors with different blood groups [60]. In this case, the electrophoresis of the cells caused the retardation of cell movement driven by the electroosmotic flow of the medium within the capillary towards the cathode (-). Capillary cell electrophoresis can be automatized, and further improvements are expected [56, 60-64].

CELL ELECTROPHORESIS AS A METHOD FOR SEPARATING CELLS FROM MIXED CELL POPULATIONS, ACCORDING TO THEIR PHYSICAL PROPERTIES (ELECTROKINETIC POTENTIAL, DIAMETER)

It was realized quite early that differences in cell electrophoretic mobilities can be used to separate cell subpopulations from heterogeneous cell mixtures. For over 40 years, attempts at the preparative electrophoresis of cells in different density gradients have been made, but never with fully satisfactory results, and always requiring complex equipment. Needing to ensure thermal stabilization of the system and prevent thermal convection, vertical preparative cell electrophoresis required the proper cooling and elaborate introduction and collection of cell samples [7, 9, 26, 53, 65-67], and thus did not succeed in becoming a commonly used technique. Vertical free-flow curtain electrophoresis (FFE), invented by Hannig, Heidrich and co-workers in the 1960s, was much more successful [9, 49, 53, 68, 69]. The equipment for this method was subsequently improved upon, and is commercially available. In numerous studies, Hannig and co-workers have demonstrated the great capacity of this method to separate and characterize a variety of cells and organelles. The FFE experiments univocally confirmed the potential of electrophoresis as a method to separate specific cell subpopulations from cell mixtures. With this method, the separation of T and B lymphocytes was achieved, as was the separation of cancer and normal cells, apoptotic cells, cells altered in a variety of pathological processes, and cells with modified surface properties [48, 55, 60, 66]. This method allows for the isolation of very clean (pure) fractions of cell organelles, for example lysosomes unloaded with triton [44, 72]. This fraction showed better purity than that obtained with centrifugation. More recently, commercially available equipment was shown to efficiently separate and clean proteins [9, 73]. The results obtained with the FFE method show the excellent prospects for using this method for cell sorting; unlike the more commonly used methods, it does not require any specific antibodies to isolate the desired cell types. The main limitations on its common application are the high price of the apparatus and the method's manual difficulty, associated with the small thickness of the separation chamber (less than 0.7 mm) and the number of tubes for fraction collection [9, 52, 53].

MODES OF ENHANCING DIFFERENCES IN CELL SURFACE PROPERTIES TO IMPROVE CELL SEPARATIONS

Research carried out with methods of microscopic cell electrophoresis exemplified the variety of possibilities of enhancing specific differences in cell electrophoretic mobilities between cell types. In Fig. 1, a few examples of the ways in which the differences can be specifically increased are shown. For example, red blood cells and leukocytes differ by about 20-30% in their electrophoretic mobilities under control conditions at neutral pH [10, 18, 35, 36, 74].

When electrophoresis is carried out at decreased pH (about 6.0), the difference can reach 50 or 60%, depending upon the type of leukocytes. The electrophoretic mobility of hepatoma cells differs by not more than 10% from the value for hepatocytes from the regenerating liver. After treatment with neuraminidase, hepatoma cell mobility is decreased by more than 70%, but normal hepatocyte mobility remains unchanged. Lectins, polymers, antibodies and dyes may specifically modify the electrophoretic mobilities of cell subpopulations intended for separation. It seems that such specific modifications can effectively improve and accelerate the separation of desired cell types from mixed populations of cells with methods based on cell electrophoresis.

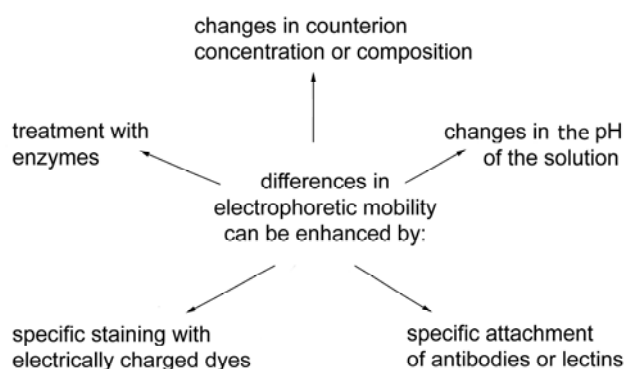


Fig. 1. Prospects for enhancing the differences in the electrophoretic mobility of cells for improved cell separation.

PROSPECTS FOR BROADER APPLICATION OF CELL ELECTROPHORESIS TO RESEARCH INTO CONTEMPORARY PROBLEMS OF CELL BIOLOGY

The heterogeneity of various cell populations is well documented [75-81]. Even in homogeneous populations, individual cells may differ in their cell cycle phases, and hence in cell activities, surface properties, enzyme activity and gene expression. A variety of types of lymphocytes, differing in functions, can be identified due to differences in cell surface antigens (CD-markers), and can be separated with a free-flow separator and the FACS (fluorescent activated cell sorter) method. Tissue cell lines cultured *in vitro* appear to be composed of a mixture of cell subpopulations, and depending upon the cell culture conditions, one or another prevails [76, 79-81]. Research on stem cells demonstrated that cells endowed with special capacities to divide and differentiate can represent a very small fraction of the cell populations in which they reside. All this creates the need for efficient methods of cell mixture separation. Excellent separation can be achieved with FACS if the separated cell subfraction can be marked with specific, fluorescently labeled antibodies. In other cases, such methods as cell elutriation, counter-current cell separation, or magnetic sorters can be used.

These methods are usually more efficient than those based on cell centrifugation or differences in cell adhesiveness. Nevertheless, even these less precise methods remain suitable for enriching cell samples in the desired cell type (e.g. isolation of crude fractions of blood cells enriched in a mixture of lymphocytes and monocytes on density gradients of Percoll or Ficoll solutions). However, cell electrophoresis has the potential for application in cell separation, as demonstrated by free-flow curtain electrophoresis [10, 82-85], and can more extensively complement the methods used to date. As already pointed out, in order to be more commonly applied, cell electrophoresis methods must be simplified and made user-friendly. The results with horizontal cell electrophoresis under near-isopycnic conditions and with capillary cell electrophoresis methods seem to be very promising [56, 61, 63, 86-88]. The correlation of research into cell surface electrochemical properties with research on a variety of cell functions and cell responses to extracellular factors that might be permitted with the use of these methods could verify many postulates and hypotheses.

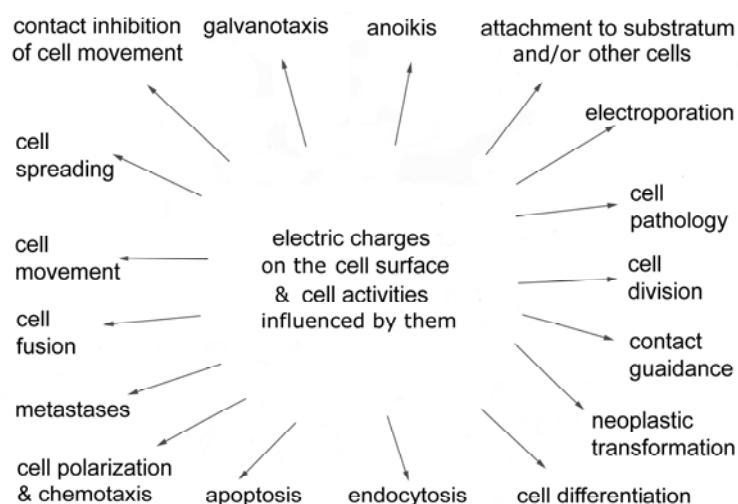


Fig. 2. The involvement of electric charges on the cell surface (which can be measured via cell electrophoresis) in some cell activities [89-94].

In Fig. 2, some examples are shown of the relationship between cell surface electric charge and electrokinetic potential and cell function, which have been postulated or already investigated with other methods. We hope that the development of simple and versatile electrophoretic methods for following and evaluating changes in cell surface electrochemical properties will result in the stimulation of further research and the reexamination of earlier postulated correlations (e.g. cell surface electric charge and galvanotaxis or electroporation).

NEW PERSPECTIVES OF ANALYTICAL AND PREPARATIVE CELL ELECTROPHORESIS

Capillary cell electrophoresis, in contrast to many other electrophoretic methods, permits very fast analysis of heterogeneous populations of cells, in the range of a few minutes instead of hours. The application of a strong electric field is possible due to the easy dispersion of Joule heat from narrow capillaries. The electrophoresis is used to modulate the passive movement of particles with an electroosmotic bulk flow of fluid, rather than to cause particle translocation. The capillary electrophoresis of cells can in future be applied for analytical purposes, but it is more limited as a preparative tool when a great amount of material needs to be separated [87, 88, 95, 96].

Recently, we described horizontal cell electrophoresis, in which cell sedimentation was reduced by near-isopycnic conditions. This permitted the separation of human and chicken red blood cells, which differ in their electrophoretic mobilities [86, 97]. A very important advantage of this method is its facility to perform simultaneous cell electrophoresis of a few samples in parallel. For the first time, this makes it feasible to directly compare electrophoretic mobilities and to separate experimental and control cell samples in one experiment. In addition, cell separation can be carried out under sterile conditions, and separated cell fractions can be collected and used for further analysis and experiments. Separation by electrophoresis does not change cell viability.

The development of horizontal analytical and preparative cell electrophoresis was based on results achieved with conventional methods of cell electrophoresis under a microscope and on free-flow curtain preparative electrophoresis. A few years ago, experiments carried out by others under "microgravity" conditions yielded very promising cell separations [98-100]. This turned our attention to problems related to cell sedimentation, the main cause of difficulties in the application of electrophoretic methods to cells, in contrast to the relatively straightforward electrophoresis used for the separation of ions and molecules. We concentrated on the prevention of cell sedimentation and gravity effects, and also on the thermal stabilization of the system.

The adaptation of horizontal cell electrophoresis under near-isopycnic conditions can allow fast and relatively easy separation of cell populations to subsets differing in cell surface properties, in electrophoretic cell mobility, or in cell diameter. The main advantage is that a few samples can be analyzed simultaneously in parallel. This permits easy comparison of modified or unknown cells (including pathological cells) with control or standard cells such as human red blood cells. The limitation of this method is associated with difficulty of measurement of the dielectric constant of the fluid in the inter zone between two liquid phases of different densities. The electrophoresis under such conditions can be used to determine the electrophoretic mobility of the cells, but it is difficult to calculate the zeta potential and surface charge densities. In spite

of the mentioned limitations, both capillary cell electrophoresis and horizontal electrophoresis under near-isopycnic conditions can facilitate research on cells and their separation.

Stabilization of the horizontal systems for cell electrophoresis requires further work, and the development of horizontal electrophoresis under near-isopycnic conditions. In this system, cell sedimentation is greatly reduced for at least a few hours by near-isopycnic conditions. Convection is tapered by the horizontal orientation of the separation chamber, and if it takes place, it occurs in a direction perpendicular to the direction of electrophoresis along a short distance of a few millimeters of the thickness of the solution in which cell electrophoresis occurs. Anticonvective conditions can be ensured by the additional insertion of anticonvective matrices made of non-adhesive materials [86]. If separated cells such as macrophages or cells with experimentally modified surfaces tend to adhere to the anticonvective matrices, a density "cushion" can be used to reduce convection and to assure near-isopycnic conditions.

CONCLUSION

In this review, we intended to turn the attention of cell biologists to methods of cell electrophoresis as complements to the more often used methods of cell separation such as flow cytometry, magnetic sorting, sedimentation (with centrifuges or at 1 g), and selective adhesion.

The goals of current studies of cell electrophoresis should concentrate on:

- i) further development of methods of cell electrophoresis for the analysis of cell surface properties (analytical cell electrophoresis), and for the effective separation and isolation of subpopulations of cells from cell mixtures (preparative cell electrophoresis);
- ii) simplification of the methods of analytical and preparative cell electrophoresis in order to avoid the requirement of specialized and expensive equipment, thus permitting broader application of cell electrophoresis in cell biology laboratories;
- iii) demonstration of the applicability of the new methods for the confirmation of earlier results achieved with microscopic cell electrophoresis and/or with free-flow curtain electrophoresis;
- iv) verification of the extensive applicability of cell electrophoretic methods to cell separations based not only on cell surface electrokinetic potential, but also on cell diameter, with these methods applied to separate small cell samples according to cell size as a complement to commonly used methods, e.g. cell elutriation, which needs a large volume of cell suspension;
- v) adaptation of electrophoresis under near-isopycnic conditions for the separation of cell organelles and macromolecules accompanied by an automatization of the method.

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