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Electrode-based AC electrokinetics of proteins: A mini-review

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ABSTRACT

Employing electric phenomena for the spatial manipulation of bioparticles from whole cells down to dissolved molecules has become a useful tool in biotechnology and analytics. AC electrokinetic effects like dielectrophoresis and AC electroosmosis are increasingly used to concentrate, separate and immobilize DNA and proteins. With the advance of photolithographical micro- and nanofabrication methods, novel or improved bioanalytical applications benefit from concentrating analytes, signal enhancement and locally controlled immobilization by AC electrokinetic effects. In this review of AC electrokinetics of proteins, the respective studies are classified according to their different electrode geometries: individual electrode pairs, interdigitated electrodes, quadrupole electrodes, and 3D configurations of electrode arrays. Known advantages and disadvantages of each layout are discussed.

Contents

1. Introduction

In biomedical sciences the application of direct current (DC) electric fields is widely established for, e.g., the characterization and spatial manipulation of cells and molecules by electrophoresis. Less widespread is the application of alternating current (AC) electric fields. Whilst the former interact only with objects carrying a net charge, AC fields in principle affect any matter. This is a consequence of their polarizing action, i.e. the separation of opposite charges, which are present in any atom and molecule. This results in induced dipoles, which then interact with the electric field. Besides this electronic polarization there exist a number of other polarization mechanisms depending on, e.g., the structure of the object and the field frequency [1]. The electric field can be employed either as a probe to gain information about the sample or as a tool to manipulate the sample. Information about macroscopic as well as molecular properties of tissues, cells, or biomolecules is accessed by dielectric spectroscopy where the frequency-dependent impedance is measured and evaluated [2,3]. An example for manipulation of the sample is the thermal impact of medical or laboratory microwave treatment. An important application of the forces that are exerted by AC fields, i.e. AC electrokinetics, is the spatial manipulation of cells and sub-microscopical objects [4,5]. Since the electrical impact differs between cell types and cell states, proper choice of frequency allows adaptation to the respective purpose. Lateral movement is used for concentrating and sorting cells [6,7], rotational movement is exploited for cell characterization [8] and tomography [9], and elongated cells are oriented by AC electrical alignment [10].

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AC electrokinetics as opposed to DC electrokinetics offers the advantage of exploiting frequency-dependent effects, which comprise attraction or repulsion of particles in positive or negative dielectrophoresis (pDEP or nDEP), respectively. Furthermore, the balance of interplaying effects like, e.g., dielectrophoresis (DEP) and AC electroosmosis (ACEO) can be shifted towards the desired effect. Moreover, electrolysis of water and electrophoresis of charged particles or proteins, which are usually unwanted side-effects, are avoided in AC fields. Comprehensive overviews of AC electrokinetic effects are given in detailed monographies by Jones [11], Hughes [12], Morgan and Green [13], Chang and Yeo [14], and by Nili and Green [15]. An important prerequisite for the progress in the spatial manipulation of cells has been the availability of electrode microstructures through lithographical techniques with typical electrode dimensions in the size range of a cell. Owing to the advances in photolithography and direct electron-beam writing ever smaller electrodes are available reaching now molecular dimensions [16,17]. Although the fabrication of nanometer sized electrodes appears rather expensive on the first sight by, e.g., electron-beam lithography, artful technologies have been developed that allow a well-priced production of both electrodes and insulating constrictions by optical lithography. This has prepared the way for selective action on biomolecules in solution.

In many AC electrokinetic experimental setups, mainly DEP is held responsible for particle movement. The dielectrophoretic force is directly dependent on the gradient of the electric field squared. It also depends on the particle volume (i.e. the third power of the particle radius), which is why the decreasing force acting on small particles like proteins has to be compensated by increased electric field gradients. High voltages, however, are generally less favorable because they promote side reactions like electrolysis of water. The first report on AC electrokinetics of a biomolecule stems from Washizu and Kurosawa, who stretched and aligned λ-DNA molecules in solution between planar electrodes [18]. Since then this combined action of gradient forces (DEP) and aligning torques has been exploited for, e.g., the visual investigation of RNA-polymerase action [19], for chromatography [20], and for the determination of DNA electrical conductivity [21]. DEP of proteins in solution has been demonstrated first by Washizu et al. [22] and then, with some years delay, by Bakewell et al. [23], and Kawabata and Washizu [24]. Whilst these studies utilized flat metal electrodes to produce the strong electric field gradients needed for molecular DEP, Nakano et al. [25] placed insulating posts into the protein solution between macroscopic electrodes. The resulting constrictions between neighboring posts distort the electric field leading to gradient forces.

Meanwhile, a number of reviews have been published dealing with DEP in general and including protein DEP. A recent article by Pethig summarizes the existing literature on DEP of cells down to molecular DEP, and provides perspectives for future applications [26]. Kuzyk gives an overview of DEP of metal and semiconducting nanoparticles and nanowires, graphene, DNA molecules and DNA origami structures [27]. Two articles discuss biomolecules, focused mostly on DNA but including also proteins [28,29]. Three reviews cover protein DEP, both insulator- and electrode-based, discussing the pros and cons of the different strategies [30,31,32]. The existing literature on AC electrokinetic experiments with proteins includes much larger protein assemblies like actin filaments or peptide nanotubes, whose dimensions often amount to several micrometers in length. The dielectric properties are assumed to be comparable to each other. Additionally, there are studies involving antibody-decorated beads [33,34] or probe–target–enhancer conjugates, e.g. biotin as the target, fluorescence-labeled antibiotin antibody (IgG, 160 kDa) as the probe, and DNA as the enhancer [24]. True molecular protein DEP, however, as opposed to streaming effects that drag along proteins, which is often the case in insulator-based DEP, still seems to be scarce. This mini-review is aimed at understanding the challenges of protein and polypeptide AC electrokinetics with a focus on the dielectrophoretic force. As the type of occurring phenomena depends more on electrode architecture than on the sample choice, this review is structured according to the different electrode types used in each study.

2. Electrode types used for protein AC electrokinetics

There are basically four different types of electrodes that are used for protein DEP (see Fig. 1). a) The first one is a simple pair of planar electrodes, where the electric field gradient that is necessary for dielectrophoretic action is generated at the adjacent edges of the two opposing electrodes (as a consequence of the electrodes' thinness). Often, electrode pairs with sharp tips are used to increase the electric field gradient. b) Interdigitated electrodes (IDE) follow the same principle as individual electrode pairs. They consist of two electrodes with interdigitated electrode fingers, thereby multiplying the number of adjacent edges, which are the regions of dielectrophoretic accumulation. Since this geometry allows covering a large area whilst keeping a high field gradient, these electrodes are quite common in AC electrokinetic experiments. c) The quadrupole electrode arrangement was presumably derived from setups for electrorotation experiments [8]. It comprises four planar electrodes that are arranged with circular symmetry. For AC electrokinetic experiments the two diagonally opposed electrodes are set to the same potential, respectively. d) The fourth electrode type has a 3D geometry, where the counter electrode is not located in the same plane. It consists of an array of electrodes that are set to the same potential and one flat counter electrode mounted in some distance. In contrast to electrode types a)–c), where the electric field has a large component parallel to the electrode plane, this 3D electrode type is characterized by the electric field being mostly built up orthogonally to the substrate.

2.1. Individual electrode pairs

Planar microelectrodes can easily be fabricated by photolithography, and as a consequence they are fairly common for AC electrokinetic experiments, especially for experiments with objects whose dimensions amount to the micrometer range. Self-assembled amyloid peptide nanotubes were aligned and immobilized on gold microelectrodes by AC electric fields [35]. These nanotubes are built up by diphenylalanines, and they have an internal diameter of 20 nm, an external diameter of 80 nm or more, and a variable length of several micrometers. Individual nanotubes as well as bundles were immobilized. A tight connection to the electrodes after immobilization allowed their electrical behavior to be measured, which yielded a very low conductivity as was expected for such a bionanostructure of peptides.

Another tubular structure is formed from polymers of the proteins α- and β-tubulin. These microtubules have an inner diameter of 12 nm and an outer diameter of 24 nm. The structures are dynamic and the tubule length can reach approximately 50 μm. Microtubules were aligned and accumulated at the edges of chrome microelectrodes in order to study kinesin motility and to establish an in-vitro assay for the investigation of microtubule dynamics and of their function in mitosis [36]. The experimental protocol for the formation of these microtubules was later extended to mimic mitotic spindles in order to investigate the dynamics of microtubule binding proteins [37].

An important problem in bionanotechnology is the assembly of nanometer sized objects into micrometer structures in a properly controlled manner. A possible approach is to exploit solutions that already exist in nature. A candidate for self-assembly are collagen fibers with a diameter of 1.5 nm and about 300 nm length. The protein building blocks, i.e. the collagen monomers, were attracted towards gold microelectrode tips by application of an AC electrokinetic field [38]. Raman measurements during trapping confirmed protein accumulation in the electrode gap. As a result of the higher protein concentration, self-assembly was triggered, and growth of the nanofibers was investigated with an AFM.