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Chapter 7

Cell Concentration Systems for Enhanced Biosensor Sensitivity

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Additional information is available at the end of the chapter

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Abstract

Concentration processes for analytical systems based on different types of biosensors are very important for many applications. The sample conditioning is oriented to enhance the sensitivity or directly to make the detection or analysis possible. Processes that may be used for concentration and conditioning of original samples are very diverse, depending on applications that may range from clinical diagnostics to industrial processes control, and there are different strategies to achieve the final goal.

This chapter presents an overview of the most important and relevant microscale techniques to produce an effective separation and concentration of cells, mostly bacterial cells. The main focus is put on the separation mechanisms as a tool for biosensing toward the development of complete devices in a lab-on-a-chip format by integrating the concentration, sample conditioning, and detection subsystems.

Keywords: Cell concentration, microfluidics, hydrodynamics, electrophoresis, dielectrophoresis

1. Introduction

The sensitivity and the detection limit of biosensors have natural limitations. It is possible to enhance these analytical parameters and the performance of the system by increasing cell concentration, reducing the sample volume in a preconcentration stage. The utilization of any

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concentration technique is crucial for the diagnostic and detection of particular cells [1–3] or metabolites [4] in clinical, food, or water environment [5].

Along with sensitivity and detection limits, another important aspect of any detection method is related to the signal-to-noise ratio and false positives, which determine the specificity of the system. This defines what percentage of the measured signal is related to our specific bacteria of interest. To enhance this signal-to-noise ratio, it is useful to apply a selective preconcentration methodology adding some specificity to detect only the cell of interest. The most selective methods to isolate specific bacteria from the complex mixture presenting a real sample are immunological systems based on the utilization of specific markers or identifiers.

Cell detection for clinical diagnostics sometimes requires detection of low abundance cells circulating in complex samples such as blood, e.g., specific detection of cancer cells from tissue or determination of small quantities of cells in noncellular samples as urine. These are only some examples of different types of bioanalysis that require the preconcentration of samples [1, 3].

Bacteria detection, especially in environments affecting the public health risks, such as food, water, and clinical, requires an efficient concentration process to guarantee low detection limits corresponding to official law regulations. This means that the objective sometimes implies the detection of few bacteria in large water volumes. The best option to solve this problem and improve detection limits of a biosensor detection method is the sample treatment by retaining all bacteria present in the sample, decreasing its volume and, in this way, increasing the concentration of bacterial cells [1].

This section will mainly be focused on the concentration of cells for biosensor applications, but we will try to present a complete view of the physical and chemical phenomena that could be potentially used for this purpose. We will provide important details of each evaluated methodology focusing on the main characteristics of the concentration system effectiveness: the capacity of sample volume processing, the velocity of processing or extraction time, and the recovery, concentration, purity, and detection factor.

The modern appeal for miniaturization requires the use of microtechnologies for sensor fabrication as well as the application of microfluidic systems. These may be regarded as crucial tools for the integration of concentration steps in sensor-based bioanalytical systems [6]. It is clear that speaking about large volumes may cause confusion in terms of relative volume amounts. In this chapter, we will focus on those concentration systems that could process volumes up to 60 mL.

The main concept of concentration is the separation. Thus, many different techniques based on various physical principles (electric, magnetic, gravitational, acoustic, hydrodynamic, affinity chemistry, etc.) have been proposed for cell and protein separation within microfluidic devices [6]. From the point of view of the flow conditions, the preconcentration techniques on chip can be classified as static and dynamic [4]. The static techniques are those used to stop or trap cells, blocking them in particular places[7], while dynamic techniques are those that separate them from the main carrier flow to lateral streams by focusing or deviating the cells, bringing them to specific places under continuous flow conditions[8].

The most interesting static concentrators are those that trap the cells at particular places where the sensing would be carried out. These systems enhance the sensitivity of the biosensor technique, in this case, the detection limit, as they perform the measurements in a volume limited by the sensor area. The dynamic concentration techniques are very interesting for their use in sequential systems or devices, build up in a modular architectural design [9].

However, along with certain advantages, both methodologies possess some drawbacks, mainly due to the relationship between the trapping forces and the dragging forces of the fluid movement. This fact imposes limitations in terms of velocity and sample processing capacity as well as proper characteristics such as efficiency, purity, and recovery factor. These limitations may be improved by using large channel dimensions to generate relatively low flow velocities, which implies high dead volumes and a low concentration ratio (samples/volume unit). Thus, it is necessary to find a compromise between design limitations and concentration volume requirements for a measurement or analysis.

In this chapter, from a general point of view, we present the main methods with the objectives to show the most interesting technical solutions and to give some numbers characterizing their performances in order to give an effective perspective in this field.

2. Filtration-based processes

Fibrous filters are routinely used in laboratory protocols for filtration of water and wastewater samples. Typical wastewater filtration methods include the use of paper filters or glass fiber filters as a mechanical trapping surface. After the trapping process, particle recovery should be performed by diffusion, interception, gravitational settling, or impaction [10].

These mechanical filtration methods remain the most easy, cheap, and effective for very large volumes. In particular, the tangential or cross-flow filtration has demonstrated a good performance for volumes over 200 mL [11]. However, they present several drawbacks if its integration into microfluidic devices is required.

Nevertheless, a filter-based bacteria preconcentrator embedded in a microfluidic system with a miniaturized sensor based on electrical impedance spectroscopy method was presented by Jiang and coworkers [12]. The design of a multistage filtering was comprised by the first layer of a silicon chip having a large array of holes with a diameter of 10 μm and the second layer of a nanoporous filter paper with submicron pore size. This direct integration of a paper filter is very interesting since the system is able to process 60 mL *Escherichia coli* samples with a detection limit of 10^2 cell/mL.

A different perspective in terms of the reutilization of the system and release capacity represents the system reported by Bao and Lu [13]. They created an array of microscale beads by pinching the polydimethylsiloxane (PDMS) channel using a pneumatic valve, which allows the formation of a conglomeration of beads that are used as a filter. The device was tested using *E. coli* cells, showing an increase in the concentration of two orders of magnitude.

Therefore, different filtration-based concentration methods may be used for the detection of low concentration cells. However, as it has been said, their integration in miniaturized systems for the sensor fabrication is still a challenge.

3. Specific interaction of cells with functionalized surfaces or particles

This approach is based on the adsorption or binding of the microbial cells to a specific material surfaces treated with special reagents. The main idea is to generate large surfaces with affinity to certain bacteria cells or class of bacteria. Basically, this methodology is considered as a static concentration process; however, the utilization of beads may be performed in dynamic processes.

3.1. Treated surfaces

This concentration concept is fully applicable to sequential systems in which trapped cells are released from the surface and resuspend in a smaller volume giving sufficient concentration to be addressed by the detection system. In this kind of concentration process, it is important to take into account the relationship between fluidic phenomena considerations and the surface reactivity, in terms of its capability to capture and retain the target cells. This technique may be modeled [14], studying the capacity of the system to transport cells or bacteria in the sample solution to the treated surface (k_0) and the probability that an association event will occur during the time that the target cells are in proximity of the surface with capture molecules. Another critical point is the relationship between the adhesion force (F_A) experienced by the target cell at the treated surface and the shear force (F_S) generated by the flow stream that is tangentially directed trying to tear off the cell.

Cells can be trapped at particular places using different specific and nonspecific affinities. Specific cell surface binding exploits selective interactions between a ligand and a target-specific receptor, for example, a membrane protein. In this case, antigen/antibody is the most extended strategy, although they present certain disadvantages such as difficulty of surface immobilization or instability. To overcome these problems, new strategies have been proposed [1], which make use of aptamers, peptides, or toxins to selectively bind and concentrate a specific microorganism or cell.

A very important application of this separation technique is aimed on the separation and concentration of rare cells or low concentration samples. Thus, we may find many different options of isolation of circulating tumor cells (CTCs) from blood [15–21]. For example, Nagrath and colleagues [15] presented in 2007 a device with 78,000 microposts coated with EpCAM antibody, increasing the interaction surface to 970 mm^2. The EpCAM antibody was demonstrated to trap different cells, such as lung, breast, prostate, and bladder cancer cells, in a whole blood. The optimization of the microfluidic design and micropost distribution permitted to minimize the shear stress at flow rates of 1–2 mL/h. This system permits to process maximum sample volumes of 5.1 mL, reaching a best capture of the 65% of the target cells (meaning ≈65 cells/mL) with a final sample containing around 50% of CTC along with blood cells. The

micropost array, forming obstacles for the cells within the flow, may be further optimized in terms of the particular post diameter and distances between posts to enhance the collision probability of cells with particular sizes, thus enhancing the purity of the concentration up to 90% [18, 21, 22].

These capture concepts also require the development of the cell release method to recover thus immobilized cells for detection. The most relevant systems use digestive enzymes like trypsin to break the bond between the ligands and the surface [15, 18, 21] or biopolymers to reduce the possibility of damaging the recovered cells [23]. Adams et al. [16] presented an integrated system where the cells are trapped and released to perform the sample concentration. The following measurement of recovered cells was carried out by a conductivity sensor.

Another interesting approach is the concentration of cells by reactive binding directly on a sensor surface. Recently, Souiri et al. [24] studied and characterized the immobilization of antibodies on an indium-tin oxide (ITO) electrode and the capture of *Legionella pneumophila*. The immobilization of the specific antibody for *L. pneumophila* was carried out by a covalent binding to a silane self-assembled monolayer (SAM). Surface characterization by AFM, confocal microscopy, and impedance spectroscopy demonstrated the deposition of different layers at the ITO surface used as an impedimetric electrode sensor for characterization. Dulay and colleagues [25] also used this silane SAM technique with a specific antibody for an electrochemical ELISA detection of *Francisella tularensis*.

Surface chemistry and different physicochemical factors, such as hydrophobicity, hydrophilicity, steric hindrance, or roughness between others, play an important role in bacterial adhesion to the surfaces [26]. Although the major part of the research in the bacterial adhesion to solid surfaces is aimed on the prevention of biofilm formation, their results may be useful for an opposite strategy [27, 28].

3.2. Treated magnetic particles

A particular case of the implementation of functionalized surfaces is the utilization of microbeads modified with antibodies to recognize certain groups of bacteria. This is a good strategy to increase the interaction surface. Besides, it offers an easy way for the manipulation of the captured cells. Thus, concentrated samples may be carried by a flow, fixing the beads in particular places or deflecting them under flow conditions by different forces.

In particular, preconcentration techniques with magnetic particles are very popular in microfluidic devices for biological applications, implementing and integrating on a chip normal bench protocols to include high specificity attributed to the utilization of immunological interaction. The use of magnetic beads is very widespread due to the facility of separation and concentration by using a magnet. The utilization of this technique was implemented in a system to extract *E. coli* from such complex samples as soil [29].

Verbarg et al. [30] presented a very promising spinning magnetic trap for automated assay systems, which gives the possibility to perform a simple protocol assays typical for any laboratory that includes catch, clean, and release steps. The system called "MagTrap" consists of a mobile disk with magnetic rods disposed radially forming rotating magnet arrays that

permit to entrap or to move the magnetic beads within the channel during processing. They demonstrated the capabilities of the system presenting a 98% of capture efficiency with a release capacity of 80%. The experiment was carried out processing from 0.1 to 1 mL sample volumes with a flow rate of 10 μL/min. The system was used for the detection of *E. coli*, *Salmonella*, *Listeria*, and *Shigella* integrated with flow microcytometer [31].

In terms of sensor sensitivity, magnetic particles may be used to increase the interaction between the sample and the sensing transducer, enhancing the sensitivity to detection levels of around 10 cfu/mL [32–34].

Some interesting examples may be presented concerning the use of microbeads for direct concentration and posterior off-chip detection. Beyor et al. [35] presented an immobilized bed of magnetic beads functionalized with specific antibodies. They processed 10, 20, and 100 μL samples with different concentrations of *E. coli* with a mean flow rate of 0.24 μL/min. Analysis was performed by using off-chip polymerase reaction and capillary electrophoresis to evaluate the trapping efficiency. The system presents a capacity of 70% capture yield from a pure sample of 10^3 *E. coli* in 100 μL PBS, while a 40% capture efficiency was achieved in a complex mixture of *E. coli* and *S. aureus* in a 1:100 ratio.

Recently, Lee and colleagues [36] presented a new device that implements the same idea of the surface coated with magnetic beads. They used specific geometry of magnets and nano-magnetic beads of around 150 nm functionalized with specific antibodies for *Salmonella* to trap bacteria in a large volume container (see Figure 1). The release of particles was implemented by removing the magnets and then flushing the particles with the captured bacteria. With this system, a complete assay of 10 mL of a real food sample may be accomplished in less than 3 min, under flow rates of 25 mL/min. The concentrated sample of released cells is then analyzed by ATP luminescence measurements, demonstrating a detection limit of 10 cfu/mL.

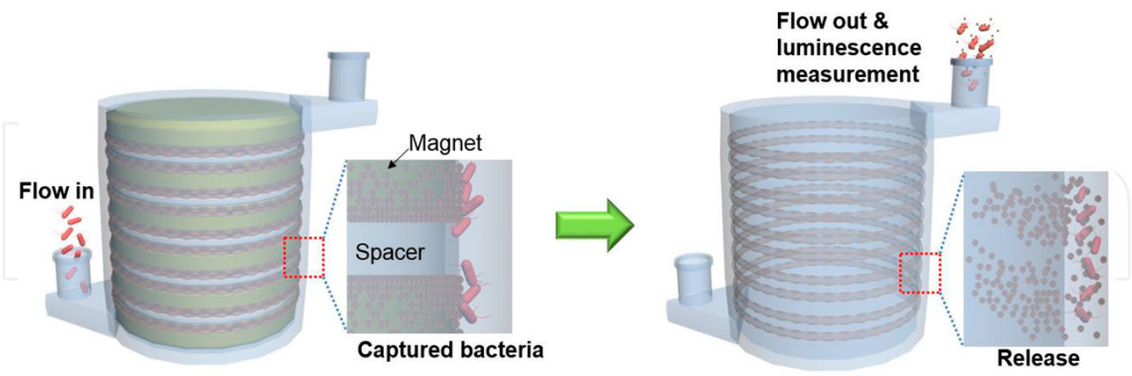

Figure 1. Schematic illustrations of 3D immunomagnetic flow assay. The magnet-spacer assembly was placed in the center opening during the capture and rinsing of the magnetic particles and the antibody–Salmonella complexes, which were trapped. After removing the magnet assembly from the center opening, the concentrated AbMNC–Salmonella solution was collected using a disposable syringe. Reprinted with permission from Lee et al. [36]. Copyright 2014, American Chemical Society.

As mentioned at the beginning of the section, trapping processes have to take into account dragging forces. With some devices, it is required to reduce the flow rates below 0.1 mL/min

[31, 35] to allow effective reaction binding, which increases the time needed to process the sample volume. However, the device presented by Lee and colleagues [36] is able to perform at high flow rates of 25 mL/min, but it presents a dead volume (≈3 mL) comparable to the initial sample volume(10 mL). Thus, working with microfluidic systems, it is necessary to consider possible dead volumes of devices, which may affect the final cell concentration even for systems with high trapping coefficients.

Most of the cited works employ immunochemical methods for cell entrapment. However, in the case of bacteria concentration, we think that this entrapment concept should be extended more, focusing on different bacteria cell capture strategies involving hydrophobic interactions that are related to the adhesion involved in microbial infections [37], biofilm formation of specific species in the presence of calcium ions [38],bacteria interactions with some known polysaccharides [39], or antibacterial peptides [40, 41].

4. Electro capture

This process is based on the capture of charged or polarizable cells or other micro-objects due to the interaction of the material with the external electric field. These phenomena generally depend on the frequency and strength of the electric field. Some bacteria are typically charged due to the presence of ionizable groups in its outer membrane and also are polarizable under an electric field. Within electro capture-based methods, we may distinguish two different groups of techniques: electrophoresis and dielectrophoresis, which can be used to perform static or dynamic separations.

4.1. Electrophoresis

Electrophoresis is defined as the motion of a charged particle within a uniform electric field. Due to such electric field of magnitude E, a particle with a charge Q will experience a Coulomb force.

Some bacteria have a net negative charge on the cell wall, although the magnitude of this charge varies from strains depending on the molecular structure of their membranes as well as on the pH of the medium [42, 43].

The velocity of a particle under applied electric field force is defined by its intrinsic mobility in a given buffer solution. This specific mobility of a particle (μ_e) could be expressed by

$$\mu_e = \frac{v}{E} \propto \frac{\xi}{\eta}, \tag{1}$$

where v is the linear velocity of the particle and E is the electric field applied. The mobility of the particle is then independent of the electric field applied and is proportional to the zeta potential (ξ) (which depends on the ionic strength of the fluid) and on the medium dynamic viscosity (η).

The static electrophoretic devices retain the particles at particular places due to the specific net charges on the surface of some bacteria. For example, Balasubramanian et al. [44] used two glass slides covered with chromium (15 nm) and gold (35 nm) with a spacer to create a microcontainer 60 mm wide, 40 mm long, and 150 μm high, for static concentration. The glass covers work as big electrodes where the bacteria may be attached depending on their net charge. A water sample with a concentration of 10^6 cfu per mL is injected into the microcontainer passing during few minutes at a very low flow rate (2–6 mL/h) while applying 1 V between the electrodes. The efficiency of the trapping container, which was measured with a negative control in agar plates, resulted in 95% of capture efficiency for *Salmonella*, *E. coli*, and *Pseudomonas* under different flow rates.

An important improvement of this idea was presented by Podszun and colleagues [45], who developed a more complex device for free-flow electrophoresis adapted for bacteria enrichment of *Enterococcus faecalis*. The system incorporated a gel container where the bacteria may be trapped and released by electrophoretic control. With this device, Podszun presented trapping efficiencies of 80% and a concentration factor of 25 over 200 μL sample. The maximum flow rate achieved was 6 μL/min.

This electrical separation concept permits to perform trapping and release steps, crucial for a concentration process. However, it presents important drawbacks related to the necessity of high voltages (>1 V) that may induce electrolysis, high temperatures associated with the current pass, and cell damage. Also, the compromise between trapping forces and flow rates should be achieved.

4.2. Nanogap: Exclusion-Enrichment Effect (EEE)

The generation of exclusion-enrichment zone effect by using nanogaps or nanopores to generate a trap for charged particles has been demonstrated to be a very promising tool for analytes static concentration [4]. The translation of this method to the preconcentration of charged particles or cells may be regarded as a good option for biosensing.

The exclusion-enrichment effect is produced when an electric field is applied along a nanochannel. Figure 2 represents a simplified scheme of this effect. If dimensions of the nanochannel are comparable to the Debye length of the electrical double layer formed at the nanochannel walls—electrolyte solution interface, then an overlap in electrical double layers occurs, resulting in a higher concentration of positive ions (for a negatively charged surface) inside the nanochannel. This effect produces unbalanced ionic flows that provide ionic enrichment at the cathode side of the nanochannel, resulting in formation of an ionic exclusion zone at the anode side [46].

Wang and colleagues [47] recently presented a preconcentrator microsystem consisting of two microchannels connected by 205 nm high nanochannels fabricated by electric breakdown of a 25-μm-thick polydimethylsiloxane (PDMS) membrane using high electric shock. Figure 2 shows the final concentration of *E. coli* produced by the ionic-enrichment effect and the electroosmotic flow. The detection of the concentrated bacteria was performed by fluorescence measurements. Using this methodology, they estimated to reach a 10^4-fold concentration factor

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within a few minutes. The main drawback of this method is similar to the classical electrophoresis, as it also requires high voltages. Even so, the capture capacity of this method seems very promising and useful.

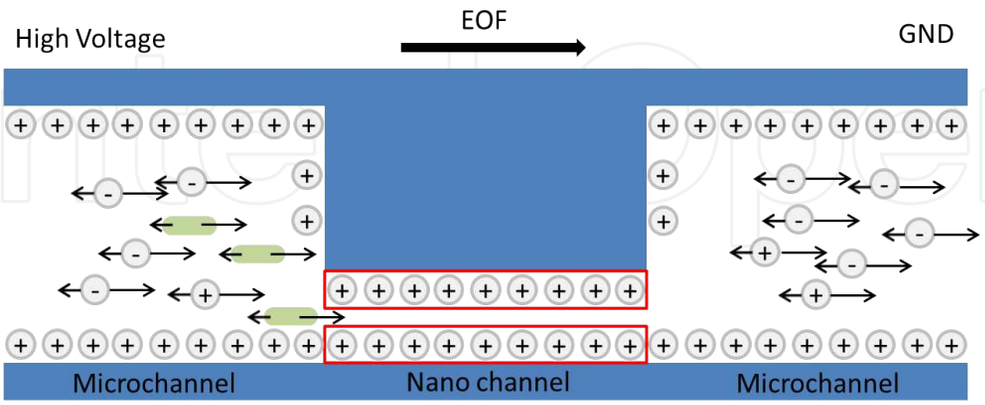

Figure 2. Shows a scheme of the distribution of charges inside and outside the nanochannel. The green ellipsoids indicate the concentration of *E. coli* cells at the cathode side.

4.3. Dielectrophoresis

Dielectrophoresis uses the effect of electrical polarization of particles under the influence of nonuniform electric fields to induce a translational motion. The inner elements of the particle under the influence of an electric field, together with the free charges and surrounding media, are polarized, forming an induced dipole. The positive and the negative extremes of the dipole are influenced by forces of different magnitude because of the nonuniformity of the electric field. These forces depend on the strength and frequency of the applied field, as well as on the conductivity of the supporting electrolyte. Thus, cells can be polarized in a highly conductive electrolyte suspension [48, 49] or in diluted solutions.

The dielectrophoretic (DEP) model for cells is normally described by a spherical particle approach [50], and the DEP force, F_{DEP}, may expressed by

$$F_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \operatorname{Re}\left[K(\omega)\right] \nabla \left|E_{rms}\right|^2, \tag{2}$$

where r is the radius of the particle, $\varepsilon_0 \varepsilon_m$ are the permittivities of the free space and suspending medium, and E_{rms} is the root-mean-square electric field. The factor $K_{(\omega)}$ is the Clausius–Mossotti factor (CM), which depends on the relationship between the particle and the medium complex permittivity, that is,

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \tag{3}$$

where ε^* is the dielectric complex permittivity, for the particle and medium, expressed by

$$\varepsilon^* = \varepsilon_0 \varepsilon - \frac{j\sigma}{\omega}, \tag{4}$$

where j is (√-1), σ is the conductivity of particle or medium, and ω is the frequency in radians.

According to the simplified model proposed by Huang et al. in 1992 [51], cells can be presented using a simple spherical concentric multishell model, composed of different spheres, shells, contained from one to another with interfaces separating the different dielectric layers, as presented in Figure 3.

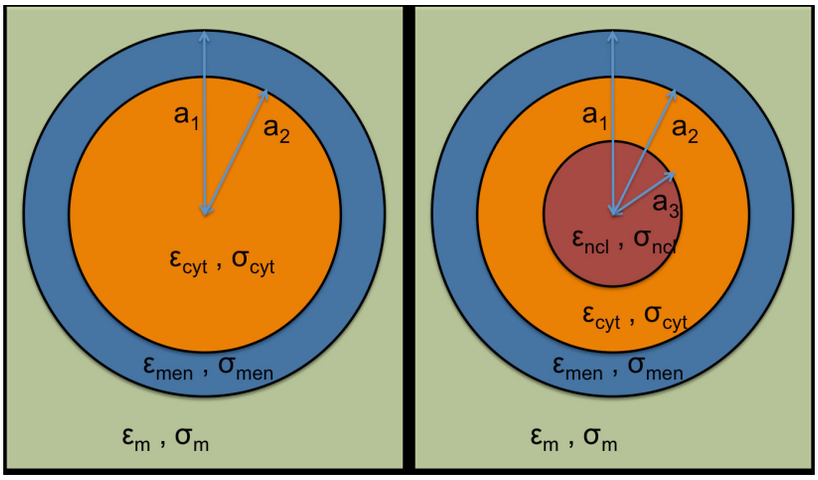

Figure 3. Schematic representation of the multishell model for a nonnucleated cell (left) and a nucleated cell (right). m indicates the medium; mem, cell membrane; cyt, cytoplasm; ncl, nucleus, a_1, a_2, and a_3, radius of the shells.

The complex permittivity of the cell is calculated by a geometrical relationship between shell geometries, and it defines the sign of the dielectrophoretic forces. When the factor is positive, the particles are attracted to the places where maximums of the electric field distribution appear, and we talk about positive dielectrophoresis (pDEP). On the other hand, when the CM is negative, the particles are repelled from the maximum of the electric field, and we talk about negative dielectrophoresis (nDEP). Figure 4 presents the evolution of the CM factor with the frequency of the applied electric field for a nonnucleated cell of 6-μm size with typical electric parameters [52] in different conductivity mediums. It is important to note the evolution of the pDEP with the solution conductivity, which disappears in high conductivity mediums. Moreover, the maximum value of CM factor for pDEP is over 0.8, whereas that for nDEP is –0.5, indicating that forces generated by negative DEP are weaker than positive DEP forces.

Despite that the bacteria multishell dielectrophoretic model [51] may be simplified to a sphere, the majority of bacteria have different morphological shapes, and it is necessary to modify the calculation of the CM factor taking into account their geometry, such as the ellipsoidal bacterial cells [53].

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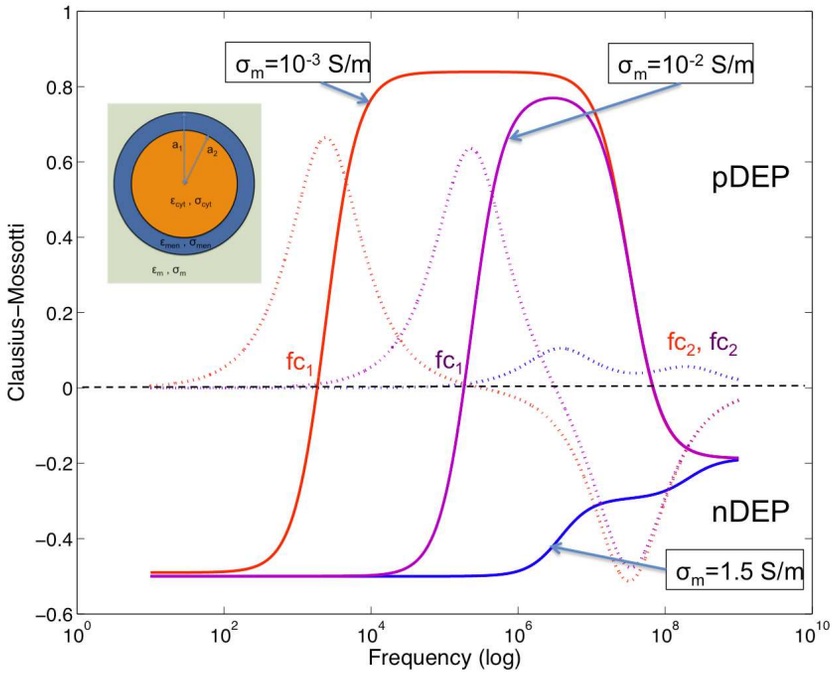

Figure 4. The solid lines represent the variation of the real part of the CM factor, and the dotted lines represent the evolution of the imaginary part of CM. The conductivity of the medium, σ_m, affects to the cutting frequencies that define the sign of the DEP force. fc1 indicates the transition from nDEP to pDEP, and fc2 indicates the transition from pDEP to nDEP. The color indicates the medium conductivity case. In high conductivity medium (blue line), the cell is under negative DEP for all frequencies.

As we said previously, a nonuniform electric field is required for produce a DEP effect. These nonuniform electric fields may be generated by electrodes of different geometrical arrangement, the most common being coplanar electrode arrays, in which the current distribution forms a maximum of electric field between the electrodes close to the electrode plain. This electrode configuration opens the possibility to exploit two different functions offered by an array of interdigitated electrodes (IDE), namely, simultaneous trapping and impedimetric detection. Ferrier et al. [54] implemented an nDEP system for yeast cells to elevate the cells over electrodes, measuring at the same time the capacitance changes associated to the cell distance from the electrodes. Dastider et al. [55] used pDEP for *E. coli* trapping to enhance impedance sensitivity and to lower detection limit down to 10^2 cfu/mL.

Another option for nonuniform electric field generation is the utilization of pore surfaces [56, 57]. Cho et al. [57] presented a system that implements a porous SU8 polymer membrane with the optimized pore shape designed to generate a maximum electric field gradient inside the pore. In this work, they optimized conditions for *E. coli* trapping in terms of frequency, media conductivity, and flow rates. The optimal parameters were found to be 300 kHz, 0.2 S/m, and flow rates below 50 μL/min. A complete concentration experiment performed with a 500-μL sample with a concentration of ≈9 × 10^3 cells/mL at 100 μL/min flow rate in 5 min consisting in capture and release of bacteria cells resulted in a trapping efficiency of more than 60% and a recovery rate of 93% in approximately 150 μL.

When it is required to work with physiological medium, normally highly conductive buffers, it is necessary to use negative DEP because, as it was shown earlier, the CM values under these medium conditions are negative for frequencies below 10^8 Hz. This implies certain limitations because the forces generated by the negative DEP are weaker than of the positive DEP [58].

A very interesting method of the *E. coli* deviation and concentration by negative DEP was implemented by Park and colleagues [59]. The complex fluidic system presented in Figure 5 involves simultaneous parallel laminar flow of two different solutions: a highly conductive sample volume (where the separation is carried out by nDEP) and a low conductive water solution (for trapping the cells by pDEP). The separation is carried out by deflecting the bacterial cells moving them from the sample stream to the low conductivity buffer stream, as it is shown in Figure 5(A). This buffer stream delivers cells to an IDE structure where the cells are trapped by a positive DEP (Figure 5(B)).

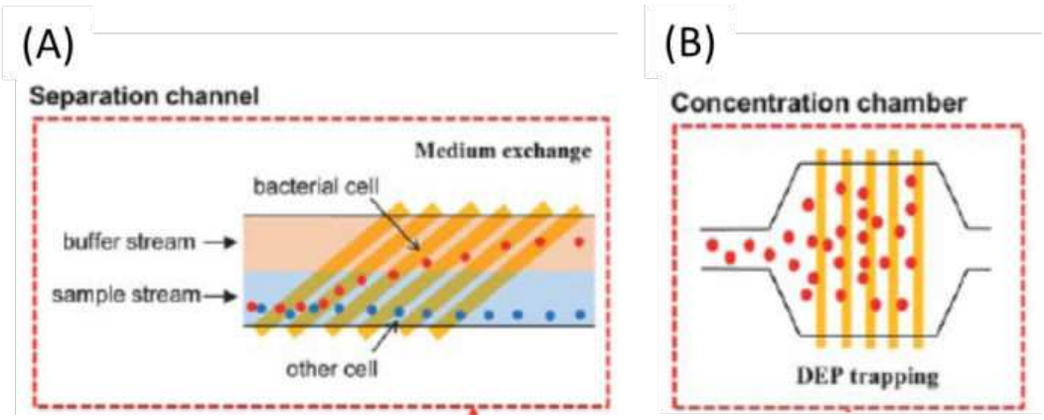

Figure 5. Scheme of the microfluidic device presented by Park et al. [59]. In scheme (A), the deviation of bacterial cells from the sample stream is represented, to be incorporated to the buffer stream that brings bacteria to the concentration chamber (B). Adapted from Park et al. [59], with permission of the Royal Society of Chemistry.

This method showed a separation efficiency of 95% at 800 μL/hand. The capture ratio in the final chamber with the volume of 0.5 μL was reported to be of 100%. The concentration ratios were unknown, and the measurements were performed by taking instant images that were analyzed by ImageJ (Image processing software). The flow rate of sample processing (≈13 μL/min) is quite low, but the importance of this system lies in the use of nDEP for separation process. The pDEP trapping permits to achieve separation at higher velocities, thanks to higher values of the CM factor as, for example, in the device presented by Cho and colleagues [57], with a retention capacity over 60% at flow rates 100 μL/min. These results were obtained by the positive DEP in a low conductivity buffer (0.2 S/m).

4.3.1. Combination of DEP with functionalized surfaces or particles for enhanced capture strategies

Dielectrophoresis is a commonly used technique for low abundance sample enrichment in many different applications, from the simple cells concentration to the differentiation of living and dead bacteria or to enhance the efficiency of bacteria binding to immune-treated surfaces [60].

The capacity of the DEP forces to attract particles to particular places gives a possibility to combine this method with cell interaction with a specially treated surface [61–63] as discussed previously. Yang and colleagues [61] used a microchannel system with interdigitated electrodes on the surface of SiO_2, where monoclonal antibodies were attached. Binding of antibodies was carried out by using a biotin/streptavidin strategy. Combining DEP with specific binding, it was possible to collect 90% of *Listeria monocytogenes* under flow conditions of 0.2 μL/min. The capture efficiency was calculated by counting colonies on plates and for concentrations from ≈3 10^7 cells/mL to ≈2.5 10^6 cells/mL was estimated to be 90%.

Koo and colleagues [63] presented the increase in the capture rate of *L. monocytogenes* by using a biotinylated Hsp60, a protein receptor responsible for cell adhesion during infection processes. The capture efficiency of the Hsp60 in the DEP system in comparison with static flow conditions was increased by 60%.

Another electric effect known as AC electro-osmotic flow could be useful to enhance the trapping efficiency of functionalized surfaces [64]. This phenomenon is related to the generation of an electro-osmotic flow at the surface by the application of AC electric fields over coplanar electrodes [52]. The net fluid flow depends directly on the electrode geometry. Using a very specific geometry (asymmetric geometry with a conical electrodes), Vaidyanathan et al. [64] were able to generated a lateral flow and microvortices that generate local shear forces that prevent weak interactions at the surface, reducing the nonspecific attachment of cells. This system permitted to capture breast cancer cells with 87% of efficiency.

Due to weakness of dielectrophoretic forces in comparison with dragging forces, the utilization of surface-modified microbeads is more advantageous as the effect of electric field is higher for a bigger particle. This is the case of the device implemented by Hu et al. [65], in which pDEP deviation under flow conditions was successfully used for separation of specific *E. coli*. In this system, target cells were attached to polystyrene microbeads modified with monoclonal antibody using a streptavidin–biotin method. The application of the positive DEP caused the deviation of modified beads from the main stream flow facilitated by an angled electrode. This method that permitted to recollect 95% of injected beads was reported under 300 μL/h flow conditions.

5. Hydrodynamic mechanism

We would like to finish this chapter, introducing technologies permitting to perform sample separation by hydrodynamics phenomena [66, 67], such as an inertial flow dynamics and Dean flows [68] or hydrodynamic focusing [69, 70]. These concepts are very interesting for sample separation and concentration in terms of sample processing capabilities. While the systems based on surface binding, magnetic interaction, electrical trapping, acoustic separation, and optical effects generate forces that are weaker in comparison to the dragging forces at flow conditions, hydrodynamic mechanisms implement forces and effects produced at relatively high flow rates.

A very useful effect at high flow rates is the formation of fluid vortices, in which the cells are trapped or focused. The generation of microvortices in microfluidic channels may be implemented using spiral channels or asymmetrical geometries, called Dean vortices, as represented in Figure 6(A) for a vortex formation on a channel curve. Under inertial conditions in curved channels, the faster fluid at the central section tends to move outward by inertial effect, while the slower parts of the fluid near to the channel walls tend to recirculate to the internal part of the curve, generating two rotating vortices. On the other hand, vortices can also be created in linear channels by a stepwise change of the channel width. Figure 6(B) shows the creation of vortices, geometry, and sizes that depend on the Reynolds number, a value proportional to the fluid velocity.

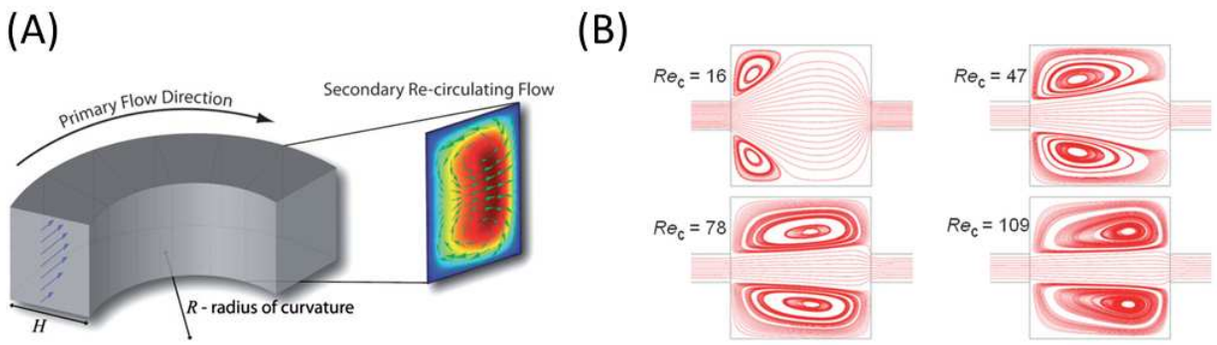

Figure 6. (A) An example of a Dean flow [68] under inertial conditions. Faster moving fluid near the channel center tends to continue outward, and to conserve mass, more stagnant fluid near the walls recirculates inward. This creates two vortices perpendicular to the primary flow direction. (B) the representation of different vortices produced by the change on the channel width at different Reynolds number (Re proportional to fluid velocity) [67]. (A) Reproduced from Di Carlo [68], with permission of the Royal Society of Chemistry. (B) Adapted from Park et al. [67], with permission of the Royal Society of Chemistry.

Using a system with a consecutive succession of width variations in long channels, Mach et al. [71] implemented a microfluidic device performing as a two-step concentration system able to capture cells at the created vortices, releasing then afterward by reducing those vortices lowering the fluid velocity. With this system, they treated a 10-mL sample of a diluted blood (5% v/v) with a ratio of 10^9 blood cells to 500 cancer cells, processing the sample with ~5 mL/min flow rates, concentrating the cancer cells into a final volume of 200 μL in less than 3 min with a purity of 40%. Moon et al. [72] used a similar system with multiorifices combined with an angled electrode DEP system to perform cell separation with high purification. The sample flow rated decay to 126 μL/min, but the purity ratios increased up to 90%.

Inertial systems are mostly used for separation based on a particle size; however, these hydrodynamic-based systems can perform a separation based on inner physical properties of cells, such as its deformability. This kind of sorting can be determinant for the diagnosis of illnesses that affect to the cell physical properties, such as cancer or diabetes [66]. Using an inertial system with a final aperture, Hur et al. [73] demonstrated that at the aperture cells suffer a lateral displacement depending on their deformability. According to this lateral displacement, the cells were collected in different reservoirs. With this method, it was possible to generate an enrichment by factor 5 with a recovery ratio of 96%, with flow rates between tens to hundreds of microliters per minute.

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6. Conclusions

We have tried to show that the application of concentration techniques may result in considerable improvement of biosensor-based analytical systems, helping to increase the sensitivity and selectivity and reduce the overall analysis time. Some examples of the most interesting and potentially useful strategies were presented. However, there are other mechanisms to handle and concentrate cell samples in microfluidic environments, such as acoustic [74], CD centrifugal microfluidic technologies [75, 76], or optical [77] methods that we have not treated in this review.

Among different existing concentration mechanisms, one should implement the most convenient, satisfying the requirements of the detection sensor used. There are many possibilities if we are looking for the concentration of large sample volumes with a very high concentration ratio. However, in many cases, a very specific system is required with high rates of purity or recovery efficiency.

	Device	Cell	Processed Volume	Initial Concentration / Detection	Final concentration	Trapping Efficiency / Separation Efficiency	Purity	Flow rates
Hydrdynamic	Hur [73]	CTC Breast cancer cells	-	1:100 (CTC:RBC) ≈$2 \cdot 10^3$ CTC/ml	≈5 times c/ml	-	96%	*≈ 100 µL/min*
	Mach [71]	CTC	10 ml	1:10^7 (CTC:RBC) 500 CTC/ml	*≈ 100 CTC/200µL ≈ 500 CTC/ml*	20%	40%	5 ml/min
Dielectrophoresis	Yang [61]	*Listeria Monocytogenes*	-	-	-	90%	*Pure samples*	0.2 µL/min
	Park [59]	*E. Coli*	-	10^8 c/ml	-	94%	*Pure samples*	13 µl/min
	Cho [57]	*E. Coli*	500 µL	≈ $9.3 \cdot 10^3$ c/ml	≈ $4 \cdot 10^4$ c/ml	60%	*Pure samples*	100 µL/min
Filter	Filter [12]	*E. Coli*	60 ml	10^2 c/ml	-	-	*Pure samples*	-
Surface	Nagrath [15]	CTC	5.1 ml	10^2 CTC/ml in whole blood	-	65%	50%	≈ 30 µl/min
Magnetic	Beyor [35]	*E. Coli*	100 µL	*≈ 10^4 c/ml*	-	70% (Pure samples) 60% (mixtures 1:1) 40% (mixtures 1:100)	-	0.24 µL/min
	Lee [36]	*Salmonella*	10 ml	10 cfu/ml	-	-	*Pure samples*	25 ml/min
Electrophoresis	Podszum [45]	*E. Faecalis*	200 – 100 µL	10^2 c/ml	Calculated factor ≈ x 25	80%	*Pure samples*	3 - 6 µL/min
	Wang [47]	*E. Coli*	-	10^4 c/ml	Calculated factor ≈ x 10^4	-	*Pure samples*	

Table 1. Summary of some experimental parameters of different reported methods of cell concentration and separation.

Table 1 summarizes experimental parameters of different reported methods of cells concentration and separation, including processed sample volumes, initial and final concentration, trapping efficiency, purity of the sample after separation, and respective flow rates. Some of

the table data marked in cursive are our own estimations based on the direct data presented by the authors and were introduced to provide the possibility to perform direct comparison of different methods. However, due to the differences in the methodologies and measuring setups and sometimes poor details on the release and final concentration obtained, this comparison should be performed with care.

In general, to cover the real necessities of industry and the public health system in micrototal analysis systems and for the wide application of concentration techniques for biosensor applications, three main problems should be resolved:

- integration of known mechanisms into complete microfluidic systems
- capacity of sample processing and velocities of the most specific methodologies
- recovery performances and the purity of the recovered analyte

It seems that to enhance the performance of these systems, it is reasonable to look for a combination of different capture and separation methods combining them effectively with detection routines. This will open a future option to implement efficient concentration and sensing on a chip for real-life applications, providing high levels of integration and velocity, high volumes of sample processing, and high ratios of concentration and purity.

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