

Chapter 2

Microfluidic Systems

Sandra Skorupska, Elzbieta Jastrzebska, Michal Chudy,
Artur Dybko and Zbigniew Brzozka

2.1 Introduction

Microfluidic technology has a rapidly growing meaning in many fields of science. It results from advantages which offer microtechnology with comparison to conventional analysis methods. Small volumes of reagents, low power consumption, flexibility, and adaptability to different experimental conditions and purposes these are some of benefits. Additionally, the microfluidic systems are easy to use and have a great potential for automation as well as they are compatible with a commercially available laboratory equipment. They are characterized by less material consumption, less waste production as well as saving time and costs (Zhang et al. 2016).

Analytical analysis using microdevices was performed in 1979 for the first time. Terry et al. created a miniature gas chromatograph made of silicon wafer (Terry et al. 1979). After that, many researchers put an impact on developing miniaturized separation devices for gas as well as liquid chromatography. There was also a rapid progress in high-throughput on-chip capillary electrophoresis systems for several purposes, for instance separation amino acids or deoxyribonucleic acid (DNA) analysis (Harrison et al. 1993; Woolley and Mathies 1994). The researchers started to expand applications of the microsystems for new purposes: inorganic synthesis (Lee et al. 2008), proteomic analysis (Dodge et al. 2006), or cell cultures (Leclerc et al. 2003). Nowadays, the microdevices are widely used in chemistry, biology, and pharmacology. In microscale, there is a possibility to carry out inorganic synthesis (for instance quantum dots) or determine a concentration of analyte (for instance uric acid) (Grabowska et al. 2008). The researchers developed the microsystems for molecular biology purposes (for instance sequencing DNA

S. Skorupska (✉) · E. Jastrzebska · M. Chudy · A. Dybko · Z. Brzozka
The Chair of Medical Biotechnology, Faculty of Chemistry,
Warsaw University of Technology, Warsaw, Poland
e-mail: sskorupska@ch.pw.edu.pl

(Gan et al. 2014)) and enzymology (Reif et al. 2010). Moreover, there are many microdevices for cellular applications such as cytotoxicity tests (Becker et al. 2017), research on single cell (Altinagac et al. 2016), cell metabolism study (Lee et al. 2016), or effectiveness of new therapy (Ahn et al. 2016; Jastrzebska et al. 2016a). The microfluidic systems for cell cultures will be widely discussed in this chapter.

Nowadays, in vitro cell cultures are used in many fields of science such as molecular biology, biotechnology, analytical chemistry, and biosensors. They also play an essential role in pharmaceutical industries. In vitro cell cultures are used to design models of native tissues and cell-based assays for drug screening and investigation on cell response to external factors. Furthermore, they fulfill the 3Rs rule (i.e., replacement, reduction, refinement), which requires minimizing a number of tests on animals, propagating alternative methods such as in vitro cell cultures. Conventional in vitro cell cultures are performed as two-dimensional (2D, monolayer) model on a flat surface. In an organism, cells are exposed to multiple signals, which are changing in time and space, such as nutrients, growth factors, ions, and biochemical signals. Furthermore, there are also presented direct cell–cell and cell–extracellular matrix (ECM) interactions. Conventional in vitro cell culture do not provide proper environment to mimic state that occurs in living organisms what certainly influences on the cellular response. This can cause differences in drug efficiency between in vitro research and human responses. Moreover, analyzes of cells in macroscale have several important drawbacks. First of all, such studies require a great number of cells and large volumes of reagents. Additionally, a single cell analysis is complicated and time-consuming in macroscale. The microfluidic systems for cell engineering (*Cell-on-a-chip*) are one of the solutions, which can improve current biological techniques or develop a novel method to investigate cellular interactions and responses to external factors. First microsystems were based on cell sorters and detection molecules secreted by the cells (Fu et al. 1999). The microsystems for 2D and three-dimensional (3D) cultures as well as analysis of cell lysis, proliferation, viability, migration, and interactions were developed in the next years. Advanced in vivo-like culture models for investigation of various cell types are being developed nowadays.

The microfluidic systems for cell culture have several advantages. Firstly, benefits from physiological point of view of using microfluidic systems for cell culture will be considered. The organism is built by billions of cells that communicate each other by many networks. These cells are organized in functional units such as tissue, organs, and organs' systems. The nutrients are delivered to cells by a complex network of blood vessels. The nutrition occurs at the cellular level what correspond to the microscale. Due to unique properties and broad possibilities of modification of the microdevices, it is possible to mimic natural cells' environment. Micrometer-sized chambers where cells are seeded and narrow channels with laminar flow of medium correspond to nature physiological environment. Furthermore, it is possible to create a model of interaction between different kinds of cells representing various tissues and organs by culturing them in one microdevice. That kind of the microsystems is called *Organ-on-a-chip* or *Human-on-a-chip* (Cho and Joon 2017; Jastrzebska et al. 2016b). They allow to

communicate between distant cells, what is difficult or even impossible in macro-scale. Such systems provide more information about cells response to many external factors. Moreover, they can be used in personal medicine to study individual responses to drugs to establish the most effective treatment.

Secondly, cell cultures *in vitro* can be realized in static as well as dynamic conditions. In both, nutrients are delivered through microchannels where the fluid flow is always laminar. The mass transport occurs only in diffusion way. This feature makes appropriate conditions for many applications such as cells separation, immunoassays, and various analyzes on cells. Furthermore, it is well known that cells communicate each other by many signals such as autocrine, paracrine, and endocrine factors. It is possible to manipulate cell–cell interactions by the formation of gradient concentration of solutions in a static cell culture or applying various values of a flow rate under perfusion conditions. Moreover, dynamic cell culture enables the imitation of physiological processes, for instance by mechanical stimulation of cells. Due to a wide range of materials that can be used to produce the microdevices and various geometry of the designed channels and chambers, the interactions between cells and ECM can be investigated. It is a huge step to better mimicking physiological environment. Furthermore, it is possible to make 3D cell culture what truly imitate *in vivo* conditions (Cho and Joon 2017; Khademhosseini and Langer 2016).

At last, economical advantages of using the microfluidic systems for cell culture are also important. The microfluidic systems operate on very small volumes of fluids. Therefore, less volumes of reagents are consumed and also the amount of chemical wastes is reduced. Moreover, the reaction time is shortened, due to the fact that there is large surface-to-volume ratio (SAV) in a microsystem. There are various methods of production of the microdevices—some of them are not complicated and do not require advanced equipment. Currently, there is a wide range of materials for microdevices' manufacturing, which are inexpensive and commonly used (for instance poly(dimethyl siloxane)—PDMS). Furthermore, it is possible to make several tests simultaneously using a single microdevice. It is a first step to make high-throughput platform for biological and pharmaceutical research. Moreover, many different analyzes can be integrated on a single device, what also reduces costs and minimizes a risk of mistakes made by an operator during analytical process (Bhise et al. 2014; Cho and Joon 2017).

There are no doubts that the microfluidic systems have a great application potential in many fields. Due to their unique properties they become more and more popular. Currently, the microdevices are mostly used by academic researchers but it is desired to commercialize the microsystems to make faster analyzes in medical laboratories. Recently, there are some companies that offer standard and customized microdevices for biological as well as analytical applications (Halldorsson et al. 2015). The most important parameters of microfluidic systems will be discussed below.

2.2 Microfluidic Systems for Cellomics

2.2.1 Materials and Sterilization

Materials used for the fabrication of the microdevices for cell cultures should have specific properties. Various types of materials utilized for cell engineering and techniques of microstructure fabrication in these materials are presented in details in Chap. 3. Here, we shortly discuss this aspect. First of all, the materials used for developing *Cell-on-a-chip* systems should be non-toxic and biocompatible. The first microfluidic systems were made of silicon as a substrate. Nowadays, glass and polymers are most commonly used (Minerick and Swalm 2008). Glass has several advantages in comparison to silicon. Firstly, it is transparent, what allows to observe analysis in a real time. Additionally, glass is a hydrophilic material, which provides proper conditions for cell adhesion (for 2D culture). Moreover, glass can be easily bonded to another material. Polymers such as: poly(methyl methacrylate) (PMMA), polylactic acid (PLLA), polycarbonate (PC), and poly(dimethyl siloxane) (PDMS) are widely used for fabrication of the microsystems for cell cultures. PDMS is the most popular of them. It is transparent and non-toxic to the cells. Furthermore, PDMS is permeable to gases what allows their exchange. Different geometries of microchambers and microchannels can be designed for the flow regulation. PDMS can be bonded to materials such as silicon, glass, and other polymers. A great advantage of PDMS is also low price and a simple method of microstructure fabrication (Hashimoto et al. 2013). However, PDMS is a hydrophobic material, which impedes adhesion of the cells. This is advantage for 3D cell cultures and disadvantage for 2D cultures. In this case, surface modifications such as gas-phase processing and chemical modification of PDMS are needed. The most popular methods are plasma treatment, ultraviolet (UV) irradiation, and introducing different proteins, i.e., poly-L-lysine, gelatin, fibronectin, and collagen (Zuchowska et al. 2016).

The microfluidic systems for cell cultures need to be sterilized to ensure aseptic conditions and avoid microbiological contamination (Kakac et al. 2010). The most widely used method of sterilization is autoclaving. It is characterized by a simplicity and a high efficiency. This technique is used for sterilization in both macroscale and microscale. Despite the several advantages, autoclaving can not be applied to sterilize the microdevices very often. There is a risk of the destruction or the geometrical deformation of microchambers and microchannels, because of their very small dimensions. There are several different sterilization techniques that can be used with the microfluidic systems. The exposition of a microdevice to UV light as well as oxygen plasma treatment is widely used. The aseptic conditions in the microdevices are being achieved by flushing it with ethanol, hypochlorite, or sodium hydroxide. It is really important to remove chemicals after sterilization by rinsing a microdevice with phosphate buffer and then with culture medium.

2.2.2 Geometry

The microdevices have several advantages and one of the most important of them is a possibility to manipulate of the microstructure geometry. Due to this feature, it is possible to create appropriate conditions for many different cell cultures as well as various assays. The conditions in the microfluidic systems are characterized by several unique properties. Firstly, the ratio of cell volume to medium volume is generally greater than one. Therefore, the scale of the microstructures corresponds with natural environment of cells. This phenomenon contributes to create conditions that better mimic the *in vivo* environment. Moreover, small dimensions of microchambers require a lower number of cells to single experiment and also lower volume of reagents. The SAV ratio is another important feature of microsystems' geometry. SAV ratio is high in the microsystems, and therefore, the transport of molecules (e.g., gases and nutrients) by diffusion is more effective. It is very significant feature, due to the fact that cells require continuous gas exchange such as oxygen and carbon dioxide. Furthermore, the control of temperature in the microdevices is also facilitated by high SAV ratio, because of effective heat transport. Temperature of cells environment plays a key role in cell culture and has influence on their morphology and viability. In the microfluidic systems, it is possible to realize uniform thermal conditions and precise temperature control. On the other hand, high SAV ratio contributes to rapid liquid evaporation if the environment is not properly humidified. PDMS, which is most commonly used material for microdevices fabrication, is permeable to gasses. Due to the fact that, the microfluidic systems contain very small amount of fluids, uncontrolled liquid evaporation is dangerous and may lead to changes in cellular microenvironment. In this case, the cellular response could be modified and the results of the experiments could be unreliable. Furthermore, high SAV ratio can contribute to nonspecific adsorption of proteins. The culture medium comprises a wide range of proteins, which play an important role in cellular nutrition and have huge impact on cellular growth. The protein adsorption results in inhomogeneous cellular environment. It can cause differences in cellular growth conditions, and therefore, the cellular response could be altered. This problem can be solved by modification of the microstructure surface with hydrophilic compounds (e.g., poly(ethylene oxide)—PEO) (Wu et al. 2010). Walker and coworkers introduced a new indicator of cell environment in the microfluidic systems—an effective culture volume (ECV). It indicates a cell ability to regulate their microenvironment (Fig. 2.1). The ECV takes into account the influence of mass transport, rate of diffusion, and level of protein adsorption on the surface of microstructures. The ECV is smaller in microscale than in macroscale, and therefore, cells can better control their microenvironment in microscale (Walker et al. 2004).

In the microfluidic systems, cell cultures could be carried out in static (with periodic medium exchanges) or perfusion conditions (with constant medium flow). The most common problems during cell cultures are: how often culture medium has to be changed in a static culture and how to determine a proper flow rate in a

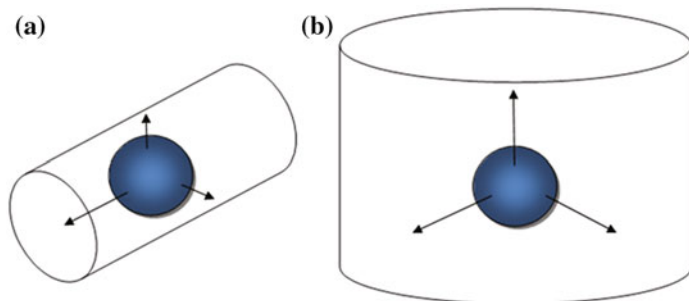


Fig. 2.1 Scheme of effective culture volume (ECV) of a cell: **a** in microsystem—small ECV; **b** in macroscale—large ECV

perfusion culture. To resolve these issues there are another two parameters describing cell culture in microscale: an effective culture time (ECT) and critical perfusion rate (CPR). ECT helps to adjust the time interval between media changes, and CPR indicates appropriate perfusion system to cell culture in the microfluidic systems (Young and Beebe 2010).

2.2.3 Mass Transport

2.2.3.1 Laminar Flow

There are two main types of fluid flow: laminar and turbulent. First one is characterized by calm fluid flow in parallel layers. Due to a high order of the motion of the molecules, there is no any mixing process except diffusion. On the other hand, the turbulent flow describes the chaotic movement of the molecules that contributes to lateral mixing (Whitesides 2006). The type of flow is determined by the value of the Reynolds number (Re) (see Eq. 2.1):

$$\text{Re} = \frac{ud\rho}{\mu} \quad (2.1)$$

where: Re—the Reynolds number, u —fluid velocity, d —a diameter of capillary, ρ —density of the fluid, μ —dynamic viscosity of fluid (Walker et al. 2004).

If the Reynolds number is lower than 2000, the fluid flow is always laminar. In *in vivo* environments, predominantly laminar flows occur. Therefore, it is important to mimic that condition in research carried out *in vitro*. Due to small dimensions of microchannels in the microfluidic systems there are very low Reynolds numbers (usually $\text{Re} < 10$) what determines the laminar flow. There are no turbulences and the mass transfer occurs only by diffusion. It was difficult to create that conditions in macroscale; therefore, the fabrication of the microfluidic systems is a huge step to

better mimicking natural cell environment. Different cellular microenvironments even in a single microsystem can be created thanks to the laminar flow. However, it should be noted that laminar flow can be inconvenient in cases where mixing is required (Wu et al. 2010).

2.2.3.2 Diffusion

Diffusion is a phenomenon that describes the molecule movements from regions of higher concentration to regions characterized by a smaller amount of molecules. The flux of molecules is characterized by the first Fick's law of diffusion (Eq. 2.2):

$$J = -D \frac{dC}{dx} \quad (2.2)$$

where: J —flux of molecules, D —diffusion coefficient, C —concentration of molecules, x —position.

Diffusion dominates over other transport mechanisms only on short distances. This condition occurs in microscale where the mass transport takes place only in this way. Macroscale cell culture with huge volumes of media requires stirring to deliver nutrients to every cell and to distribute the metabolites and waste. Due to the fact that diffusion is only present in cell cultures carried out in microscale, less homogenous conditions of molecules are also observed (Husain et al. 2010).

2.2.3.3 Gas Transport

In vitro cell cultures are carried out under appropriate conditions, such as temperature and gas concentrations. Usually, the microfluidic systems are stored in a special equipment, such as incubators, where proper conditions are maintained. However, there are some microdevices with an additional equipment for temperature regulation (Tian and Finehout 2008). It is also necessary to provide oxygen and remove carbon dioxide from cellular environment. The regulation of concentration of gasses has an influence on cells metabolism and viability. Gas exchange depends on a microdevice geometry, construction material, cell type, and cell density. The gas concentration is mainly regulated by the usage of external devices (Meyvantsson and Beebe 2008). Workstations and specialized chambers have been used to study cell behavior in different levels of gasses till now. They suffer several disadvantages such as ability to generate only single uniform concentrations of gasses, slow balance, and difficulties in live-cell microscopic observations. Nowadays, more and more microsystems have been developed to allow control of gasses levels in cell culture. The microfluidic systems seem to be a great platform for exploring the effect of gasses concentrations on the cellular responses. They are used for creation a native cell environment and investigating the mechanism of different diseases as well. The microdevices are based on diffusion from fluid,

mixing or using chemicals producing gasses directly on chip. They allow to control the cell environment including the generation of gradients of gasses. The microfluidic systems are high throughput and compatible with cell imaging platforms, which allow to generate multiple conditions with rapid equilibration times (Brennan et al. 2014).

2.2.4 *Mixing*

Mixing is required in many chemical and biochemical analyzes. It is very important to ensure homogenous conditions that can be repeated every time in every kind of experiments. In macroscale, mixing can be done using simple equipment, such as magnetic stirrers or vortex. In microscale, the mixing is not easy to obtain because there is laminar flow, where mass transport is achieved by the diffusion. To obtain mixing in the microdevices two main strategies have been developed: passive and active mixers. The first is performed based only a microstructure geometry, second one needs an external energy/factor to work.

2.2.4.1 *Passive Mixing*

The simplest examples of passive mixing are T- or Y-shaped microchannels where the mixing occurs only due to diffusion. A more advanced passive mixer divide main stream into several narrower where the diffusion process is faster. Then every single flux is merged into a common microchannel (Jeon et al. 2000). To obtain disorderly advection multiple changes in microchannel geometries (Fig. 2.2a, b) or some barriers in the mixers are used. Advection is efficient even at low Reynolds number and may cause fluid re-circulations (Novotny and Foret 2017). There are also 3D mixers, which contain C-, L-, or F-shaped microchannels to make rotational motion of flow on several levels (Liu et al. 2000). The next kind of mixers is based on Coanada effect (Fig. 2.2c). They used the phenomena that fluids flowing from a narrow channel to large space are attached to the curved surface and simultaneously they produced counter-flow what contributes to advection (Hong et al. 2001). Another kinds of passive mixers are vortex micromixers (Fig. 2.2d). They consist of one chamber and several tangential inlets. Due to the geometry, the influx of fluid makes rotations in a chamber and improves the diffusion (Lin et al. 2005).

2.2.4.2 *Active Mixing*

There are several kinds of active mixers, in which different principles of physics have been used. Firstly, pressure perturbation is used to achieve pulses in the fluid flow and then some deformation in a flux. For instance, the chaotic mixing in the

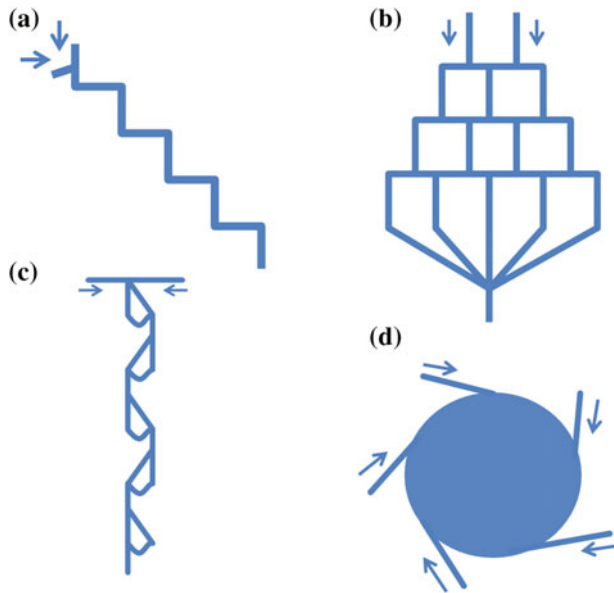


Fig. 2.2 Examples of passive mixers: **a** a zigzag mixer, **b** a microfluidic network, **c** a coanda-effect mixer, **d** a vortex micromixer

main microchannel was accomplished by the application the pressure between opposite channels (Niu and Lee 2003). Another kinds of mixers utilize absorption of acoustic waves by fluid what induces disorderly flux (Fig. 2.3a). This phenomenon was utilized in a microdevice based on trapped air bubbles, which were exposed to acoustic waves. This generated oscillations on the air/liquid surface and finally mixing of the fluids (Ahmed et al. 2009). Third kind of active mixers is actuated by an external magnetic field, which causes oscillations of magnetic microparticles placed in a fluid (Fig. 2.3b). For this purpose, magnetic particles or ferrofluids can be used. The motion of the particles leads to chaotic mixing of fluid (Wang et al. 2007; Oh et al. 2007). Lorentz force can also be used to actuate mixing in the microsystems (Fig. 2.3c). It can be done by subjecting conductive fluids to an external magnetic field, what promotes fluid flow and enhances fluid mixing (Bau et al. 2001).

2.2.5 Concentration Gradient Generators (CGGs)

Many biological and chemical analyzes require an application of a concentration gradient generator (CGG) to examine different phenomena such as chemotaxis or cell stress. There are two main types of gradient generators developed in the microdevices:

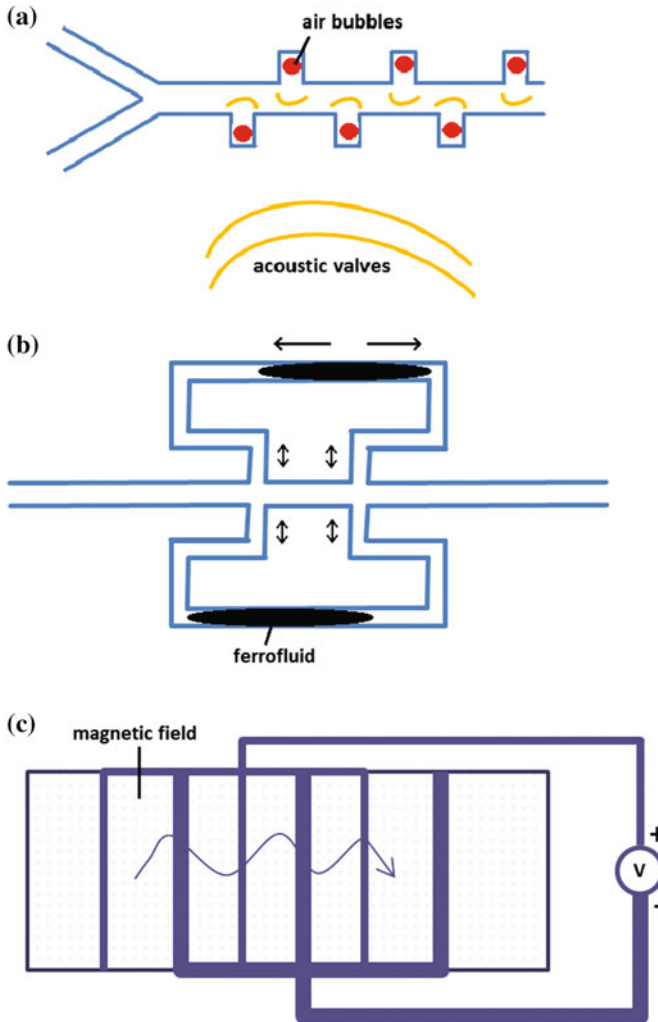


Fig. 2.3 Examples of active mixers: **a** a mixer utilizing absorption of acoustic waves, **b** a mixer with magnetic microparticles oscillating under external magnetic field, **c** a mixer utilizing Lorentz force

- flow-based gradient generator—a fluid flow is used to improve mass transport by convective contribution and advection,
- diffusion-based gradient generator—without a fluid flow, mass transport depends on the diffusion.

Both of them are commonly used in biological research because they allow mimicking of natural cellular environment (Fig. 2.4).

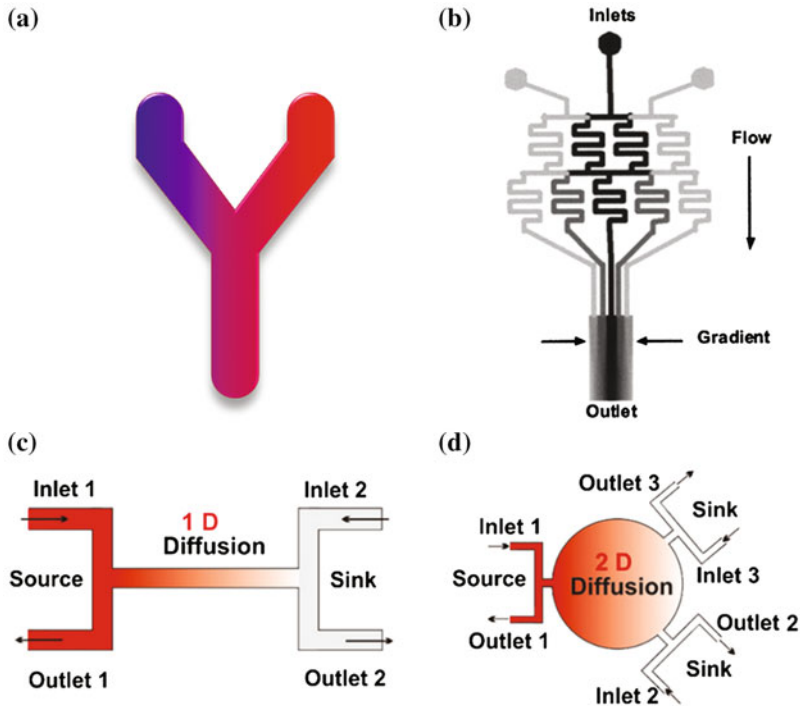


Fig. 2.4 Scheme of different concentration gradient generators (CGGs): **a, b**—flow-based gradient generators, **c, d**—diffusion-based gradient generators. **b–d**: Reprinted with permission from Nguyen et al. 2013. Copyright 2013 Elsevier

Flow-based gradient generators use a direct contact between two flowing streams. The flow velocity increases the diffusion process and generates the advection or convection. In a simple geometry, like T- or Y-shaped microchannels, the gradient region is not homogeneous. To make uniform gradient region a different networks of microchannels are used where streams are separated and mixed repeatedly. These kinds of gradient generators are particularly interesting because dynamic conditions that accurately mimic the natural cellular environment can be obtained. Furthermore, the usage of flow-based gradient generators gives possibility to monitor cellular responses under shear stress.

Diffusion-based gradient generators are based on the diffusion as only one mechanism of mass transport. Usually, a region of the diffusion is connected to several symmetric inlet microchannels with different concentrations of solutions. There are many geometric approaches to develop this kind of gradient generators. An application of diffusion-based gradient generators makes possibility to mimic natural static cellular environment. In such a type of CGG, no shear stress is generated. Therefore, they are mostly used to study: migration of cells at gradient

concentration, pharmacodynamic drug effects, and cellular response to different chemical and biochemical factors.

Flow-based and diffusion-based gradient generators are attractive and powerful tools for making appropriate conditions for wide range of analysis in the microfluidic systems. It is possible to create a cellular environment similar to in vivo and investigate a cellular response to various external factors what was impossible in macroscale. It is especially important for drug industry as well as for understanding the cellular behavior from biological point of view (Nguyen et al. 2013; Oliveira et al. 2016).

2.2.6 Shear Stress

Shear stress is present everywhere, where the fluid flow is applied (Fig. 2.5). This factor is especially important for perfusion cell cultures carried out in the microfluidic systems. It was reported that shear stress can enhance or inhibit cell proliferation, but it is dependent on shear stress value and cell type. To determine shear stress value for 2D perfusion cell culture carried out in the microsystems it is possible to use some equation:

$$\tau = \frac{6\mu Q}{h^2 w} \quad (2.3)$$

where: τ —shear stress, μ —viscosity, Q —flow rate, h —chamber height, w —chamber width.

Several methods are used to obtain the value of shear stress, which is proper for cell culture (e.g., low value of fluid velocity and high depth of culture microchambers are used). Thanks to that culture microenvironment is created, in which affective nutrient delivery and a low concentration of the secreted factors are obtained. Micropillars and microwells are also used for mechanical cell protection. On the other hand, a high level of shear stress is used to examine its influence on some biological aspects such as cell adhesion or function of protein (Kim et al. 2007). Some researchers investigated the effect of fluid flow and shear stress on cell proliferation, changes in cytoskeleton rearrangement, vessels formation, and ability

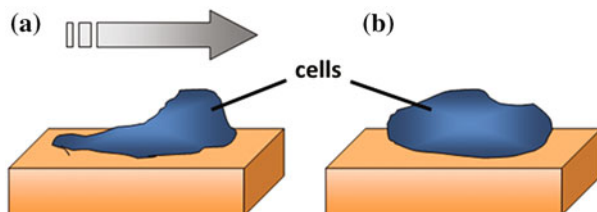


Fig. 2.5 Scheme indicating the shear stress: **a** perfusion cell culture—shear stress is present, **b** static cell culture—no shear stress

of tumor cells to metastasize. Shear stress reduces the number of new sprout vessels formed by endothelial cells. It is a simulation of cancer angiogenesis model. Although further research is needed, this is a huge step toward understanding the phenomenon of cancer metastasis (Song and Munn 2011).

2.3 Perfusion and Static Cell Cultures

The development of the microsystems created new opportunities to improve the biological studies. For instance, it has been possible to create a perfusion cell culture, which better mimics natural cellular environment *in vivo*. There are two different ways to carry out cell cultures performed in the microsystems: under static or perfusion conditions (Fig. 2.6). Both of them have some benefits and limitations.

Static cell cultures are simply to carry out and widely used and in biological research. The experiments are usually carried out in multi-well plates or Petri dishes but also in the microsystems. The cells are seeded in the culture microchamber, and the medium is exchanged periodically. This kind of cell culture is not preferred to long-term analysis due to the possibility of contamination. Additionally, in this type of culture there are periodic changes in cellular environment due to medium replacement processes. It can cause differences in cellular response. Furthermore, even small alteration in cells environment can affect the cell physiology. Therefore, it is very important to provide stable conditions during all steps of cell-based research.

Perfusion cell culture has several advantages compared with the static culture. Firstly, it is more appropriate for long-term analysis due to sterility resulting from less manual interventions. Furthermore, it is possible to continuously provide nutrients and remove waste what makes cellular environment more stable. It has positive influence on cell physiology and their response to external factors. On the other hand, perfusion cell culture could impede cell-to-cell communication due to

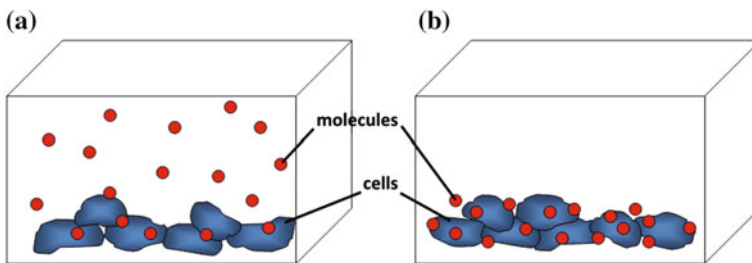


Fig. 2.6 Scheme of perfusion (a) and static (b) cell cultures: **a** nutrients are continuously provided and waste removed—cellular environment is stable, **b** culture medium with nutrients and waste is periodically changed—cellular environment is changed in time and space

elution of relay molecules. Even that, most of the researches used perfusion conditions because they better mimics natural cellular environment. The cells can be continuously fed and can be exposed to shear stress. Moreover, specific physical environment can be created such as gradient concentrations. These facilities allow to make in vivo-like conditions. Despite many advantages of perfusion cell culture over static cell culture both of them are equally popular and willingly used in scientific research. Choosing a cell culture method depends on the purposes and assumptions of the study and should be carefully thought out (Wu et al. 2010).

2.4 Monolayer and Spatial Cell Cultures

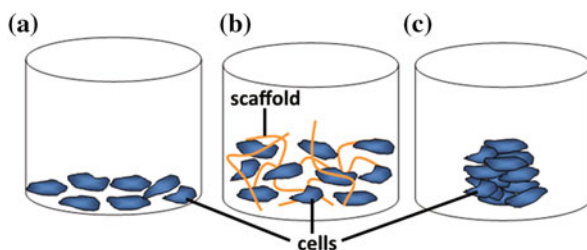
Cell cultures are commonly used in biological research instead of animals and tissue cultures due to easy handling and lower costs. There are two main techniques to carry out cell cultures: two-dimensional (2D, monolayer) and three-dimensional (3D, spatial) cell cultures. Scheme of most popular cultures types formed in the microsystems are shown in Fig. 2.7. The most important question is: does in vitro cell culture can mimic thoroughly the in vivo conditions?

Cellular assays such as drug research are mainly carried out in 2D cultures. In this case, the cells are seeded as a monolayer on the surface. 2D cell culture is a simple model in terms of both the manual operations and cellular behavior. It is well known that standard monolayer is not quite good model of the in vivo environment due to the absence of several biological factors such as signaling molecules (hormones, cytokines, etc.), cell–cell and cell–ECM interactions. All of these agents could affect on cell proliferation, viability or responding to external factors. Furthermore, cells in 2D cultures are exposed to more homogeneous conditions—in vivo the concentrations gradient is often present. For instance, in a tumor, there is cellular heterogeneity due to differences in mass transport. Therefore, there could be differences between cell responses in 2D and 3D cultures.

3D cell cultures better mimic in vivo conditions than monolayer. There are different methods of spatial cell cultures in the microfluidic systems, e.g., cell multilayers, spheroids, hydrogels, scaffolds, bioprinting (Costa et al. 2016; Sung and Beebe 2014; Tomecka et al. 2018; Zuchowska et al. 2017). 3D culture model is really promising due to the fact that it creates appropriate cell culture conditions and

Fig. 2.7 Scheme of different cell cultures performed in the microsystems:

a two-dimensional monolayer
b spatial by usage of hydrogels
c spatial—spheroid model



gives really good approximation of cellular environment in vivo. 3D cell culture allows to create ECM, which has a huge impact on cell behavior. Mechanics and dynamics of microenvironment affect cell viability and resistance to external factors. Moreover, in spatial cell culture, cell–cell interactions and signaling molecules are presented. ECM usually contains collagen, glycoproteins, and polysaccharides. To create ECM in the microsystems, most often are used hydrogels: natural forms and their derivatives such as collagen, chitosan, or alginate. Synthetic hydrogels, for instance poly(ethylene glycol) (PEG)-based, are also widely used due to their biocompatibility. Natural and synthetic hydrogels have some advantages and disadvantages. Firstly, natural hydrogels are components of native extracellular matrix what lead to their bioactivity and provide many adhesion sites. On the other hand, their mechanical properties are complicated and not explained at all. Furthermore, the compositions of them are various due to their different natural sources. Synthetic hydrogels are more reproducible and their chemical composition is well determined. Oppositely, there could be a problem with their bioactivity and adhesion. Sometimes, it is necessary to make additional treatment to promote cell adhesion and proliferation on this kind of materials (Wu et al. 2017). It is worth mentioning that there are also non-scaffold-based 3D cell cultures, named spheroids. Spheroids are micro-sized aggregates of cells. They are widely used as a model of solid tumor in many cancer drug research (Zuchowska et al. 2017).

Possibility to create 3D cell culture allows to take an attempt to mimic organ microarchitecture. *Organ-on-a-chip* systems, which contained cells simulating organ-level physiology, are developed in recent years. In such systems, different conditions of tissues such as spatiotemporal chemical gradients, mechanical forces, strains, and fluid shear stresses can be created. To design *Organ-on-chip* systems, it is necessary to take into account the following issues (Sung and Beebe 2014; Wu et al. 2017):

- Spatial (scaffold/hydrogel) material

First of all, material used for spatial arrangement of the cells should be biocompatible. It cannot affect on cell viability and morphology. The interaction between spatial material and cells can not change the cellular response to external factors. Furthermore, mechanical stability of material should be known to assure appropriate cell microenvironment. It is important to consider permeability of spatial material to enable migration of molecules.

- Microstructure geometry

Geometry and dimensions of culture microstructures are very important to make proper conditions to cell growth. ECV value should be selected in this way that the cells are able to control culture microenvironment. Furthermore, the microstructure should be specifically designed for each tissue culture. The proper geometry of the microchambers and microwells should be fabricated, when non-scaffold 3D cell cultures (spheroids) are performed in the microsystems. Microstructure should provide possibility to form single spheroid in one microstructure.

- Delivery of fluid flow

Fluid flow is an essential factor which mimics *in vivo* conditions. Continuous flow regulates the cell-to-cell signaling and also cell-to-ECM interactions. Fluid flow is responsible for delivery of the nutrients and removal of the waste as well. Additionally, it influences cell proliferation (mechanical stimulation).

- Compatibility with analytical method

It is necessary to establish analytical method that could be used to determine cell function in the designed microfluidic system. It is desired to develop the microdevices, which allow high-throughput analyzes.

2.5 Conclusions

The microfluidic systems are more and more popular in chemistry, biotechnology as well as molecular biology. They are also widely used in cell engineering. The microfluidic-based assays for cell study have several advantages compared with macroscale processes. They make a possibility to create more *in vivo*-like culture environment. This contributes to better mimicking conditions present in a living organism. The microfluidic systems for cell culture are promising tools for drug researches and cytotoxicity studies. It is desired to use them not only in scientific research but also commercially for analytical tests and personal medicine. The microfluidic systems are high-throughput platforms for drug testing which are characterized by better prediction of the human response to external factors. There are some limitations which impede practical applications of the microdevices. Firstly, potential user should be trained in the use of tools and the procedure of analyzes should be simple and clear. Secondly, the results from analyzes must be easy to interpret—the validation and careful verification are needed. Thirdly, receiving results should be fast and high throughput with using widespread analytical techniques. Taking everything into account, the most important challenge for widespread applicability of the microfluidic systems is to simplify the using of them.

Acknowledgements This work was realized with the frame of project SONATA 5 program No. UMO-2013/09/D/ST5/03887.

References

- Ahmed D, Mao X, Juluri BK, Huang TJ (2009) A fast microfluidic mixer based on acoustically driven sidewall-trapped microbubbles. *Microfluid Nanofluid* 7:727–731
- Ahn K, Kima SH, Lee GH, Lee S, Heo YS, Park JY (2016) Features of microsystems for cultivation and characterization of stem cells with the aim of regenerative therapy. *Stem Cells Int* 2016:6023132_1–6023132_13

- Altinagac E, Taskin S, Kizil H (2016) Single cell array impedance analysis in a microfluidic device. *JPCS* 757:012010
- Bau HH, Zhong J, Yi M (2001) A minute magneto hydro dynamic (MHD) mixer. *Sensor Actuat B-Chem* 79:207–215
- Becker H, Hansen-Hagge T, Kurtz A, Mrowka R, Wölfl S, Gärtner C (2017) Microfluidic devices for stem-cell cultivation, differentiation and toxicity testing. Paper presented at the SPIE BiOS, BioMEMS and medical microsystems XV, San Francisco, 28 Feb 2017
- Bhise NS, Ribas J, Manoharan V, Zhang YS, Polini A, Massa S, Dokmeci MR, Khademhosseini A (2014) Organ-on-a-chip platforms for studying drug delivery systems. *J Control Release* 190:82–93
- Brennan MD, Rexius-Hall ML, Elgass LJ, Eddington DT (2014) Oxygen control with microfluidics. *Lab Chip* 14:4305–4318
- Cho S, Joon JY (2017) *Organ-on-a-chip* for assessing environmental toxicants. *Curr Opin Biotech* 45:34–42
- Costa EC, Moreira AF, de Melo-Diogo D, Gaspar VM, Carvalho MP, Coreia IJ (2016) 3D tumor spheroids: an overview on the tools and techniques used for their analysis. *Biotechnol Adv* 34:1427–1441
- Dodge A, Brunet E, Chen S, Goulpeau J, Labas V, Vinh J, Tabeling P (2006) PDMS-based microfluidics for proteomic analysis. *Analyst* 131:1122–1128
- Fu AY, Spence C, Sherer A, Arnold FH, Quake SR (1999) A microfabricated fluorescence-activated cell sorter. *Nat Biotechnol* 17:1109–1111
- Gan W, Zhuang B, Zhang P, Han J, Li CX, Liu P (2014) A filter paper-based microdevice for low-cost, rapid, and automated DNA extraction and amplification from diverse sample types. *Lab Chip* 14:3719–3728
- Grabowska I, Chudy M, Dybko A, Brzozka Z (2008) Uric acid determination in a miniaturized flow system with dual optical detection. *Sensor Actuat B-Chem* 130:508–513
- Halldorsson S, Lucumi E, Gomez-Sjoberg R, Fleming RMT (2015) Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. *Biosens Bioelectron* 63:218–231
- Harrison DJ, Fluri K, Seiler K, Fan Z, Effenhauser C, Manz A (1993) Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* 261:895–897
- Hashimoto M, Tong R, Kohane DS (2013) Microdevices of nanomedicine. *Mol Pharmaceut* 10:2127–2144
- Hong CC, Choi JW, Ahn CH (2001) A novel in-plane passive micromixer using Coanda effect. In: Ramsey JM, van den Berg A (eds) *Proceedings of the 5th micro total analysis systems μ TAS symposium*, Monterey, 21–25 Oct 2001
- Husain AA, Starita C, Hodgetts A, Marshal J (2010) Macromolecular diffusion characteristics of ageing human Bruch's membrane: implications for age-related macular degeneration (AMD). *Exp Eye Res* 90:703–710
- Jastrzebska E, Bazylińska U, Bulka M, Tokarska K, Chudy M, Dybko A, Wilk KA, Brzozka Z (2016a) Microfluidic platform for photodynamic therapy cytotoxicity analysis of nanoencapsulated indocyanine-type photosensitizers. *Biomicrofluidics* 10:014116-1–014116-15
- Jastrzebska E, Tomecka E, Jesion I (2016b) *Heart-on-a-chip* based on stem cell biology. *Biosens Bioelectron* 75:67–81
- Jeon NL, Dertinger SKW, Chiu DT, Choi IS, Stroock AD, Whitesides GM (2000) Generation of solution and surface gradients using microfluidic systems. *Langmuir* 16:8311–8316
- Kakac S, Kosoy B, Li D, Pramuanjaroenkij A (eds) (2010) *Microfluidics based microsystems fundamentals and applications*. Springer, Dordrecht
- Khademhosseini A, Langer R (2016) A decade of progress in tissue engineering. *Nat Protoc* 11:1775–1781
- Kim L, Toh YC, Voldman J, Yu H (2007) A practical guide to microfluidic perfusion culture of adherent mammalian cells. *Lab Chip* 7:681–694

- Leclerc E, Sakai Y, Fujii T (2003) Cell culture in 3-dimensional microfluidic structure of PDMS (polydimethylsiloxane). *Biomed Microdevices* 5:109–114
- Lee WB, Weng CH, Cheng FY, Yeh CS, Lei HY, Lee GB (2008) Biomedical microdevices synthesis of iron oxide nanoparticles using a microfluidic system. *Biomed Microdevices* 11:161–171
- Lee JS, Romero R, Han YM, Kim HC, Kimj CJ, Hong JS, Huh D (2016) Placenta-on-a-chip: a novel platform to study the biology of the human placenta. *J Matern-Fetal Neo M* 29:1046–1057
- Lin CH, Tsai CH, Fu LM (2005) A rapid threedimensional vortex micromixer utilizing self-rotation effects under low Reynolds number conditions. *J Micromech Microeng* 15:935
- Liu RH, Stremmer MA, Sharp KV, Olsen MG, Santiago JG, Adrian RJ, Aref H, Beebe DJ (2000) Passive mixing in a three-dimensional serpentine microchannel. *J Microelectromech Syst* 9:190–197
- Meyvantsson I, Beebe DJ (2008) Cell culture models in microfluidic systems. *Annu Rev Anal Chem* 1:423–449
- Minerick AR, Swalm DC (2008) The rapidly growing field of micro and nanotechnology to measure living cells. *AIChE J* 54:2230–2237
- Nguyen NT, Shaegh SAM, Kashaninejad N, Phan DT (2013) Design, fabrication and characterization of drug delivery systems based on a lab on a chip technology. *Adv Drug Deliver Rev* 65:1403–1419
- Niu X, Lee YK (2003) Efficient spatial-temporal chaotic mixing in microchannels. *J Micromech Microeng* 13:454
- Novotny J, Foret F (2017) Fluid manipulation on the micro-scale: basic of fluid behavior in microfluidics. *J Sep Sci* 40:383–394
- Oh DW, Jin JS, Choi JH, Kim HY, Lee JS (2007) A microfluidic chaotic mixer using ferrofluid. *J Micromech Microeng* 17:2077
- Oliviera AF, Pessoa ACSN, Bastos RG, de la Torre LG (2016) Microfluidic tools toward industrial biotechnology. *Biotechnol Prog* 32:1372–1389
- Reif RD, Aguas C, Martinez MM, Pappas D (2010) Temporal dynamics of receptor-induced apoptosis in an affinity microdevice. *Anal Bioanal Chem* 397:3387–3396
- Song JW, Munn LL (2011) Fluid forces control endothelial sprouting. *Proc Natl Acad Sci* 108:15342–15347
- Sung KE, Beebe DJ (2014) Microfluidic 3D models of cancer. *Adv Drug Deliver Rev* 78–79:68–78
- Terry SC, Jerman JH, Angell JB (1979) A gas chromatographic air analyzer fabricated on a silicon wafer. *IEEE T Electron Dev* 26:1880–1886
- Tian WCh, Finehout E (eds) (2008) *Microfluidics for biological applications*. Springer, New York
- Tomecka E, Zukowski K, Jastrzebska E, Chudy M, Brzozka Z (2018) Microsystem with micropillar array for three- (gel-embaded) and two-dimensional cardiac cell culture. *Sensor Actuat B-Chem B* 254:973–983
- Walker GM, Zeringue HC, Beebe DJ (2004) Microenvironment design considerations for cellular scale studies. *Lab Chip* 4:91–97
- Wang Y, Zhe J, Chung BTF, Dutta P (2007) A rapid magnetic particle driven micromixer. *Microfluid Nanofluid* 4:375–389
- Whitesides GM (2006) The origins and the future of microfluidics. *Nature* 442:368–373
- Woolley A, Mathies R (1994) Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. *Proc Natl Acad Sci USA* 91:11348–11352
- Wu MH, Huang SB, Lee GB (2010) Microfluidic cell culture systems for drug research. *Lab Chip* 10:939–956
- Wu J, Chen Q, Liu W, He Z, Lin JM (2017) Recent advances in microfluidic 3D cellular scaffold for drug assays. *TrAC* 87:19–31

- Young EWK, Beebe DJ (2010) Fundamentals of microfluidic cell culture in controlled microenvironments. *Cheml Soc Rev* 39:1036–1048
- Zhang Y, Ge S, Yu J (2016) Chemical and biochemical analysis on lab-on-a-chip devices fabricated using three-dimensional printing. *TrAC* 85:166–180
- Zuchowska A, Kwiatkowski P, Jastrzebska E, Chudy M, Dybko A, Brzozka Z (2016) Adhesion of MRC-5 and A549 cells on poly(dimethylsiloxane) surface modified by proteins. *Electrophoresis* 37:537–544
- Zuchowska A, Kwapiszewska K, Chudy M, Dybko A, Brzozka Z (2017) Studies of anticancer drug cytotoxicity based on long-term HepG2 spheroid culture in a microfluidic system. *Electrophoresis* 38:1206–1216