# Chapter 8
# *Heart-on-a-chip* Systems

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## 8.1 Cardiac Cell Cultures in *Lab-on-a-chip* Systems

*Heart-on-a-chip* systems are developed to create culture models, which are able to mimic functional heart tissue (Selimović et al. 2013). Such cellular models could lead to a better understanding of mechanisms responsible for heart diseases. In vivo-like cardiac cultures obtained in *Lab-on-a-chip* systems can be utilized for (1) the investigation of cardiac cell physiology under conditions close to in vivo, (2) the evaluation of the cardiotoxicity of drugs used for CVD therapy, (3) the investigation of side effects generated by different types of drugs, and (4) the study of mechanisms responsible for heart regeneration (Jastrzebska et al. 2016; Ribas et al. 2016; Zhang et al. 2016a, b).

Two-dimensional (2D) cell cultures (monolayers) are the most common in vitro models of the heart and cardiovascular system. However, they do not mimic the conditions present in a living organism accurately. Conventional cardiac cell cultures are based on a monolayer culture, where the cardiac cells are cultured on a flat surface of the culture flask. In this case, the culture is carried out under static conditions, where a culture medium is exchanged periodically (Ralphe and de Lange 2013). Additionally, the arrangement of the cells to each other is usually random in a conventional monolayer culture. The cells are arranged parallelly to each other in living organisms (Bhaarathy et al. 2014; LeGrice et al. 1995). It should be noted that such cell arrangement is essential in a cell model. The orientation of myocardial cells is determined by many factors. Mechanical factors play the most important role. In the native environment, heart tissue is constantly in contact with body fluids (blood, lymph) (Kujala et al. 2016). The continuous stimulation of heart cells by blood flow and physical forces in vivo (i.e., shear

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stress) is crucial for the determination of cardiac cell structure, phenotype, and alignment to the other cells. Therefore, the dynamic conditions should be provided to in vitro cardiac cell cultures. The *Lab-on-a-chip* systems are suitable tools, in which biochemical, mechanical, and physical factors of heart tissue can be mimicked (Chen et al. 2017; Giaseddin et al. 2017; Simmson et al. 2012). Key signaling parameters used to mimic native myocardium in the microsystems are described in detail in Chap. 7.

Heart and cardiovascular systems are composed of different cell types: e.g., cardiomyocytes (CMs), fibroblasts, endothelial cells, smooth muscle cells, and pericytes (see Chap. 5 and Fig. 8.1). CMs are myocardial cells, which are responsible for heart contractions. These cells have contractile proteins such as sarcomeres and myofibrils. Fibroblasts are responsible for producing an extracellular matrix (ECM) and the arrangement of other heart cells (Ugolini et al. 2016). Endothelial cells build heart atriums and produce atrial natriuretic peptide (ANP), which prevents heart failure (Maksimov et al. 2015). Pericytes build small blood vessels. Their function is very important, because they strengthen the blood vessels. Pericytes can also differentiate into other cells (fibroblasts or smooth muscle cells) depending on tissue requirements (Hall et al. 2014).

Cardiac and vascular cells are characterized as having a high dependency on the external environment (Conant et al. 2017). They exhibit morphological and physiological changes as a result of mechanical, biological, chemical, and electrical stimulations (Dahl et al. 2010; Simmson et al. 2012). CMs are the most commonly used for in vitro studies. Cardiomyoblasts (precursor of CMs) are the next cell type often utilized for in vitro experiments. Cardiomyoblasts are mesodermal cells, which can differentiate into transverse striated heart muscle cells (Simmson et al. 2012). The cells, utilized for in vitro assays, can be mainly derived from human, mouse, or rat. However, it should be noted that there are significant differences

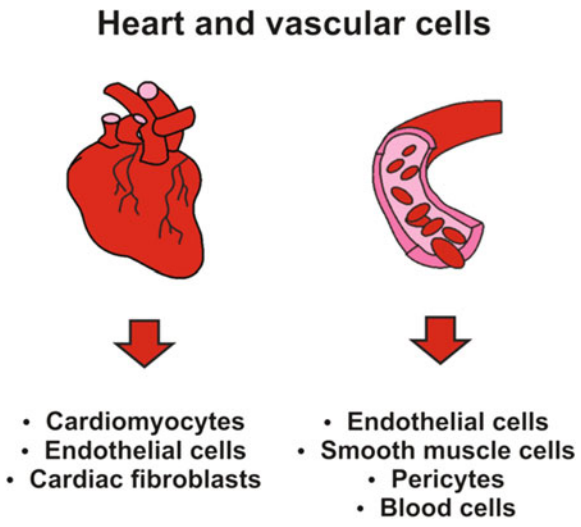


**Fig. 8.1** Types of cells, which build in vivo cardiovascular system

between human and animal cardiac cells. Rat CMs have a higher percentage of protein chains, which build filaments fibers (e.g., α-myosin) and a fivefold higher resting heart rate than human CMs. A calcium transport through the rat cell membrane is also carried out by other mechanism than in human cells. Mouse CMs differ from human heart cells even more than rat CMs. The resting heart rate of mouse CMs is tenfold higher than in human cells. Moreover, the way of potassium repolarization in mouse CMs is different than in human cardiac cells. This can influence the permeability of chemical compounds (e.g., drugs) through cell membranes. Finally, different drug cytotoxicity could be obtained depending on cell origin (Simmson et al. 2012). For this reason, stem cell-derived cardiomyocytes (SC-CMs) have been used for in vitro studies in recent years (Au et al. 2009). Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSC) are predominantly differentiated in CMs using conventional methods (macroscale). Moreover, SC differentiation into CMs using different types of stimulation (biochemical, mechanical, and electrical) can be performed in microscale. These methods are described in detail in Chap. 9.

The *Lab-on-a-chip* systems used to create both a beating heart culture model and a whole vascular system have been presented in the literature in the last few years. Two-dimensional (2D) and three-dimensional (3D) cell cultures are obtained in the developed microsystems (Cheng et al. 2006; Ges et al. 2008; Horiguchi et al. 2009; Yue et al. 2014). A scheme of culture types, which can be obtained in *Heart-on-a-chip* systems, is shown in Fig. 8.2.

Poly(dimethyl siloxane) (PDMS) and glass are most commonly used for fabrication of *Heart-on-a-chip* systems. It results from the properties of these materials (see Chap. 3). It should be noted that PDMS is highly hydrophobic. 2D culture of CMs on PDMS surface is limited, because its hydrophobic properties inhibit cell attachment. In turn, a hydrophobic surface of PDMS enhances 3D cultures

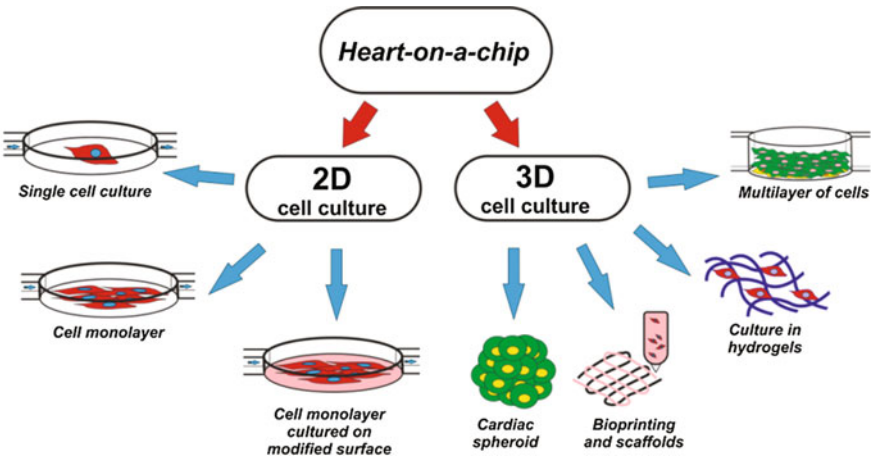


**Fig. 8.2** Types of cardiac and vascular cell cultures performed in *Heart-on-a-chip* systems

(spheroid formation). There are many physical and chemical methods, which can be used to increase PDMS hydrophilicity (Zuchowska et al. 2016). PDMS surfaces utilized for CM cultures are often modified by coating them with proteins such as collagen type I, fibronectin, laminin, or gelatin (Ugolini et al. 2016). The addition of proteins to the culture environment mimics a natural extracellular matrix (ECM). Moreover, proteins have an impact on the spatial organization of the CMs, their shape, contractility, and transport of calcium ions (Boudou et al. 2012). The culture surface can also be modified by changing the surface topography. For this purpose, the microgrooves with controlled depth and width are formed. Additionally, nano- and microfibers are used to change properties of the surface for CM cultures (Simmson et al. 2012; Tomecka et al. 2017). Modifications of culture surfaces are mainly carried out to stimulate anisotropic orientation of cardiac cells. Because of this, the cells can be parallelly arranged as in the native environment.

As was mentioned in the previous chapters, there are significant differences between 2D and 3D cultures. Both of them have important benefits and disadvantages to use. 3D cultures are similar to the in vivo microenvironment, whereas 2D cell models enable fast and precisely controlled analysis. A single-cell culture is considered to be a 2D culture. There are not physical and biochemical intercellular interactions in such a culture model. Single-cell culture is free of tissue-specific intercellular connections, intercellular spaces and gaps with natural ECM. The natural structure and morphology of the cytoskeleton are also not preserved in a single cell culture. However, the simplicity of such cultures allows for quick analysis of cell functions. It is possible to measure the contraction of a single cell. Kaneko et al. (2007) presented an interesting solution for single-cell analysis (Fig. 8.3a). The microplatform was composed of a glass plate with three layers: chromium, collagen type I, and agarose. Single cardiomyocytes isolated from 13- to 14-day-old mouse embryos were trapped into each microchamber fabricated in the agarose layer. Next, a CM contraction after introduction of haloperidol was measured using a microscope and a video image recording method. The results indicated that the direct single-cell-based measurement enabled precise and quantitative control of cytotoxic effects of the tested drug. The device for trapping a single cardiac myocyte and the measurement of pH in the extracellular environment was also presented in the literature (Ges et al. 2005, 2008). To trap a single cell, the microsystem was equipped with an integrated system of microchannels and microvalves. Each trapped cell was cultured in the microsystem for 1–2 h. Next, the influence of chemical compounds on cell physiology was studied based on the formation of metabolites (pH changes). The microsystem was made of a glass plate covered with a thin layer of two pH-sensitive iridium oxide (IrOx) electrodes and PDMS layer containing microstructures (Fig. 8.3b). Wild-type (WT) single CMs isolated from a mouse were tested in the microsystem. The proposed microsystem can be applied to study ischemia, reperfusion injury, or disorders of other biological systems.

2D monolayer culture is the next model often used to investigate CMs in microscale. Tanaka et al. (2007) have started research based on 2D cardiac cell culture in the microsystems. A microplatform (bio-microactuator) was made of

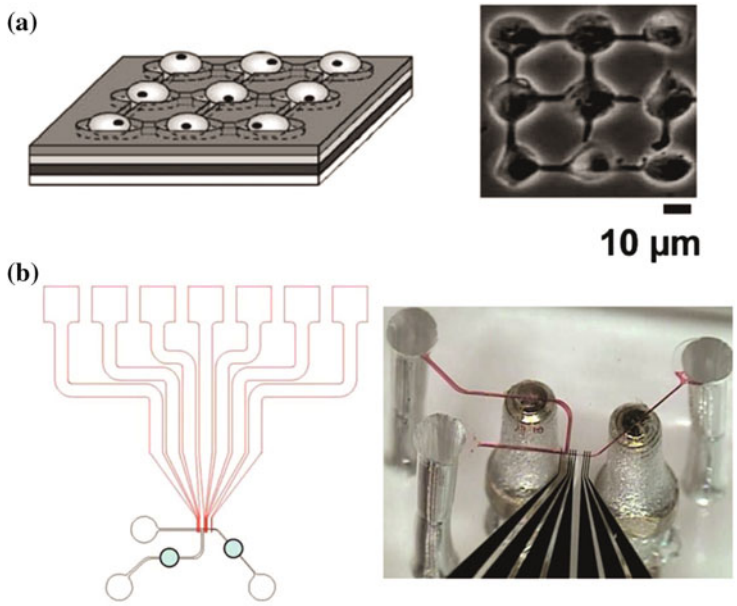


**Fig. 8.3** Geometries of the microsystems for single cardiac cell analysis. **a** The microsystem for the single-cell-based cardiomyocyte culture with nine single microchambers (left) and microscopic image of the nine growing cardiomyocytes (right). Reprinted with permission from Kaneko et al. (2007). Copyright 2007 Royal Society of Chemistry. **b** A scheme of platinum microelectrode array and image of microchannel network in the microsystem. Reprinted with permission from Ges et al. (2008). Copyright 2007 Springer

PDMS using a replica molding technique. The microplatform consisted of a series of specific micropillars (10 µm high) designed for CM placement (Fig. 8.4a). Additionally, the PDMS surface was modified by fibronectin to enhance cell attachment to the micropillars. Primary neonatal rat CMs were cultured in the designed microplatform for 3 days. After that time, heart cell attachment to the micropillars and cell spontaneous beating was noticed. Cell contractions caused micropillar dilatation/deflection (1.4 Hz, ~3 µm displacement). The results showed that continuous cell-to-cell contacts and interactions indicated spontaneous and regular cardiac contraction without the use of any additional biochemical agents. The proposed bio-microactuator is a simplified type of an actuator, in which chemical energy generates mechanical energy (associated with cardiac muscle contractions).

To improve culture and beating of cardiomyocytes in the microsystems, the cells are exposed to additional factors. For example, methacrylated tropoelastin (MeTro) and methacrylated gelatin (GelMA) hydrogels were used for 2D CM cultures (Annabi et al. 2013). The cellular attachment, alignment, and beating of neonatal rat CMs cultured on the modified PDMS layers were compared. The obtained results indicated that PDMS coated with MeTro hydrogel had a high impact on cardiac cell

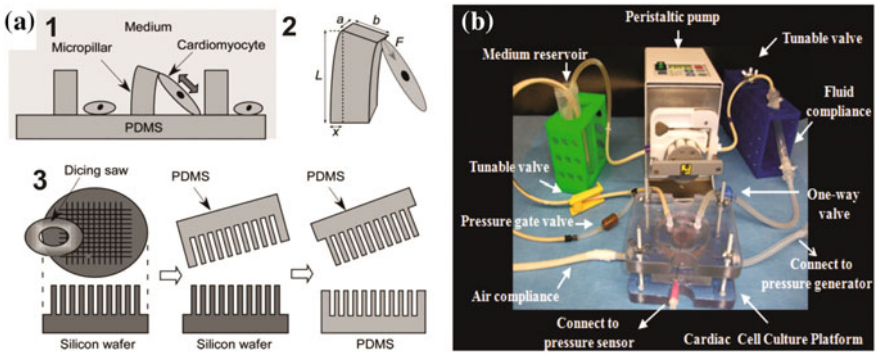


**Fig. 8.4** **a** 1 PDMS micropillars in the microsystem for monolayer cardiomyocytes culture, 2 Scheme of cardiomyocyte adhesion to the micropillar. 3 A scheme of the micropillar fabrication. Reprinted with permission from Tanaka et al. (2007). Copyright 2006 Royal Society of Chemistry. **b** An experimental setup of 2D cardiac cell culture platform. Reprinted with permission from Nguyen et al. (2015). Open access

proliferation. Moreover, this method of surface modification could be useful for the culture of other cell types derived from the cardiovascular system (e.g., blood vessel cells).

The cells in heart tissue are exposed to dynamic flow conditions and stretching. Therefore, it is important to mimic these features in the *Heart-on-a-chip* systems. The microsystems, in which perfusion conditions are simulated, were often presented in the literature (Chen et al. 2017; Kobuszewska et al. 2017; Kujala et al. 2016; Nguyen et al. 2015). It results from the fact that flow conditions play a critical role in the early development and functional maturation of CMs. Moreover, the flow affects cell organization, intercellular interactions, and the transmission of chemical and physical signals (e.g., responsible for contraction) (Mannhardt et al. 2017). For example, Kobuszewska et al. (2017) studied how the geometry of a microsystem and microenvironmental conditions (static and perfusion) influence the proliferation, morphology, and alignment of rat cardiomyoblasts—H9C2 cells. Three different microsystems with a circular chamber, a longitudinal channel, and three parallel microchannels separated by two rows of micropillars were used in the experiments. It was found that perfusion conditions enhanced cell proliferation and induced parallel arrangement of the cells more than static conditions. Additionally, it was observed that the parallel orientation and elongation of the cells are dependent on microchamber geometry.

Stimulation of the cells using two different external agents enhanced CM proliferation. The usage perfusion conditions and mechanical stimulation resulted in increasing of cardiac gene expression (e.g., α-actin sarcomere, cardiac troponin T) and protein synthesis for calcium transport. For example, Nguyen et al. (2015) presented a fully automated platform for 2D cell culture of the embryonic ventricular chick CMs (Fig. 8.4b). The cells were cultured under perfusion conditions and cyclic mechanical stimulation. The obtained results showed that mechanical

stimulation of embryonic CMs is crucial to enhance cell proliferation and to create calcium transporting proteins, which are necessary in the process of cell contraction. It was investigated that mechanical conditions can be essential for the development of functional cardiac fragments (implants), which could replace damaged parts of the heart.

A muscular thin film (MTF) platform consisted of an anisotropic fragment of heart muscle cell tissue is the next interesting solution proposed as a functional cellular model (Grosberg et al. 2011). Such a 2D culture can compete with complex and advanced 3D models. Deformable elastic and flexible thin films made of PDMS, which are the component of MTF, were utilized to culture the neonatal rat ventricular myocytes. To enhance cell organization into an anisotropic form, thin PDMS layers were modified with ultraviolet (UV) light and fibronectin. The modification of culture surfaces can significantly increase cell viability and proliferation, and it can influence parallel cell orientation to each other. The CMs cultured on the designed microplatform were additionally electrical stimulated (square wave pulse, 5–20 V, 2 Hz, 10 ms duration). Thanks to the measurement of the curvature radius of MTF, the cell response to the external stimuli and cell contraction could be established.

Cocultures and multilayers are more advanced models of cardiac cell cultures, which mimic in vivo conditions better than monolayer cultures (Akins et al. 1999; Cheah et al. 2010; Horiguchi et al. 2009). CM cocultures with other types of heart cells allow enrichment of ECM with additional proteins and improvement of a 2D cell model (Gupta and Grande-Allen 2006; Liu et al. 2017). Cell coculture stimulates cell proliferation and increases the efficiency of cell signaling. Endothelial cells and cardiac fibroblasts are the cells, which are capable of producing complex ECM (Garzoni et al. 2009; Hussain et al. 2013; MacKenna et al. 2000; Saini et al. 2015). Additionally, because stem cells (SCs) play a very important role in regenerative medicine, these cells are also used in coculture with cardiac cells. They are utilized as a potential method to regenerate CMs (Garbern and Lee 2013; Ou et al. 2016). However, three-dimensional (3D) heart cultures are the most advanced cellular models used for heart research at the laboratory level. Spatial culture using heart tissue fragment (biopsy) can be performed in a microfluidic device (Cheah et al. 2010). The example of such a microsystem is shown in Fig. 8.5a. The microsystem consisted of a single flow chamber with a diameter of 7 mm. Right ventricular tissue from rat and right atrial tissue biopsies from patient were tested in the presented microdevice. The samples were placed in the microchamber, which was equipped with platinum electrodes for electrical stimulation. The presented microfluidic device was successfully used for real-time electrochemical monitoring of reactive oxygen species (ROS) release from a fragment of heart tissue. Additionally, cell damage was determined by measuring lactate dehydrogenase (LDH).

The scientists have also developed other methods for 3D cardiac cell cultures. 3D structures of CMs can be obtained by electrospinning, which uses the aligned biopolymer fibers as spatial scaffolds. Arrangement of CMs has been tested on nanofibers made of materials such as: poly(l-lactid-co-ε-caprolactone) [P(LLA-CL)]

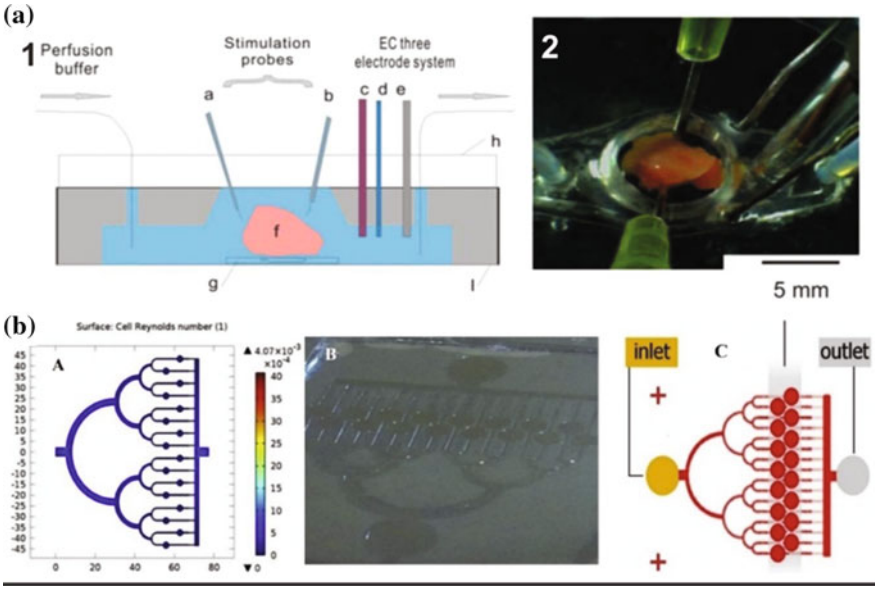


**Fig. 8.5** **a** The microsystem for 3D tissue fragment analysis (a, b—stimulation electrodes, c, d, e—working, reference, and counted electrodes, f—holder, g—layer of PDMS, h—petri dish lid, i—petri dish). Reprinted with permission from Cheah et al. (2010). Copyright 2010 Royal Society of Chemistry. **b** The geometry, mold, and pattern of microsystem for cardiac cell culture in a hydrogel. Reprinted with permission from Ghiaseddin et al. (2017). Copyright 2017 Elsevier

copolymer, poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone) (PCL), poly (hydroxybutyrate) (PHB), chitosan–polycaprolactone, polymethylglutarimide (PMGI) (Mannhardt et al. 2017; Rogozhnikov et al. 2016; Tomecka et al. 2017; Visone et al. 2016). Multilayers without the use of scaffolds can also be used to obtain 3D cell cultures. For this purpose, thermo-sensitive polymers (e.g., poly (N-isopropylacrylamide) can be applied. They are disintegrated at a cell safe temperature, at the last stage of cell culture (Kikuchi and Okano 2005; Shimizu et al. 2002, 2003). A laser microablation is also used to create 3D structure in biodegradable polymers [e.g., poly(glycerol sebacate)], characterized by high porosity and elasticity (Simmons et al. 2012). Hydrogels, which can be gelled under the influence of various external factors (ultraviolet irradiation, temperature, chemical factors), are also utilized for creation of CM spatial arrangement. For example, 3D cultures with controlled size and architecture can be performed using, e.g., fibrin-based or collagen-based hydrogel matrix generated by soft lithography technique (Ghiassedin et al. 2017; Visone et al. 2016; Zhang et al. 2016c). A micro-bioreactor with a network of microchannels, in which cardiac cells with chitosan hydrogel were successfully cultured, is shown in Fig. 8.5b. A high density of mice cardiac progenitor cells (CPC) were loaded into the microchambers and cultured for 10 days. Based on this method, spatial forms of cardiac tissue were created. Marsano et al. (2016) as one of the first examined how the simultaneous

mechanical and biochemical stimulations affected the 3D culture of heart cells. A fibrin gel matrix was used to create 3D culture in a microsystem consisted of two PDMS layers with micropillars and a PDMS membrane. The micropillars in a top layer were used to create spatial models from both neonatal rat and human-induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM). A bottom layer with the micropillars and a PDMS membrane was used to induce homogeneous cyclic strains of 3D cell constructs. It was noted that the cyclic strain enhanced cardiac differentiation. High expression of cardiac markers such as cardiac troponin I and sarcomere α-actin was measured. Mechanical stimulation also influenced higher spontaneous cell beating. The proposed *Heart-on-a-chip* device was also used for evaluation of drug cytotoxicity.

The main challenge for the scientists is to develop an in vitro culture model, which has the ability to contract spontaneously. Additionally, heart cell models integrated with a microvessel network should be elaborated to mimic native heart tissue more. Such a network is responsible for proper distribution of oxygen and nutrients as well as removal of waste products (Gao et al. 2015). Bioprinting is a new technique used for tissue engineering. Compared with other tissue engineering approaches (e.g., the usage of scaffolds or hydrogels), bioprinting is an attractive method thanks to which fabrication of complex tissues also integrated with a vascular network is possible (Kolesky et al. 2016). A microsystem based on bioprinting can be a new approach in regenerative medicine, drug screening, and modeling of CVDs (Jia et al. 2016; Murphy and Antala 2014; Ozbolat 2015). There are a few bioprinting methods: bioprinting of cell-laden hydrogel 3D structures (Dhariwala et al. 2004), cell sheet lamination (Haraguchi et al. 2012), inkjet bioprinting (Boland et al. 2006), laser-assisted bioprinting (Yan et al. 2013), and extrusion-based bioprinting (Beyer et al. 2013). The materials used for bioprinting have specific properties. Functional inks based on piezo-resistive, high-conductance, and biocompatible soft materials (e.g., alginiane, gelatin methacryloyl) are most often used in this technology. Lind et al. (2016) presented an interesting microphysiological device, which was made using 3D bioprinting. To fabricate the microdevice, six inks with different properties were utilized. The sterilized and fibronectin-modified microsystem was used to culture neonatal rat ventricular myocytes (NRVMs) and human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs). The cells were exposed to the isoproterenol and verapamil as well as mechanical strain. The fabricated microsystem enabled the electrical measurement of cell functions and microscopic observation of the immunostained cells (i.e. sarcomere α-actin).

Zhang et al. (2016b) developed a microfluidic bioreactor with the network of blood vessels. Two PDMS layers created a main chamber with bioprinted scaffolds. The main chamber was additionally equipped with four micropillars, which were used to avoid scaffold movement during the flow of a culture medium. Alginate, gelatin methacryloyl (GelMA), photoinitiator Irgacure 2959 were the components of the bioink used for the bioprinting. Human umbilical vein endothelial cells (HUVECs) mixed with the bioink were bioprinted in the scaffolds. Next, CMs (neonatal rate CMs or hiPSC-CMs) were seeded on the created scaffolds. Finally,

cell contraction and high expression of cardiac markers (e.g. sarcomere α-actin, connexin-43) were observed. The bioprinting procedure scheme is shown in Fig. 8.6a. It was noticed that HUVECs migrated toward the peripheries of the microfibers and formed a layer of a confluent endotheliumin. Coculture with cardiac cells created a complex and technologically advanced 3D heart model. The developed microsystem based on bioprinting scaffolds can also be successfully used for screening the efficacy and toxicity of cardiovascular drugs.

Multicellular spheroids are the next known 3D models of cell culture. Spheroids exhibit a number of characteristic in vivo features such as: spatial physical and biochemical intercellular interactions, morphology of the cytoskeleton similar to in vivo morphology, the presence of ECM as well as nutrient and oxygen gradients (Hamilton 1988). Although spheroids are most often considered as tumor model, they are also used to spatial cardiac cell culture (Lee et al. 2013; Oliveira et al. 2013; Ota et al. 2010). Garzoni et al. (2009) presented a 3D spheroid coculture of murine embryos with endothelial cells or bone marrow-derived mesenchymal stroma cells (Fig. 8.6b). New approach for cardiac spheroid formation was also

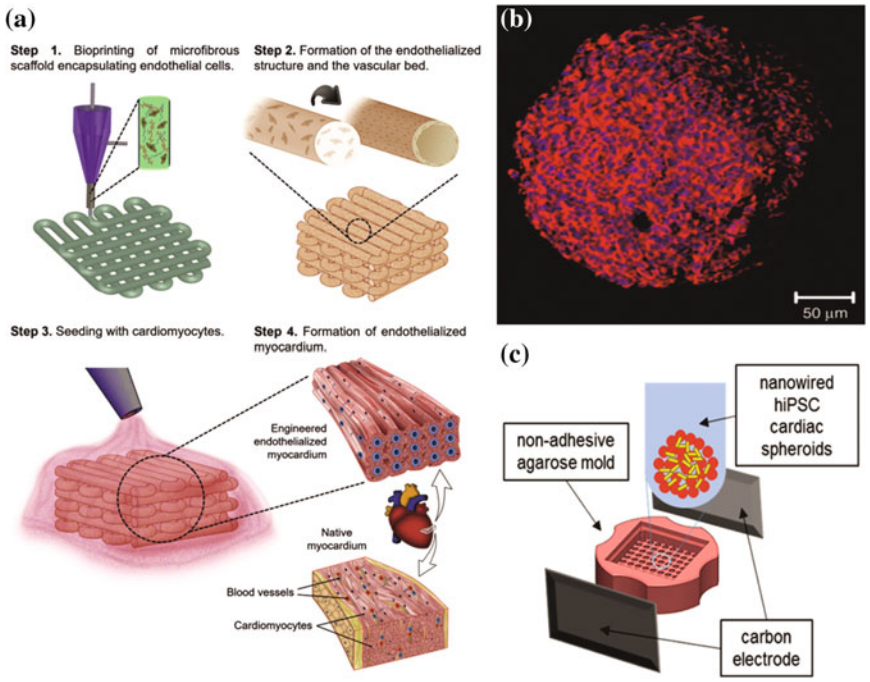


**Fig. 8.6** **a** A scheme of a bioprinting procedure utilized to obtain 3D cardiac cell model. Reprinted with permission from Zhang et al. (2016b). Copyright 2016 Elsevier. **b** A 5-day-old cardiac spheroid stained with fluorescent dyes (DAPI and Evan's blue). Reprinted with permission from Garzoni et al. (2009). Copyright 2009 Elsevier. **c** The microsystem for cardiac spheroid culture with nanowires. Reprinted with permission from Richards et al. (2016). Copyright 2016 American Chemical Society

proposed by Richards et al. (2016). They developed a microsystem for spheroid culture of hiPSC-CMs with silicon nanowires (Fig. 8.6c). Electrical stimulation was used to form the intercellular connections and the spontaneous contraction of CMs. The formed cardiac spheroids were used as a 3D model for in vitro investigation of heart muscle contraction.

## 8.2 Toxicity Assays

The pharmaceutical industry spends a lot of money on implementing new drugs in the market. In addition, it is a long-term process (about 10–15 years). Nonetheless, many of the implemented drugs are removed from the market due to their side effects, which are very often associated with cardiotoxicity. Some of the nonsteroidal anti-inflammatory drugs (NSAID) (e.g., rofecoxib, cisapride, tegaserod) belong to this group of compounds (Mordwinkin et al. 2013). Preclinical drug development can be limited by high cardiotoxicity of compounds developed to treat CVDs and other diseases. Detecting the potential side effects of drug action can reduce the cost of drug production and implementation. Therefore, in vitro cardiac cell models have two main purposes: (1) evaluating cardiotoxicity of new and approved drugs used for the treatment of various diseases, (2) evaluating therapies and drugs used to treat CVDs (Fermini and Fossa 2003). Toxicological and pharmacological tests performed on cardiac models play an important role in the development of personalized medicine (Zhang et al. 2016d). In vitro studies are conducted to investigate both the degree of cardiotoxicity and the preliminary assessment of the drug dose, which is safe for the heart. There are many classes of drugs, including non-cardiovascular ones, that cause significant side effects to heart tissue (e.g., anthracyclines and other cytostatic antitumor drugs, some antipsychotic drugs, and NSAIDs). Anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin) as well as mitoxantrone, dasatinib, imatinib, and trastuzumab are anticancer drugs, which exhibit high cardiotoxicity (Kim et al. 2011; Zhang et al. 2017). Antipsychotics (e.g., clozapine, droperidol, thioridazine) can also disturb heart functions. They can cause heart inflammation, delay of cellular depolarization or tachycardia (Nozaki et al. 2014). Based on in vivo and in vitro studies, it is also known that analgesic drugs (e.g., naproxen, diclofenac, celecoxib, ketoprofen, ibuprofen) may also increase the risk of CVDs (Force and Kolaja 2011).

So far, cardiotoxicity is most often studied based on conventional, macroscale tests (Esch et al. 2014). To test the effect of chemical compounds and drugs, the viability of the cells is evaluated. Additionally, expression of cardiac markers is analyzed. Because CMs have a specific feature (a spontaneous contraction), the influence of compounds on cell beating is also investigated. Parameters such as the level of calcium ions and ROS generation are also monitored in CMs after drug exposure (Cheah et al. 2010; Saric et al. 2016). An important goal of cellular engineering is the development of in vivo-like heart culture models, in which cardiac cell functions as well as cardiotoxicity of various drugs will be possible to

measure (Chan et al. 2013). There are several reports on *Heart-on-a-chip systems,* which have been reported to be promising tools for drug testing (Boudou et al. 2012; Kaneko et al. 2007; Sidorov et al. 2017) (see Table 8.1). Drugs used for CVD therapy, e.g., isoproterenol (nonselective β-adrenergic agonist used to treat bradycardia) and verapamil (calcium channel blocker with antiarrhythmic properties) have often been investigated in *Heart-on-a-chip systems* (Agarwal et al. 2013; Marsano et al. 2016; Tomecka et al. 2018). Agarwal et al. (2013) presented a microfluidic system based on muscular thin films (MTFs) as a new approach for cardiotoxicity measurements (Fig. 8.7a). The microsystem was integrated with a transparent polycarbonate layer for recording MTF deformation, aluminum heating elements, and platinum electrodes for electrical stimulation. Neonatal rat ventricular myocytes were cultured on a thin flexible PDMS film, which was microprinted with fibronectin. Cardiac contraction was investigated after isoproterenol exposure (1 nM–100 μM). It was observed that cell contraction is dependent on drug dosage and concentration. The proposed microplatform is a promising tool possible to use as a commercial platform for cardiotoxicity analysis of various drugs. Cytotoxicity assays performed on spatial cultures could have a high impact on development of heart research. Such approach has been presented by Marsano et al. (2016). They investigated cardiotoxicity of isoprotenerol on human cardiac microtissues (3D culture) in a *Heart-on-a-chip* system. Mechanical and electrical stimulations were used to mimic in vivo-like conditions during the experiments. An increase in the cardiac cell contraction rate was observed after cell exposure to isoproterenol ($10^{-12}$–$10^{-6}$ M) and electrical stimulation (square pulse, a duration of 4 ms, a frequency of 1 Hz). A microscopic observation is most often used to measure cell viability in the microsystems. Therefore, electrical impedance spectroscopy was proposed as the noninvasive, real-time, and quantitative method to study cardiotoxicity in a microdevice (Zhang et al. 2016a). The device consisted of polystyrene chambers and nonconductive substrate with gold electrodes (Fig. 8.7b). Verapamil (in the range of 0–2 μM) as a drug used for CVD treatment and doxorubicin (in the range of 0–15 μM) as an anticancer drug were selected for the experiments. The viability and contractions of CMs isolated from neonatal rats after exposure to the tested compounds were studied. The results showed that impedance spectroscopy can be successfully used for real-time determination of drug cardiotoxicity in the microsystems.

As it was mentioned above, there are drugs, which cause significant side effects to heart tissue. Based on this, the *Heart-on-a-chip* systems applied for cardiotoxicity analysis of antipsychotic and anticancer drugs are more and more developed (Grosberg et al. 2011; Hansen et al. 2010; Kaneko et al. 2007). For example, Grosberg et al. (2011) used a static culture of neonatal rat ventricular myocytes to study the cardiotoxicity of epinephrine—a catecholamine neurotransmitter with properties that stimulate myocardial contractility. Kaneko et al. (2007) presented a microsystem for investigation of the cardiotoxicity of haloperidol (antipsychotic drug) on a single cardiac cell culture. It should be noted that a new microplatform based on engineered heart tissue (EHT) was also developed to test drug

**Table 8.1** Toxicity assays based on *Heart-on-a-chip* systems

| Type of cells | Type of culture | Culture conditions | Investigated drug | Drug properties | Assay(s) | References |
|---|---|---|---|---|---|---|
| Neonatal rat ventricular myocytes | Multicellular cell culture (3D) | Perfusion | Isoproterenol (1 nM–100 μM) | Nonselective beta-adrenergic agonist, used for treatment of heart disease (e.g., heart attack, congestive heart failure)<br>Possible side effects:<br>• tachycardia,<br>• palpitations,<br>• ventricular arrhythmias,<br>• tachyarrhythmias | Optical analysis of contraction (conductivity measurements) and immunostaining | Agarwal et al. (2013) |
| Neonatal rat heart cells | Multicellular cell culture (3D) | Static | Chromanol 293 B (1–100 μM)<br>Doxorubicin (0.1–1 μM) | Antiarrhythmic drug, blocker of the slow delayed rectifier $K^+$ current via potassium channels.<br>Anticancer drug, anthracyclines antibiotic with cytostatic effect | Optical analysis of contraction and relaxation time | Hansen et al. (2010) |
| Neonatal rat ventricular myocytes | Multicellular cell culture (3D) | Static | Epinephrine ($10^{-12}$–$10^{-4}$ M) | Sympathomimetic amina, hormone and neurotransmitter, drug for stimulating cardiac contraction. Used to treat anaphylaxis and cardiogenic shock | Measurement of cell contraction, and quantification of action potential propagation, immunostaining | Grosberg et al. (2011) |
| Embryonic mouse cardiomyoblast | Single cell | Static | Haloperidol (1 μM) | Antipsychotic drug<br>Possible side effects:<br>• ventricular arrhythmias<br>• abnormal beating state of cardiac tissue | Measurement of beating rhythm with a video image recording method | Kaneko et al. (2007) |

(continued)

**Table 8.1** (continued)

| Type of cells | Type of culture | Culture conditions | Investigated drug | Drug properties | Assay(s) | References |
|---|---|---|---|---|---|---|
| Neonatal rat cardiomyocytes | Multicellular cell culture (2D and 3D) | Perfusion | Isoprenaline (Isoproterenol) ($10^{-12}$–$10^{-6}$ M) | Adrenoreceptors ($\beta_1$ and $\beta_2$) agonist, drug used for the treatment of bradycardia (slow heart rate), heart block, and rarely for asthma | Viability tests: fluorescent staining with calcein AM and ethidium homodimer-1. microscopic observation, measurement of beating rhythm with a video image recording method, immunostaining | Marsano et al. (2016) |
| Rabbit ventricular myocytes | Multicellular cell culture (3D) | Perfusion | FCCP | Protonophore ($H^+$ ionophore) and uncoupler of oxidative phosphorylation in mitochondria, inhibit a background $K^+$ current and induce a small inward current and induce a rise of intracellular $Na^+$, stimulates $Mg^{2+}$-ATPase activity; used to simulate heart hypoxia | Monitoring myocyte shortening and intracellular $Ca^{2+}$ transients (using fluo-3 fluorescence) | Klauke et al. (2003) |
| Rat heart myocardium | Multicellular cell culture (3D) | Perfusion | FCCP | As above | Analysis of mitochondrial membrane potential and caspase-3 activity, fluorescent staining and microscopic observation | Ren et al. (2013) |

*2D* two-dimensional, *3D* three-dimensional, *FCCP* cyanide-p-trifluoromethoxyphenylhydrazone

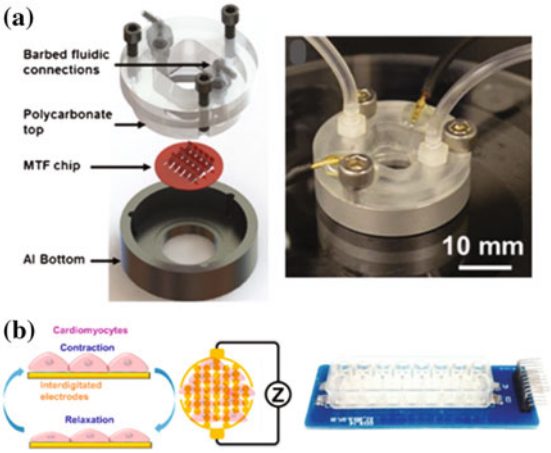


**Fig. 8.7** **a** A scheme and photograph of the MTF (muscular thin films)-based microsystem. Reprinted with permission from Agarwal et al. (2013). Copyright 2013 Royal Society of Chemistry. **b** A scheme and a photograph of *Heart-on-a-chip* system with integrated electrodes for verapamil testing using impedance detection method. Reprinted with permission from Zhang et al. (2016a). Open access

cardiotoxicity (Hansen et al. 2010). The microplatform was used to study compounds such as: chromanol 293B (potassium channel blocker), quinidine and erythromycin (both used to treat arrhythmia), and doxorubicin (an anticancer drug). To create EHT-like culture model, neonatal rat heart cells were mixed with fibrinogen and Matrigel with thrombin, and they were cultured in the microdevice. Cell contraction was observed 8–10 days after cell seeding. Such cultures were exposed to the above listed drugs. Cell response (e.g., contraction) to the drugs was monitored using a video-optical technique. The authors confirmed that chromanol, quinidine, and erythromycin (depending on the concentration) increased the relaxation time, and doxorubicin decreased the contraction force. The developed microplatform can be useful for clinical pharmacology studies.

*The Heart-on-a-chip* systems can be used not only for analysis of drug cardiotoxicity, but also for mimicking CVDs. Heart diseases such as hypoxia, arrhythmia, ischemia, or myocardial infarction have been analyzed in the *Heart-on-a-chip* systems (Chen et al. 2017; Grosberg et al. 2011; Klauke et al. 2003; Ren et al. 2013; Sidorov et al. 2017). Ren et al. (2013) presented a PDMS microsystem for the dynamic study of hypoxia-induced myocardial injury in a controlled microenvironment. The microsystem consisted of three microchannels: central one used for culture and investigation of rat myocardium cells (H9C2) and two side microchannels for introduction of a culture medium and tested solutions (Fig. 8.8a). Hypoxia-induced myocardial injury was simulated using FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone) (50 μM for 2 h). To study the hypoxic injury dynamics of H9C2 cells, the mitochondrial membrane potential and caspase-3 activity of the cells were analyzed. The usage of FCCP caused disintegration of the cytoskeleton and loss of mitochondrial membrane potential of the myocardium cells. It was noticed that the developed microsystem can be successfully used to mimic physiological and pathological conditions in heart tissue, and it has a high potential to study heart regeneration. The second microsystem, which can have also high impact on heart-based research, is a microsystem mimicking the

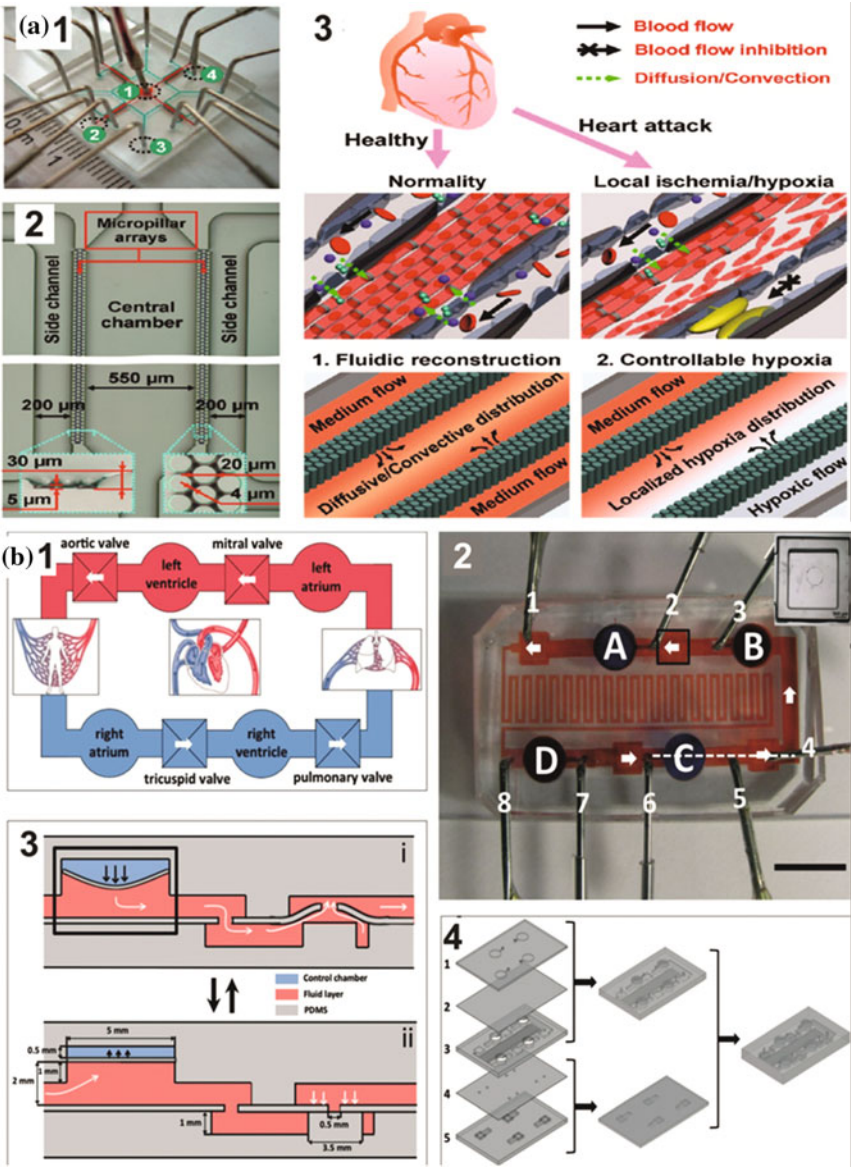


**Fig. 8.8** **a 1**, **2** Geometry and photograph of the designed microsystem. **3** A scheme of processes performed in the microsystem for studying controllable myocardial hypoxia. Reprinted with permission from Ren et al. (2013). Copyright 2013 American Chemical Society. **b 1** Scheme of the microfluidic circulatory system. **2** A photograph of the fabricated microsystem. **3** A scheme of pumps and valves system. **4** The geometry of the microsystem and layers forming the microsystem. Reprinted with permission from Chen et al. (2017). Copyright 2017 Royal Society of Chemistry

function of a whole cardiovascular system (Chen et al. 2017). The designed microsystem was integrated with a cardiac-like, on-chip pumping system. It consisted of four pumps and valves, which imitated heart atriums, ventricles, and valves. It generated a one-way, circular flow of a culture medium (Fig. 8.8b). HUVECs were cultured in the microsystem, and their response to mechanical forces generated inside the microchannels was investigated. The fabricated microsystem can also be used as a microtool for modeling vascular diseases such as: bradycardia (a lower heart beat rate compared to healthy tissue) and hypotension (lower peak pressure compared to healthy tissue). The methods based on the pressure changing inside the microchannels were used to mimic these diseases in the microsystem.

## 8.3 Electrical Field

CMs in the native heart tissue form a complex network of neighboring and contacting cells. The pacemaker cells, which are 1% of cardiac cells, regulate the transmission of electrical pulses to the other cells of the heart. These cells are responsible for the generation of electrical pulses or action potentials that maintain electrical connectivity across the tissue (Dorn et al. 2015). Because electrical pulses are presented in the native heart tissue, such conditions should also be mimicked in vitro. For this purpose, an external electrical field is utilized. Electrical stimulation influences the rate, duration, and number of CM action potentials. It induces CM contraction and increases the number of spontaneously beating cells. Moreover, the electrical field affects the transport of calcium ions between the cells. Electrical stimulation of CMs was studied in both macro- and microscale (Barash et al. 2010; Maidhof et al. 2012; Zhang et al. 2013a; Vacek et al. 2011). Studies have shown that electrical stimulation influences CMs at the molecular level. It has influence on the formation of gaps and intercellular connections and the increase of the expression of cardiac markers. An electrical field was used to stimulate cell migration and orientation (parallel to each other) (Mannhardt et al. 2017; Shin et al. 2016).

Parameters such as electrical field value, signal type, frequency, pulse duration, and exposure time have to be optimized during electrical stimulation (Tandon et al. 2010, 2011). Biphasic square pulses, in the range of 1–20 V $cm^{-1}$, at a frequency of 1–10 Hz and duration impulse between 1 and 4 ms are parameters most often used for CM stimulation. There are two main methods utilized for generation of electrical field in the *Heart-on-a-chip* systems. The first is based on the placement an anode and a cathode in a culture medium. Thanks to this, a uniform electrical field is obtained (Ribas et al. 2016; Serena et al. 2009). Although this method can be easily integrated with the microsystems, it has a few disadvantages. For instance, a single cell is not well characterized during the stimulation and it is possible to generate a pH gradient in the culture microenvironment. The integration of the microsystems with planar electrodes and multi-unit electrode arrays (MEAs) is the next method used to generate electrical field (Ma et al. 2012; Natarajan et al. 2011;

Simmons et al. 2012; Yu et al. 2012). The example of MEA used for CM stimulation is shown in Fig. 8.9a (Natarajan et al. 2011). MEA allows electrical field to be precisely regulated. Thanks to the utilization of such microarrays, it is possible to stimulate a whole cell population as well as a single cell. MEAs can be used to generate a high value electrical field (0.1–10 V) on a small working surface (instead of using high absolute voltages). An important benefit of MEAs is that they allow both stimulation of the CMs and real-time recording of CMs exposure to various external factors (Werdich et al. 2004).

Electrodes made of various types of materials are utilized for cell stimulation in *Heart-on-a-chip* systems: e.g., stainless steel, carbon platinum, gold, indium tin oxide (ITO) (Table 8.2) (Jastrzebska et al. 2016; Serena et al. 2009; Tandon et al. 2010; Wei et al. 2011). Gold or platinum wires are most often integrated with the microsystems (Chen et al. 2009; Klauke et al. 2003). Stretchable and flexible electrodes made of a thin layer of gold are also used (Rogers et al. 2010). Spiral electrodes made of carbon nanotubes (Khang et al. 2008) and silver nanoparticles (Ahn et al. 2009) have also been reported as new types of electrodes integrated with the microsystems.

Different types of cell cultures (single-cell, monolayer, and 3D cultures) were stimulated with an electric field in the microsystems. The type of culture model has

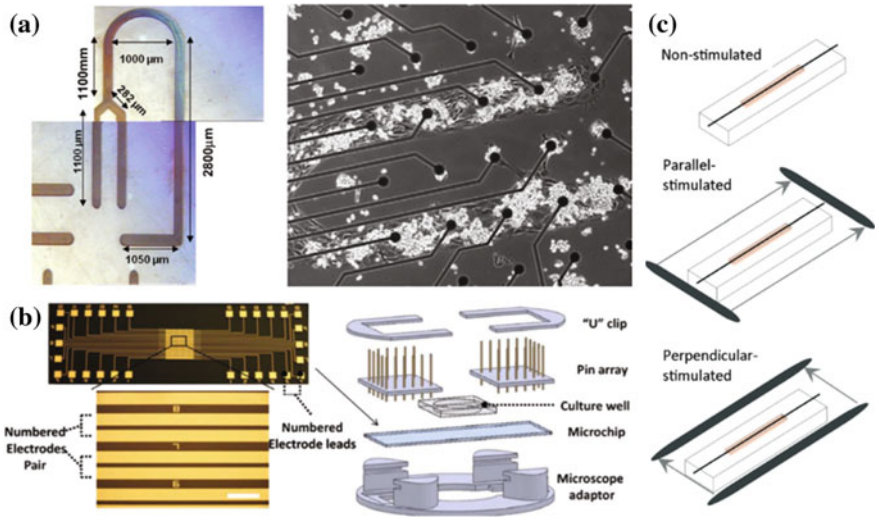


**Fig. 8.9** Examples of the microsystems integrated with electrodes with different geometries. **a** A pattern design and dimensions (electrode distance 200 µm) on microelectrode arrays (MEA) for cardiomyocyte stimulation. Reprinted with permission from Natarajan et al. (2011). Copyright 2011 Elsevier. **b** Micropatterned interdigitated gold electrodes on a glass slide. Reprinted with permission from Zhang et al. (2013b). Copyright 2013 Royal Society of Chemistry. **c** Black carbon rods in the microsystem connected to an external stimulator. They provided either parallel or perpendicular electrical field stimulation on cardiac cells. Reprinted with permission from Xiao et al. (2014). Copyright 2013 Royal Society of Chemistry

**Table 8.2** Electrical stimulation of the heart cells in the microsystems

| Cell line | Electrode material | Electrode geometry | Work parameters | Culture type | References |
|---|---|---|---|---|---|
| Neonatal rat ventricular myocytes | Carbon graphite, titanium, stainless steel, titanium nitride-coated titanium | Two rods 4 cm in length, placed 1 cm apart | Amplitude: 1–6 V $cm^{-1}$<br>Duration: 0.25–10 ms<br>Frequency: 1, 3, 5 Hz | 3D collagen scaffolds, static conditions | Tandon et al. (2011) |
| Neonatal rat cardiomyocytes | Gold | Two rods, placed 1 cm apart, oriented either parallel or perpendicular to the cultured cells | Amplitude: 1.15 V $cm^{-1}$<br>Duration: 1 ms<br>Frequency: 1 Hz | 2D monolayer cell culture | Au et al. (2009) |
| Rat cardiac myocytes | Gold | Array of interdigitated microelectrodes (comb-like geometry) | Duration: 8 ms<br>Frequency: 1 Hz | Single cells | Zhang et al. (2013b) |
| Neonatal rat cardiomyocytes | Carbon | Two parallel rods | Amplitude: 6 V $cm^{-1}$<br>Duration: 1 ms<br>Frequency: 0.2 Hz | 3D multicellular culture | Boudou et al. (2012) |
| Neonatal rat ventricular myocytes | Platinum | Array of electrodes—microcombs | Bipolar square pulse: 10–15 V<br>Duration: 10 ms<br>Frequency: 2 Hz | 3D multicellular culture | Agarwal et al. (2013) |
| Neonatal rat cardiomyocytes | | Planar 10 μm electrodes, 200 μm separation, microelectrode arrays (MEA) type | Bipolar pulse: 500 mV<br>Duration: 1 ms<br>Frequency: 2 Hz | 2D monolayer cell culture | Natarajan et al. (2011) |
| Neonatal rat cardiac cells | Carbon | Two integrated rods | Intensity: 74.4 mA $cm^{-2}$<br>Duration: 2 ms<br>Frequency: 1 Hz | 3D multicellular culture | Barash et al. (2010) |

(continued)

**Table 8.2** (continued)

| Cell line | Electrode material | Electrode geometry | Work parameters | Culture type | References |
|---|---|---|---|---|---|
| Neonatal rat cardiac cells | Carbon | Two parallel rods | Duration: 2 ms<br>Frequency: 10 kHz | 3D scaffolds | Maidhof et al. (2012) |
| Neonatal rat cardiomyocytes | Carbon | Two rods placed 2 cm apart | Amplitude: 3.5–4 V $cm^{-1}$<br>Duration: 1 ms<br>Frequency: 1.2 Hz | 3D multicellular culture | Xiao et al. (2014) |
| Adult rabbit ventricular myocytes | Platinum | Two types of microelectrodes into the arrays: a pair of 20 μm wide and 1.5 mm long line electrodes (200 μm apart) | Amplitude: >50 V $cm^{-1}$<br>Duration: 1–2 ms<br>Frequency: 1 Hz | Single cells | Cheng et al. (2010) |

an influence on the phenotype and functions of CMs. Several reports based on 2D cultures have been presented in the literature (Agarwal et al. 2013; Au et al. 2009; Cheng et al. 2010; Natarajan et al. 2011). Single adult rabbit ventricular myocytes isolated from the left ventricle were cultured and stimulated in a microsystem consisting of 15 microwells (Cheng et al. 2010). Each microwell was integrated with a set of five microelectrodes (two Pt stimulating electrodes, a Pt working electrode, a Pt counter electrode, and an Ag/AgCl reference electrode). Au et al. (2009) developed a microfluidic platform for CM culture and their electrical stimulation. The microsystem made of polystyrene (PS) was used for investigation of neonatal rat CMs. The microsystem consisted of microgrooves and microridges with a precisely defined depth (400 nm), width (0.5 or 3 μm wide grooves and 0.5 or 1 μm wide ridges), and periodicity (1 and 4 μm). Smooth polystyrene surfaces were used as control samples. The designed microsystem was integrated with two gold electrodes. The electrodes were oriented in such a way that they were placed either parallelly or perpendicularly to the microgrooves. Such an electrode arrangement enabled the investigation of the dependence between topographical factor and electrical signal. The cells were treated with symmetric biphasic pulses (electrical field of 1.15 V $cm^{-1}$, a frequency of 1 Hz, a duration of 1 ms) for 7 days. Sarcomere α-actin staining showed that such stimulation caused elongation and alignment of the cells along the microgrooves. It was also observed that cellular orientation was greatly determined by the topographical signals. Electrical field stimulation further enhanced cardiomyocyte elongation, when microgrooves were oriented parallelly to electrical field. The obtained results showed that the presented microsystem can be a useful tool for drug development (tests for verapamil were also performed). Zhang et al. (2013b) presented a microsystem used for electrical stimulation of adult cardiac myocytes (Fig. 8.9b). The developed microsystem was used to study the intercellular electromechanical transduction by measuring the contractile performance of the stimulated and non-stimulated cells. The microsystem was integrated with micropatterned gold electrodes (a width of 200 μm). CMs isolated from the left heart ventricle were exposed to an electric voltage pulse with a frequency of 1 Hz and a duration of 8 ms. The measurements were performed on the cells, which were placed between the two neighboring electrodes. Cell contraction was determined by measuring the change of cell length. Researches demonstrated that the presented microsystem is useful in studying the efficiency of gap junctions in adult cardiac myocytes.

Electrical field can also induce synchronous contractions in spatial cell cultures (Radisic et al. 2004). 3D cultures performed in the microsystems and application of electrical field are recognized as a useful method in regenerative medicine. The *Heart-on-a-chip* systems based on a 3D culture are utilized for investigation of electrical stimulation more and more (Barash et al. 2010; Boudou et al. 2012; Hirt et al. 2014; Lind et al. 2016; Schroer et al. 2017; Xiao et al. 2014). Boudou et al. (2012) developed an interesting microplatform for measurement and manipulation of 3D cardiac cell models using carbon electrodes. Cardiomyocytes isolated from neonatal rat, cultured as cardiac microtissues (CMTs) in collagen and fibrin 3D matrices, were used in the experiments. Two parallel carbon electrodes placed on

both sides of the microplatform were used for cell stimulation. The CMTs were stimulated using biphasic pulses (6 V $cm^{-1}$, 0.2 Hz, 1 ms). The effect of electrical stimulation on cell alignment and force generation within CMTs was investigated in this study. It was noticed that electrical stimulation improves both the structure and the function of CMTs. It is also important that the developed microplatform can become a potential microtool for monitoring the action of drugs on electrically stimulated 3D cardiac cell models.

The influence of electrical field, and dependence of electrodes arrangement, was investigated in 3D micro-tissue biowires (Xiao et al. 2014). The biowires integrated with carbon rod electrodes were utilized for investigation of primary neonatal rat CMs and human embryonic stem cell-derived CMs (hESC-CMs). Different electrical stimulation (biphasic, rectangular, 1 ms duration, 1.2 Hz, 3.5–4 V $cm^{-1}$, for 4 days) conditions were applied dependent on a cell type. Rat cardiac biowires were stimulated using both the parallel stimulation chambers (two carbon rods placed 2 cm apart and perpendicular to the biowires) and the perpendicular stimulation chambers (two carbon rods placed 1 cm apart and parallel with the biowires) (Fig. 8.9c). Cell function was analyzed based on the immunostaining of cardiac Troponin T and connexin-43 as well as the mechanical properties of the cells. The obtained results showed that the proposed microdevice can be successfully utilized for investigation of the influence of electrical stimulation on cell functions.

Understanding the role of electrical stimulation in cell metabolic pathways is important in the field of heart tissue engineering. Although many reports based on electrical stimulation of CMs in the microsystems have been presented in the literature, there are still research areas, which have to be deeply studied: e.g., imitation of fully functioning heart tissue, mimicking of CVDs, and regeneration of the CMs based on electric fields (Schroer et al. 2017).

## 8.4 Monitoring CM Functions

Developing a microsystem especially for culture and investigation of heart cells is associated with selection of methods for analysis of cell functions (Fig. 8.10). Based on the features, which characterize heart cells (especially CMs) parameters such as the expression of cardiac markers, cell contraction/beating, and the level of calcium ions, are often determined in the *Heart-on-a-chip* systems. Besides the above-mentioned assays, cell proliferation, cell viability as well as cell morphology are studied (Mordwinkin et al. 2013). To monitor the CM functions listed above, various instrumental (electrochemical and optical) techniques are utilized.

The examples of *Heart-on-a-chip* systems described in previous sections showed that they are appropriate tools to perform both heart cell cultures and cardiotoxicity studies. Analysis of growth and proliferation of the CMs are mainly studied in the microsystems used for cell culture, toxicity assays, and electrical stimulation. The proliferation and viability of the cells can be determined by differential staining with fluorescent dyes (e.g., Calcein AM and Propidium iodide and

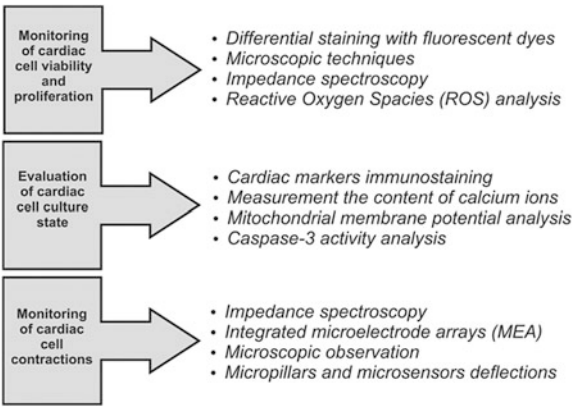


**Fig. 8.10** Most common methods used for monitoring cardiac cell viability, proliferation, and contractions in the microfluidic systems

Ethidium homodimer-1) and microscopic observations (e.g., confocal microscopy, optical and fluorescence microscopy) (Ghiaseddin et al. 2017; Marsano et al. 2016; Ren et al. 2013). Besides that, cell proliferation and CM maturation after an external stimulus can be determined by analyzing the expression of cardiac markers. Expression of typical cardiac markers and expression of structural proteins such as sarcomere α-actin, cardiac troponin I, cardiac troponin T, connexin-43, myosin heavy chain 6 (MYH6), and myosin light chain 2a are the most often monitored in heart cellular models (Belaguli et al. 2000; Serena et al. 2009; Tandon et al. 2010). These markers are determined by immunofluorescent staining with a fluorochrome (most often Alexa Fluor family). Grosberg et al. (2011) evaluated cell structure by immunofluorescent staining with Alexa Fluor 488-conjugated Phalloidin (F-actin, green dye) and Alexa Fluor 594-conjugated clone EA-53 (sarcomeric α-actin, red dye). Agarwal et al. (2013) proposed similar immunostaining for analyzing the formation of the anisotropic monolayer of CMs. They used immunostaining with Alexa Fluor 633-conjugated Phalloidin (F-actin, red dye) and Alexa Fluor 546-conjugated clone EA-53 (sarcomeric α-actin red dye). To determine the expression of cardiac markers, similar parameters were used by other research groups.

Cyclic voltammetry can also be utilized to determine viability and proliferation of cardiac cells cultured in the microsystems (Cheah et al. 2010). Such a technique allows the amount of reactive oxygen species to be measured in real-time. The calcium ion level in the cells was used for monitoring cardiac cell viability and proliferation. For this purpose, fluorescent dyes can be used (fluo-3 or fluo-4) (Klauke et al. 2003). The amount of calcium ions can be evaluated for normal (healthy) as well as hypoxia-induced cardiac hypertrophy (disease) conditions. Additionally, the mitochondrial membrane potential (using JC-1 indicator) and caspase-3 activity of the cells can be analyzed after an external stimulus. These parameters are used to evaluate culture states (He et al. 2014; Ren et al. 2013). Transmission electron microscopy is also applied to analyze micro-tissue cell models. Such a technique is used, e.g., to determine the morphology and pore

structure of the prepared hydrogels placed in the microsystems (Ghiaseddin et al. 2017). Cell morphology and arrangement are monitored using microscopy techniques. Typical cardiomyocyte structural properties such as glycogen granules, mitochondria, myofibrils, sarcoplasmic reticulum striated sarcomeres, A-bands, I-bands, and Z-lines can be successfully determined using microscopic techniques (Mordwinkin et al. 2013).

In the recent years, impedance spectroscopy (IS) is intensively developing technology for analysis of cell proliferation in the microfluidic systems (Zhang et al. 2016a). IS is a noninvasive electrochemical method, which can be used for real-time monitoring of cell proliferation, viability as well as contraction (Dean et al. 2007; Min et al. 2003; Qiu et al. 2008, 2009). Contraction is the most specific feature of CMs. This parameter is determined using techniques such as: microscopic observation, impedance spectroscopy and MEA techniques, micropillar and microsensor deflections (Marsano et al. 2016; Natarajan et al. 2011; Tanaka et al. 2007). Many of analytical techniques, applied to monitor CM functions in the microsystems, are based on qualitative analysis. Such methods are especially used for spatial cell cultures. Therefore, it is important to elaborate quantitative, repeatable methods, which can be validated and implemented not only for 2D but also for 3D cell cultures in the *Heart-on-a-chip* systems.

## 8.5 Summary and Perspectives

The *Heart-on-a-chip* systems are used to create in vivo-like culture models. They provide new possibilities in many biological and preclinical studies. Properly designed microstructures of the microsystems provide an opportunity to perform rapid drug screening and analysis of the effects of external stimulation. Thanks to this, new mechanisms and cardiac cell functions can be discovered and can consequently be useful in regenerative medicine. Many examples of heart culture models have been presented in the literature. There are *Heart-on-a-chip* systems for single, monolayer, and spatial cell cultures, in which the cardiotoxicity of different drug groups as well as the influence of external stimulation on cardiac cell cultures were studied.

Because heart cells have specific features (dynamic conditions, stretching and electrical impulses), the *Heart-on-a-chip* systems have to be equipped with the elements, which ensure such properties. This brings many challenges during the development of the microsystems for cardiac cell culture. The most important problem is the origin of the beating cardiomyocytes. To obtain such cells, some embryo and neonatal specimens are utilized. Therefore, cells coming from animals are the most often used. Human cardiac cells from adults are also investigated; however, they are often characterized by the lack of the beating. Therefore, beating stem cell-derived cardiomyocytes (SC-CMs) have been increasingly cultured. Although a number of the microsystems for heart cell cultures (e.g., for single, 2D, and 3D cell cultures) is developed, they present not fully functioning heart model.

The research, in which these cardiac models are used, still is in the early stage. However, the combination of different solutions, the microsystems and the culture methods, proposed by many research groups and described in this chapter, could improve investigation based on the *Heart-on-a-chip* systems and could implement such microsystems in a personalized medicine in the future. Some aspects should still be investigated and improved in the microsystems proposed so far. The combination of a whole vascular system with a 3D beating heart cellular model and study physiological and pathological conditions in such a fully mimicked cardiovascular system is a perspective for *Heart-on-a-chip* system developing. The usage of a spatial and vascular model for a detailed investigation of CM regeneration is also an important step which should be developed. So far, microscopic observations are mainly used to evaluate the state of 3D culture in the microsystems. Therefore, the elaboration of quantitative microfluidic methods for examination of biochemical processes in cardiovascular system is strongly important. This approach for *Heart-on-a-chip* study can be based on the usage of a perfusion microsystem enriched with additional structural elements for cellular spatial culture and components for automated analytical measurements (e.g., electrodes, integration with commercially available equipment). Moreover, the usage of a digitally controlled module can allow automated dosage of all fluids, and finally it can increase usefulness of such a microsystem in a personalized medicine.

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