Chapter 7
Microfluidic Systems for Cardiac Cell Culture—Characterization

Elzbieta Jastrzebska and Zbigniew Brzozka

7.1 Research Issue—Heart Diseases

Biomedical studies play a significant role in both science and daily life. Deep study in this research field, development of medical products and fabrication of medical devices, repeatedly contribute to save human lives. To diagnose and cure a particular illness, it is important to have a specific method, which enables scientists to detect and defeat a given ailment. However, the available methods for diagnosis and treatment of diseases are often not enough to recognize and diagnose all mechanisms responsible for an illness (Fryburg et al. 2014). Additionally, selecting the best treatment in clinical cases requires earlier investigation of the disease mechanisms. In vivo or in vivo-like assays are the best ways to test new drugs and treatment methods (Salyers 2009; Zhang et al. 2012). The existing in vitro testing models have many disadvantages (Katt et al. 2016). Consequently, it is necessary to conduct research for the development of new testing models, tools and methods, which could improve disease treatments. The developed solutions could be used by doctors not only to test new drugs but also to optimize treatment parameters. The cardiovascular system is one of such a research area, in which such solutions are needed.

Statistical analysis shows that cardiovascular diseases (CVDs), next to cancers, are the most common cause of death all over the world (World Health Organization 2014). CVDs increase the loss of cardiomyocytes (CMs), which simultaneously deprive cells of their ability to regenerate (Laflamme and Murry 2011). Treatment of heart diseases may include the usage of: various medicines, medical and surgical procedures as well as cardiomyocyte (CM) regeneration (Mampuya 2012; Sheng et al. 2013). The goals of the above methods are, among others, to relieve symptoms and reduce risk factors, which can cause heart attack as well as prevent

E. Jastrzebska (✉) · Z. Brzozka
The Chair of Medical Biotechnology, Faculty of Chemistry,
Warsaw University of Technology, Warsaw, Poland
e-mail: ejastrzebska@ch.pw.edu.pl

Z. Brzozka and E. Jastrzebska (eds.), *Cardiac Cell Culture Technologies*,
https://doi.org/10.1007/978-3-319-70685-6_7

CVD complications (Hobbs 2004). Lifestyle changes (healthy diet, no smoking, physical activity) are also crucial in treating and preventing CVDs. There are various kinds of drugs with different mechanism actions, which are used in cardiac therapy (Lundin et al. 2016). The common cardiac medications are divided into such groups as: anticoagulants, beta blockers, calcium channel blockers, digitalis preparations, cholesterol-lowering medications, angiotensin-converting enzyme (ACE) inhibitors, diuretics, and vasodilators. The effects of these drugs include, among others: the regulation of blood clotting (coagulating), decreasing blood pressure, decreasing low-density lipoprotein (LDL) cholesterol, reducing swelling from excess buildup of fluid in the body, and mitigation of chest pain (angina) (Lundin et al. 2016). The nonsurgical procedure—percutaneous coronary intervention (PCI, angioplasty) that opens blocked or narrowed coronary arteries is also used for the treatment (Hoyt et al. 2013). Another method is coronary artery bypass grafting (CABG). CABG involves removing arteries or veins from other areas of the body and using them to bypass narrowed or blocked coronary arteries (Iqbal et al. 2013). There are also advanced methods for treating heart failure such as heart transplants, artificial hearts, and mechanical devices supporting left ventricular function (Kozar-Kaminska 2012). However, these treatments are not widely available, and their usage is limited to a small group of patients. Because heart failure is becoming increasingly common, great emphasis is placed on the development of new methods, which improve and restore heart functions. Regenerative medicine has been playing an important role in cardiology. At the end of the previous century, it was stipulated that with the use of acquired knowledge and experience in tissue engineering, it should be possible to grow a fully functioning heart. However, engineered heart tissue (EHT) is still being developed. Stem cells (SCs) are more commonly used as an alternative therapy for heart diseases (Beeres et al. 2007; Zhang et al. 2015). SCs have the ability to regenerate and differentiate into other types of cells. Appropriate growth factors and external stimuli (electrical, mechanical, optical, or magnetic pulses) can differentiate SCs to the cardiac cells (Batalov and Feinberg 2015; Nadal-Ginard et al. 2014). Therefore, SCs constitute the material filling the destroyed CMs, which lack the ability to regenerate (Cambria et al. 2016). Additionally, scaffolds and multilayer cell cultures are used to improve in vivo conditions. EHT provide an in vitro model reproducing heart tissue, in which therapies for patients with CVDs can be investigated (Doppler et al. 2013; Schaaf et al. 2011; Sondergaard et al. 2012). Research conducted so far has shown that heart regeneration is a long and complex process. It could result from the fact that conventional in vitro methods have some obstacles restricting their application (Bernstein and Srivastava 2012; Lovell and Mathur 2010). Despite long and advanced research based on EHT development and improvement in regenerative medicine there are many aspects, which should be solved and improved. First of all, a large amount of SCs is required. The creation of a heart model with an appropriate system of vascularisation (angiogenesis) responsible for supplying nutritive substances is also difficult. Therefore, scientists have developed new methods for cardiac cell analysis and propose new in vivo-like models to understand processes present in a fully functioning heart.

7.2 *Lab-on-a-chip* Systems—A New Approach for Heart Investigation

Elaboration of new therapeutic methods for heart diseases is a great challenge for scientists and doctors dealing with this type of ailment. Therefore, to avoid problems which appear during research conducted in conventional laboratories, *Lab-on-a-chip* systems were developed (Mehling and Tay 2014; Ziolkowska et al. 2011). The aim of the fabrication of such systems is to develop an in vivo-like cardiac model, in which the investigation of cardiac cell processes, as well as the elaboration of new therapies for heart failure will be possible. In such microsystems, fully functioning and vascularized heart tissue could be mimicked (Jastrzebska et al. 2016; Lee et al. 2015). These models, called *Heart-on-a-chip* systems, are specific types of *Organ-on-a-chip* systems. The usage of the microsystems for cardiac cell engineering has many advantages. First of all, miniaturization allows for a reduction in volumes of reagents used for experiments. The microsystems allow also to shorten the time of assays and to automate the whole process of biological sample studies (Halldorsson et al. 2015; Sackmann et al. 2014; Tehranirokh et al. 2013). Additionally, it is possible to control precisely the spatio-temporal phenomena present in a microsystem. This is particularly important when difficult-to-isolate or expensive cells (e.g., primary and stem cells) are examined (Visone et al. 2016). However, the most important advantage of *Heart-on-a-chip* systems is the possibility to mimic in vivo conditions better than in conventional (two-dimensional, 2D) culture methods (Jastrzebska et al. 2016; Lee et al. 2015). A network of microstructures can be designed in such a way that the microenvironment created in Lab-on-a-chip systems is similar to natural cell growth. Additionally, microstructure dimensions are similar to the dimensions of the cells (Bhatia and Ingber 2014; Mehling and Tay 2014). The in vivo cardiovascular system is characterized by blood circulation, which allows necessary nutrients, oxygen, and hormones taken from components of the metabolism to be supplied to the cells (Pittman 2011). Because the microsystems provide dynamic conditions, culture models obtained in microscale brings even more to in vivo. The flow environment is an important property especially for cardiac cell cultures (Kobuszewska et al. 2017). The next feature is that the cells in tissue are surrounded by an extracellular matrix (ECM), the mixture secreted by the cells and filling the space between them (Alberts et al. 2002). ECM plays a significant role in cell behavior. It regulates intercellular communication and a dosage of various cellular growth factors absorbed in this matrix. Moreover, ECM maintains a spatial (three-dimensional, 3D) arrangement of the cells. Scaffolds, hydrogels, and multicellular spheroids are used to culture cells in such conditions (Bray et al. 2015; Nath and Devi 2016; Tomecka et al. 2018; Yan et al. 2015). However, conventional techniques (macroscale) have still not been optimized.

Cell culture methods used so far in conventional biological laboratories (e.g., using 96-well plates) do not ensure the conditions described above (Yamada and Cukierman 2007; Ziolkowska et al. 2011). A scheme of cell environments provided

in vivo and in vitro monolayer cultures is shown in Fig. 7.1. Monolayer cell culture does not correspond well to natural tissue conditions. However, the usage of the microsystems for spatial heart culture can allow to mimic in vivo environment and to control culture conditions precisely.

The systems based on *Heart-on-a-chip* have two main approaches (i) the creation of a beating heart culture model, which mimics heart tissue and (ii) creation of a whole vascular system, which mimics blood flow in vessels (Fig. 7.2) (Lee et al. 2015; Ribas et al. 2016; Simmons et al. 2012). The development of a beating heart model allows to investigate cardiomyocyte contraction under various conditions (static and dynamic). *Heart-on-a-chip* systems can also be utilized for investigation of an external stimuli influence on cardiomyocyte growth as well as stem cell cardiogenesis. Parameters such as oxygen concentration, pH value, hydrodynamic

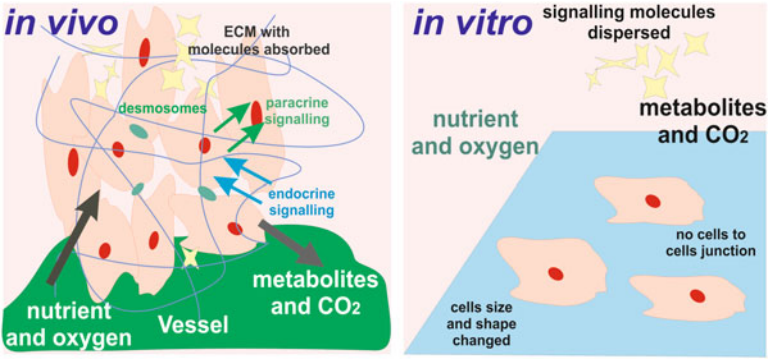

Fig. 7.1 A scheme of microenvironments provided in vivo and in vitro two-dimensional (2D) culture

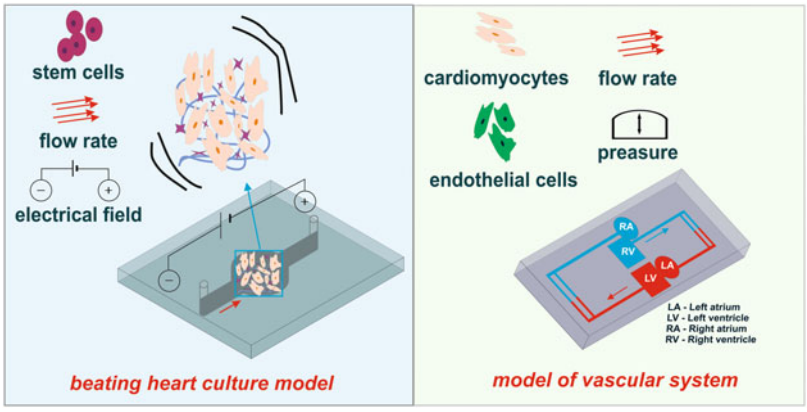

Fig. 7.2 Types of *Heart-on-a-chip* systems: model of beating heart culture, which mimic heart tissue (left) and model of a whole vascular system, which mimic blood flow in vessels (right)

stress, uptake of intracellular calcium ions, and lactic oxidase level were monitored in cardiac cultures maintained in microscale. Additionally, cytotoxicity of new compounds with cardiac therapeutic activity can be investigated. The microsystems mimicking either heart tissue or the whole vascular system can be good models for the understanding of heart phenomena as well as to verify drug usefulness in heart disease treatment (Jastrzebska et al. 2016; Lee et al. 2015; Simmons et al. 2012; Zhang et al. 2016a).

7.3 *Heart-on-a-chip* Systems—What Is Specific?

Organ-on-a-chip systems are in vivo-like models, which mimic functioning organs as well as which are used for investigation of both tissue disorders and drug cytotoxicity (An et al. 2015; Young 2013). Each in vitro organ model has parameters which are specific for that tissue. Therefore, to create a fully working heart model in microscale, it is necessary to know the properties of the specific tissue. Native myocardium is characterized by both parallel cardiac muscle fiber and complex electrochemical dynamics. Therefore, these facts are considered during the development of *Heart-on-a-chip* systems (Ribas et al. 2016). Electrochemical signals present in heart tissue are important because they regulate heart function and demonstrate spontaneous beating of CMs. A laminar flow and a pulsatile flow in the vasculature system are the next essential feature. The electrical field, stretching, the usage of hydrogels, or nanofibers are the key signaling parameters used to mimic native myocardium. Additionally, heart cell culture in the microsystems is often used to simulate heart diseases and investigate heart regeneration using stem cells (Fig. 7.3) (Ghafar-Zadeh et al. 2011; Visone et al. 2016).

7.3.1 Vasculature and Dynamic Conditions

The microenvironment occurring in the vasculature system, provided by blood flow, is characterized by dynamic conditions. Mimicking these conditions in vitro is important to study the influence of shear flow and shear stress on cell proliferation, morphology, and viability (Cheng et al. 2003; Dahl et al. 2010; Li et al. 2005; World et al. 2006). However, the imitation of a vascular environment is difficult. It results from the fact that components of such a system (arteries, veins, venules, and capillaries) have varying compositions. Because of the dynamic microenvironment presented in a body, the microfluidic systems are excellent tools for mimicking these conditions (Wong et al. 2012). This results from the fact that laminar flow is provided in the microsystems (Young and Simmons 2009). A low Reynolds number and shear stress profile in the microsystems are similar to these, which are presented in vivo. Another feature of microscale, which helps in developing

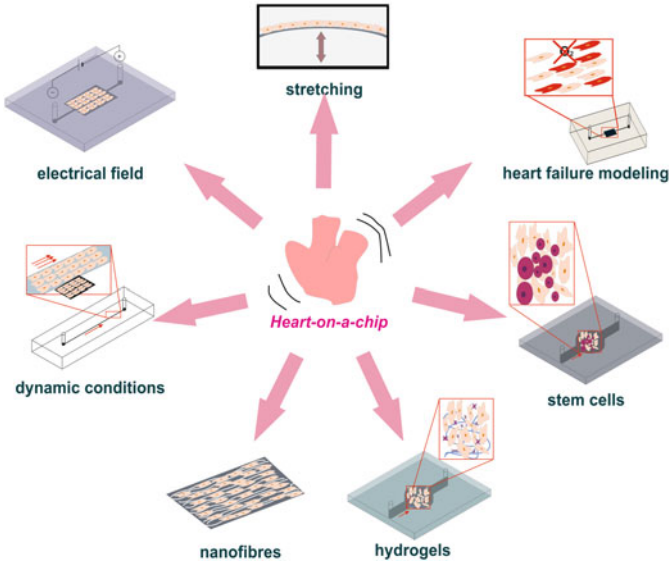

Fig. 7.3 Key signaling parameters used to mimic native myocardium in *Heart-on-a-chip* systems

Heart-on-a-chip systems is the fact that microtechnology allows various flow profiles to be precisely designed. Thanks to this, a network of microstructures can be designed in such a way that in various areas of a microsystem different cell types can be cultured and simultaneously different shear stress values can be generated (Rossi et al. 2009). Endothelial cells cushion the inner heart chamber and blood vessels, therefore these cells are most often used to mimic the vasculature system in microscale (Hasenberg et al. 2015; Morgan et al. 2013). So far several types of the microsystems have been developed to mimic vessel connections and angiogenesis formation. Additionally, the microsystems for chemotaxis study and vascular disease modeling have been fabricated (Ribas et al. 2016).

Perfusion is also an important factor in fabricating a model of a beating heart culture. Due to the fact, that the heart is still simulated by a flow rate, assays based on the comparison of static and dynamic conditions are performed in the microsystems. These tests are able to check how a flow rate influences on proliferation and contraction of CMs. Additionally, perfusion can enhance parallel CM arrangement, which is specific for native myocardium (Kobuszewska et al. 2017; Xiao et al. 2014).

7.3.2 Materials Versus Cell Alignment

Mimicking of cardiac tissue microenvironment is also related to the selection of construction materials of *Heart-on-a-chip* systems. To fabricate such systems, poly

(dimethyl siloxane) (PDMS) and glass are often used (Halldorsson et al. 2015; Ren et al. 2013). It results from the beneficial properties of these materials, which have been described in Chap. 3. To obtain a suitable microstructure/surface for cardiac cell culture, PDMS and glass are additionally modified. Native myocardial tissue is characterized by a parallel arrangement of the cells. Lack of such cell orientation is the main distinction between in vivo and in vitro cultures. Therefore, parallel cardiomyocyte alignment should be simulated in 2D and 3D in vitro models performed in microscale. Microcontact printing is a method used for obtaining ECM proteins on PDMS or glass plates and parallel CM alignment (Guillemette et al. 2010). Proteins such as collagen, laminin, and fibronectin have been used for this purpose. A fabrication of microgrooves in the culture surface has also been used to CM align (Motlhagh et al. 2003; Yang and Ma 2012). Scaffolds and hydrogels are other methods used to create a native myocardium environment. This can be achieved by the usage of regular nanofibers. Nanofibers have many advantages, which are useful for cell cultures: they have a high surface-to-volume (SAV) ratio and high porosity. Moreover, their structure and nanofiber organization influences the parallel orientation of CMs (Ashammakhi et al. 2012; Carletti et al. 2011). CM arrangement has been tested on nanofibers made of materials such as: poly(L-lactide-co-ε-caprolactone) [P(LLA-CL)] copolymer, poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone) (PCL), poly(hydroxybutyrate) (PHB), chitosan-polycaprolactone, polymethylglutarimide (PMGI) (Tomecka et al. 2017). The above mentioned materials can be placed on PDMS or glass surfaces as well as inside of the microsystems. Other method used for the creation of a spatial cardiac cell arrangement in the microsystems is the use of hydrogels. They allow to obtain uniform distribution of nutrients in 3D culture (Annabi et al. 2013). Hydrogels which are gelled under the influence of various external factors (ultraviolet irradiation, temperature, chemical factors) were used for the 3D culture in the microsystems (Hoffman 2012; Zhang et al. 2016b; Zuppinger 2016).

7.3.3 Stretching

Cell stretch, is the next feature, simulated in the microfluidic devices (Simmons et al. 2012). Regulating/moving of a surface, on which the cells are cultured, is used to stretch the cells. This is obtained by changing the pressure. Longitudinal, in-plane and out-of-plane mechanical simulations are used for this purpose. However, pressure microchannels are most often used to stretch a thin membrane made from elastic material (Huh et al. 2010; Moraes et al. 2010). This allows to mimic native conditions of heart cells.

7.3.4 Electrical Field

Electrical depolarization begins in Purkinje fibers and next propagates between the cardiomyocytes. This phenomenon is responsible for heart beat. Electrical stimulation is one method, which can be applied to cardiomyocyte depolarization and stimulation for contraction (Maidhof et al. 2012). Different types of electrical stimulation can be used for tissue engineering. It can be monophasic or biphasic stimuli, in a form of sinusoidal or square waves. Electrical signals can be delivered in pulses or continuously (Balint et al. 2012). The placement of an anode and cathode in the culture medium is the simplest method of inducing cell depolarization (Ribas et al. 2016; Serena et al. 2009). This allows to obtain a uniform electrical field across the cell culture. Either rods or microelectrodes are used in microscale. The integration of the microfluidic devices with planar electrodes and multi-unit electrode arrays (MEA) is the next method, which allows cell stimulation (Ma et al. 2012). In this case, the stimulation can be performed between two or more electrodes on which cells are cultured. Electrodes made of various types of materials are utilized for cell stimulation: stainless steel, carbon, platinum, gold, indium tin oxide (ITO). The integration of the microsystems with the electrodes allow to obtain controllable conditions, which influences cell contraction, alignment as well as differentiation.

7.3.5 Heart Failure Modeling

Lab-on-a-chip systems are used most often to analyze cell proliferation, migration, and interactions. Cytotoxicity assays of new drugs administered into the cells in a form of either solution or nanoparticles are also performed in the microsystems. Additionally, the mimicking of different organs is performed in microscale (Bhise et al. 2014). However, disease modeling is most often performed in *Heart-on-a-chip* systems. Arythmia, ischemia, and myocardium infarction were simulated and tested in microscale (Ren et al. 2013; Ribas et al. 2016). Ischemia was simulated by limiting the oxygen level or by the usage of a specific oxygen consumption blocking reagent: cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The microfluidic systems provide many possibilities to investigate the repair of "damaged" cardiac cells. Additionally, processes responsible for heart diseases can be studied in detail using in vivo-like models. Modeling of heart failure could be helpful in better understanding drug development and heart regeneration. The utilization of electrical fields, mechanical stimulation, SCs, or myocytes could be used to test cardiac cell regeneration in microscale.

7.3.6 *Co-culture with Stem Cells*

The microsystems used especially for heart cell cultures have also been used for co-culture. Because of the high importance of SC usage in regenerative medicine, these cells are most often used in co-culture with cardiac cells. They are utilized as a potential method to regenerate CMs (Garbern and Lee 2013). SCs can be differentiated into cardiac cells using different biochemical, mechanical, and electrical methods. Although, differentiation to various types of the cells is described in the literature there still are not too many reports about SC differentiation into cardiac cells (Ghafar-Zadeh et al. 2011; Jastrzebska et al. 2016). Most often such differentiation is started in macroscale, using conventional cultures and later differentiated cells are introduced and cultured in the microsystems (Mathur et al. 2015). The potential interaction between the cells as well as repair properties has been investigated using SCs (He et al. 2014).

7.4 Summary

Lab-on-a-chip systems for mimicking and studying heart cells are more often presented in the literature. Microtechnology allows in vivo conditions well to be mimicked. Therefore, the microsystems are increasingly used to simulate the vascular system, to culture cardiomyocytes (CMs) and to test their action after stimulation with various external factors. The microsystems for heart cell cultures have properties, which are the same as in another microsystems used especially for cell engineering, e.g., similar microstructure dimensions to cell dimensions, a laminar flow, high surface-to-volume (SAV) ratio, and effective culture volume (ECV). Besides that *Heart-on-a-chip* systems have additional features, which are specific for heart cells. Developing *Heart-on-a-chip* systems brings many challenges. It results from the fact that dynamic conditions, stretching, and electrical stimulation should be obtained in such microsystems. In the literature, various approaches and solutions have been presented, e.g., utilization of hydrogels and nanofibers, simulation of heart diseases. The microsystems presented so far have also a good potential to test the heart's function and regeneration with SCs. However, there are many aspects, which should still be investigated and improved in microscale, e.g., long-term CM culture, SC differentiation.

Acknowledgements This work was realized with the frame of projects LIDER No. LIDER/026/573/L-4/12/NCBR/2013 and SONATA 5 program No. UMO-2013/09/D/ST5/03887.

References

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) Molecular biology of the cell, 4th edn. Garland Science, New York

An F, Qu Y, Liu X, Zhong R, Luo Y (2015) Organ-on-a-chip: new platform for biological analysis. Anal Chem Insights 10:39–45

Annabi N, Selimovic S, Acevedo CoxP J, Ribas J, Afshar Bakooshli M, Heintze D, Weiss AS, Cropek D, Khademhosseini A (2013) Hydrogel-coated microfluidic channels for cardiomyocyte culture. Lab Chip 13:3569–3577

Ashammakhi N, Ndreu A, Yang Y, Ylikuppila H, Nikkola L (2012) Nanofiber-based scaffolds for tissue engineering. Eur J Plast Surg 35:135–149

Balint R, Cassidy NJ, Cartmell SH (2012) Electrical stimulation: a novel tool for tissue engineering. Tissue Eng Part B Rev 19:48–57

Batalov I, Feinberg AW (2015) Differentiation of cardiomyocytes from human pluripotent stem cells using monolayer culture. Biomark Insights 10:71–76

Beeres SL, Bengel FM, Bartunek J, Atsma DE, Hill JM, Vanderheyden M, Penicka M, Schalij MJ, Wijns W, Bax JJ (2007) Role imaging in cardiac stem cell therapy. J Am Coll Cardol 49:1137–1148

Bernstein HS, Srivastava D (2012) Stem cell therapy for cardiac disease. Pediatr Res 71:491–499

Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772

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

Bray LJ, Binner M, Holzheu A, Friedrichs J, Freudenberg U, Hutmacher DW, Werner C (2015) Multi-parametric hydrogels support 3D in vitro bioengineered microenvironment models of tumour angiogenesis. Biomaterials 53:609–620

Cambria E, Steiger J, Günter J, Bopp A, Wolint P, Hoerstrup SP, Emmert MY (2016) Cardiac regenerative medicine: the potential of a new generation of stem cells. Transfus Med and Hemother 43:275–281

Carletti E, Motta A, Migliaresi C (2011) Scaffolds for tissue engineering and 3D cell culture. Methods Mol Biol 695:17–39

Cheng CP, Herfkens RJ, Taylor CA (2003) Inferior vena caval hemodynamics quantified in vivo at rest and during cycling exercise using magnetic resonance imaging. Am J Physiol Heart Circ Physiol 284:H1161–H1167

Dahl KN, Kalinowski A, Pekkan A (2010) Mechanobiology and the microcirculation: cellular, nuclear and fluid mechanics. Microcirculation 17:179–191

Doppler SA, Deutsch M-A, Lange R, Krane M (2013) Cardiac regeneration: current therapies-future concepts. J Thorac Dis 5:683–697

Fryburg DA, Song DH, Laifenfeld D, de Graaf D (2014) Systems diagnostics: anticipating the next generation of diagnostic tests based on mechanistic insight into disease. Drug Discov Today 19:108–112

Garbern JC, Lee RT (2013) Cardiac stem cell therapy and the promise of heart regeneration. Cell Stem Cell 12:689–698

Ghafar-Zadeh E, Waldeise JR, Lee LP (2011) Engineered approaches to the stem cell microenvironment for cardiac tissue regeneration. Lab Chip 11:3031–3048

Guillemette MD, Park H, Hsiao JC, Jain SR, Larson R, Langer R, Freed LE (2010) Combined technologies for microfabricating elastomeric cardiac tissue engineering scaffolds. Macromol Biosci 10:1330–1337

Halldorsson S, Lucumi E, Gómez-Sjöberg R, Fleming RM (2015) Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosens Bioelectron 63:218–231

Hasenberg T, Muhleder S, Dotzler A, Bauer S, Labuda K, Holnthoner W, Redl H, Lauster R, Marx U (2015) Emulating human microcapillaries in a multi-organ-chip platform. J Biotechnol 216:1–10

He J, Ma C, Liu W, Wang J (2014) On-chip monitoring of skeletal myoblast transplantation for the treatment of hypoxiainduced myocardial injury. Analyst 139:4482–4490
Hobbs FDR (2004) Cardiovascular disease: different strategies for primary and secondary prevention? Heart 90:1217–1223
Hoffman AS (2012) Hydrogels for biomedical applications. Adv Drug Deliver Rev 64:18–23
Hoyt JR, Seth M, Hanson I, Dixon S, Share D, Lalonde T, Wohns D, Moscucci M, Gurm HS (2013) Outcome of percutaneous coronary intervention following recent surgery. Am J Cardiol 112:1580–1585
Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE (2010) Reconstituting organ-level lung functions on a chip. Science 328:1662–1668
Iqbal J, Serruys PW, Taggart DP (2013) Optimal revascularization for complex coronary artery disease. Nat Rev Cardiol 10:635–647
Jastrzebska E, Tomecka E, Jesion I (2016) *Heart-on-a-chip* based on stem cell biology. Biosens Bioelectron 75:67–81
Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC (2016) In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotchnol 4:1–14
Kobuszewska A, Tomecka E, Zukowski K, Jastrzebska E, Chudy M, Dybko A, Renaud P, Brzozka Z (2017) *Heart-on-a-chip*: an investigation of the influence of static and perfusion conditions on cardiac (H9C2) cell proliferation, morphology, and alignment. SLAS Technol. doi:10.1177/2472630317705610
Kozar-Kaminska K (2012) Regeneracja serca. Postepy w Kardiologii Interwencyjnej 8:308–314
Laflamme MA, Murry ChE (2011) Heart regeneration. Nature 473:326–335
Lee J, Razu ME, Wang X, Lacerda C, Kim JJ (2015) Biomimetic cardiac microsystems for pathophysiological studies and drug screens. J Lab Autom 20:96–106
Li YS, Haga JH Chien S (2005) Molecular basis of the effects of shear stress on vascular endothelial cells. J Biomech 38:1949–1971
Lovell MJ, Mathur A (2010) Cardiac stem cell therapy: progress from the bench to bedside. Heart 96:1531–1537
Lundin A, Djärv T, Engdahl J, Hollenberg J, Nordberg P, Ravn-Fischer A, Ringh M, Rysz S, Svensson L, Herlitz J, Lundgren P (2016) Drug therapy in cardiac arrest: a review of the literature. Eur Heart J Cardiovasc Pharmacother 2:54–75
Ma Z, Lu Q, Liu H, Yang H, Yun J, Eisenberg C, Borg TK, Xu M, Gao BZ (2012) Laser-patterned stem-cell bridges in a cardiac muscle model for on-chip electrical conductivity analyses. Lab Chip 12:566–573
Maidhof R, Tandon N, Lee EJ, Luo J, Duan Y, Yeager K, Konofagou E, Vunjak-Novakovic G (2012) Biomimetic perfusion and electrical stimulation applied in concert improved the assembly of engineered cardiac tissue. J Tissue Eng Regen Med 6:e12–e23
Mampuya WM (2012) Cardiac rehabilitation past, present and future: an overview. Cardiovasc Diagn Ther 2:38–49
Mathur A, Loskill P, Shao K, Huebsch N, Hong S, Marcus SG, Marks N, Mandegar M, Conklin BR, Lee LP, Healy KE (2015) Human iPSC-based cardiac microphysiological system for drug screening applications. Sci Rep 5:1–7
Mehling M, Tay S (2014) Microfluidic cell culture. Curr Opin Biotechnol 25:95–102
Moraes C, Chen J, Sun Y, Simmons CA (2010) Microfabricated arrays for high-throughput screening of cellular response to cyclic substrate deformation. Lab Chip 10:227–234
Morgan JP, Delnero PF, Zheng Y, Verbridge SS, Chen J, Craven M, Choi NW, Diaz-Santana A, Kermani P, Hempstead B, Lopez JA, Corso TN, Fischbach C, Stroock AD (2013) Formation of microvascular networks in vitro. Nat Protoc 8:1820–1836
Motlhagh D, Hartman TJ, Desai TA, Russell B (2003) Microfabricated grooves recapitulate neonatal myocyte connexin43 and N-cadherin expression and localization. J Biomed Mater Res A 67:148–157
Nadal-Ginard B, Ellison GM, Torella D (2014) The cardiac stem cell compartment is indispensable for myocardial cell homeostasis, repair and regeneration in the adult. Stem Cell Res 13:615–630

Nath S, Devi GR (2016) Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. Pharmacol Ther 163:94–108

Pittman RN (2011) The circulation system and oxygen transport. Regulation of tissue oxygenation. San Rafael (CA), Morgan & Claypool Life Sciences, pp 3–16

Ren L, Liu W, Wang Y, Wang JC, Tu Q, Xu J, Liu R, Shen SF, Wang J (2013) Investigation of hypoxia-induced myocardial injury dynamics in a tissue interface mimicking microfluidic device. Anal Chem 85:235–244

Ribas J, Sadeghi H, Manbachi A, Leijten J, Brinegar K, Zhang YS, Ferreira L, Khademhosseini A (2016) Cardiovascular organ-on-a-chip platforms for drug discovery and development. Appl Vitro Toxicol 2:82–96

Rossi M, Lindken R, Hierck BP, Westerweel J (2009) Tapered microfluidic chip for the study of biochemical and mechanical response at subcellular level of endothelial cells to shear flow. Lab Chip 9:1403–1411

Sackmann EK, Fulton AL, Beebe DJ (2014) The present and future role of microfluidics in biomedical research. Nature 507:181–189

Salyers KL (2009) Preclinical pharmacokinetic models for drug discovery and development. In: Pearson P, Wienkers L (eds) Handbook of drug metabolism, 2nd edn. Informa Healthcare, New York, pp 659–673

Schaaf S, Shibamiya A, Mewe M, Eder A, Stohr A, Hirt MN, Rau T, Zimmermann WH, Conradi L, Eschenhagen T, Hansen A (2011) Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. PLoS One 6:e26397-1-e26397-11

Serena E, Figallo E, Tandon N, Cannizzaro C, Gerecht S, Elvassore N, Vunjak-Novakovic NG (2009) Electrical stimulation of human embryonic stem cells: Cardiac differentiation and the generation of reactive oxygen species. Exp Cell Res 315:3611–3619

Sheng CC, Zhou L, Hao J (2013) Current stem cell delivery methods for myocardial repair. Biomed Res Int 547902:1–15

Simmons CS, Petzold BC, Pruitt BL (2012) Microsystems for biomimetic stimulation of cardiac cells. Lab Chip 12:3235–3248

Sondergaard CS, Mathews G, Wang L, Jeffreys A, Sahota A, Wood M, Ripplinger CM, Si MS (2012) Contractile and electrophysiologic characterisation of optimalized self-organizing engineered heart tissue. Ann Thorac Surg 94:1241–1248

Tehranirokh M, Kouzani AZ, Francis PS, Kanwar JR (2013) Microfluidic devices for cell cultivation and proliferation. Biomicrofluidics 7:1–32

Tomecka E, Wojasinski M, Jastrzebska E, Chudy M, Ciach T, Brzozka Z (2017) Poly(l-lactic acid) and polyurethane nanofibers fabricated by solution blow spinning as potential substrates for cardiac cell culture. Mat Sci Eng C 75:305–316

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

Visone R, Gilardi M, Marsano A, Rasponi M, Bersini S, Moretti M (2016) Cardiac meets skeletal: what's new in microfluidic models for muscle tissue engineering. Molecules 21:E1128-1-E1128-22

Wong KH, Chan JM, Kamm RD, Tien J (2012) Microfluidic models of vascular functions. Annu Rev Biomed Eng 14:205–230

World CJ, Garin G, Berk B (2006) Vascular shear stress and activation of inflammatory genes. Curr Atheroscler Rep 8:240–244

World Health Organisation (2014) Global status report on noncommunicable disease. WHO library cataloguing-in-publication data. ISBN 978 92 4 156485 4

Xiao Y, Zhang B, Liu H, Miklas JW, Gagliardi M, Pahnke A, Thavandiran N, Sun Y, Simmons C, Keller G, Radisic M (2014) Microfabricated perfusable cardiac biowire: a platform that mimics native cardiac bundle. Lab Chip 14:869–882

Yamada KM, Cukierman E (2007) Modeling tissue morphogenesis and cancer in 3D. Cell 130:601–610

Yan Y, Yang X, Zou J, Jia C, Hu Y, Du H, Wang H (2015) Evaluation of photodynamic therapy efficiency using an in vitro three-dimensional microfluidic breast cancer tissue model. Lab Chip 15:735–744

Yang H, Ma Z (2012) Microsystem for stem cell-based cardiovascular research. BioNanoScience 2:305–315

Young E, Simmon C (2009) Macro- and microscale fluid flow systems for endothelial cell biology. Lab Chip 10:143–160

Young EW (2013) Cells, tissues, and organs on chips: challenges and opportunities for the cancer tumor microenvironment. Integr Biol (Camb) 5:1096–1109

Zhang D, Luo G, Xinxin D, Lu Ch (2012) Preclinical experimental models of drug metabolism and disposition in drug discovery and development. Acta Pharm Sin B 2:549–561

Zhang Y, Mignone J, MacLellan WR (2015) Cardiac regeneration and stem cells. Physiol Rev 95:1189–1204

Zhang YS, Aleman J, Arneri A, Bersini S, Piraino F, Shin SR, Dokmeci MR, Khademhosseini A (2016a) From cardiac tissue engineering to *Heart-on-a-Chip*: beating challenges. Biomed Mater 10:034006-1–034006-21

Zhang X, Li L, Luo C (2016b) Gel integration for microfluidic applications. Lab Chip 16:1757–1776

Ziolkowska K, Kwapiszewski R, Brzozka Z (2011) Microfluidic devices as tolls for mimicking the *in vivo* environment. New J Chem 35:979–990

Zuppinger C (2016) 3D culture for cardiac cells. Biochim Biophys Acta 1863:1873–1881