Building blocks of the human heart

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CHAPTER 1

Introduction of the thesis

Cell communication in the heart

The heart is a complex organ broadly structured into four chambers (two atria and two ventricles) that pumps oxygenated and deoxygenated blood throughout the body in an intricate system of arteries, veins and capillaries (Thiriet, 2007). Oxygen-poor blood enters the right atrium and flows through the right ventricle; the right ventricle pumps deoxygenated blood to the lungs, where it becomes oxygenated; oxygenated blood returns to the heart, specifically to the left atrium, through the pulmonary veins. From the left atrium, oxygenated blood flows to the left ventricle from where it is distributed via the aorta (the main and largest artery) throughout the body (Thiriet, 2007). These diverse functions need to be carried out in synchrony and this is regulated by cells of the conduction system. Each chamber is composed of different cell types, some with their own chamber-specific identity. The electrical cardiac conduction system triggers and orchestrates the excitation-contraction function of the heart ventricles; it generates the cardiac impulse and conducts it from the atria to the ventricles, making the heart chambers contract sequentially (Moorman and Christoffels, 2003). The principle cell types that make up the (mammalian) heart are illustrated in **Figure 1**.

Cardiac muscle cells, or cardiomyocytes (CMs), the fundamental contractile cells of the myocardium, occupy approximately 75% of the myocardial tissue volume; however they account for less than a third of the total cell number. The remaining non-CM fraction includes many additional cell types, including endothelial cells (ECs), cardiac fibroblasts (CFs), smooth muscle cells, other connective tissue cells and even immune system–related cells and neurons (Armour et al., 1997; Hulsmans et al., 2017; Tirziu et al., 2010; Xin et al., 2013). Atrial and ventricular CMs form the myocardium, the muscle walls of the heart. ECs form the endocardium, the interior lining of blood vessels and cardiac valves. Given the high energy and oxygen demands of the heart, every CM is in contact with minimally one EC. CFs form the main cell population in the adult heart (up to 50%). Smooth muscle cells contribute to the coronary arteries and inflow and outflow vasculature. The epicardium gives rise to the precursors of CFs and smooth muscle cells and covers the surface of the heart during development migrating from the proepicardial organ to form a single epithelial layer (Brade et al., 2013). In the adult heart, it is the epicardial cells that give rise to the CFs, which develop after myocardial infarction and form the scar tissue that prevents heart rupture (Furtado et al., 2016). Pacemaker

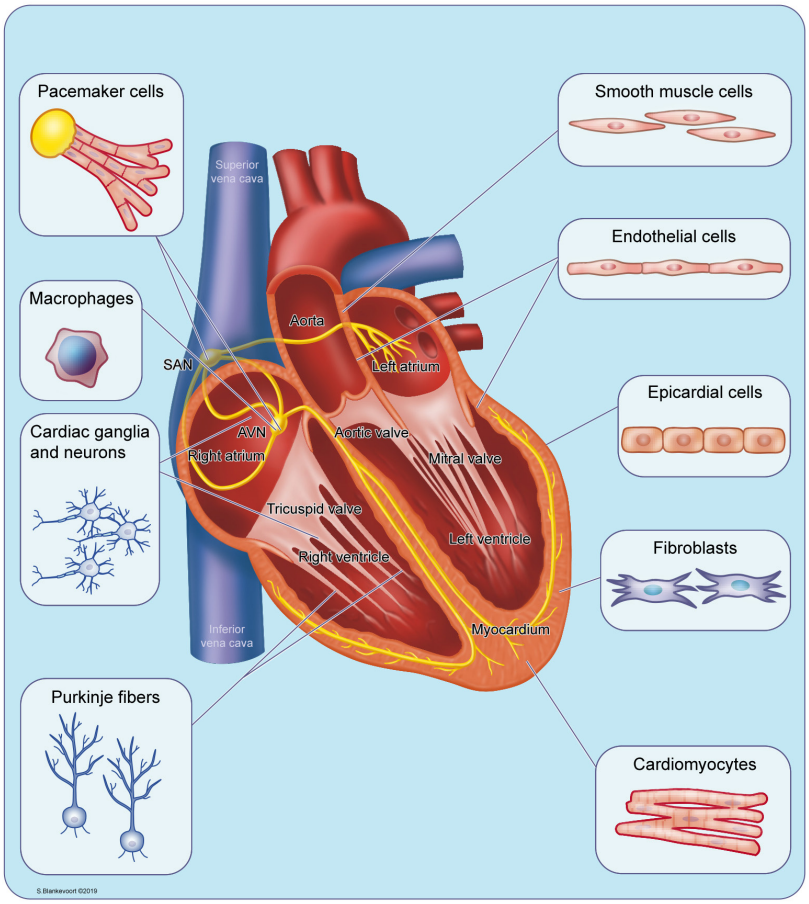

Figure 1. Principle cell types found in the mammalian heart

cells and Purkinje fibres in the conduction system are specialized CMs that generate and conduct electrical impulses. The sinoatrial node (SAN), composed of pacemaker cells, resides in the right atrium and generates impulses to initiate the contraction of the heart. The atrioventricular node (AVN), located between the atria and ventricles, conducts electrical impulses from the atria to the ventricles (Tirziu et al., 2010). Cardiac-tissue resident macrophages are spindle-like cells found abundantly in the AVN that modulate CM electrical activity through electric coupling (Harari et al., 2017; Hulsmans et al., 2017); they also play a role in atherosclerotic development and promote both injury and repair after myocardial infarction (Johnson and Camelliti, 2018). The heart

also possesses an intrinsic cardiac nervous system (ICNS) that controls heart rate, atrial and ventricular refractoriness, cardiac contractility and conduction and blood flow (Zipes et al., 2017). The ICNS is composed of sensory (afferent), interconnecting (local circuit), and motor (adrenergic and cholinergic efferent) neurons that, communicating with intrathoracic extracardiac ganglia, control cardiac function under the influence of the central nervous system (CNS) and circulating catecholamines (Zipes et al., 2017). Cardiac ganglia are located at specific atrial regions: around the SAN, roots of caval and pulmonary veins, and near the AVN (Armour et al., 1997; Zipes et al., 2017); they are also found scattered through the ventricles, although in a smaller number compared to the atria (Armour et al., 1997; Zipes and Jalife, 2013).

These distinct cell populations are not isolated from one another within the heart but instead communicate physically via a variety of soluble endocrine, paracrine and autocrine factors. The principle factors identified, mostly from transgenic or knockout model systems in the mouse, are summarized in **Figure 2** (simplified from (Tirziu et al., 2010)).

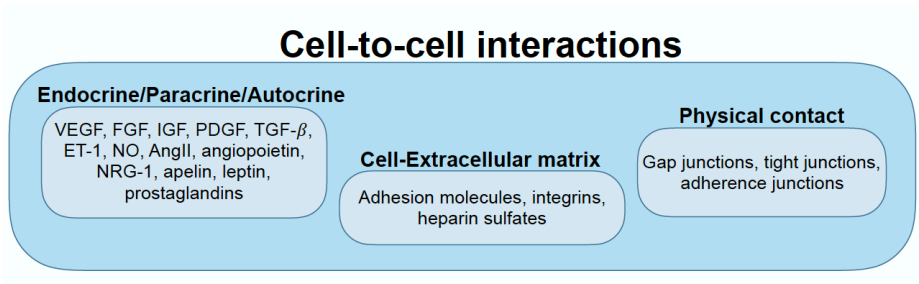

Figure 2. Schematic representation of cell-to-cell interactions in the mammalian heart

This cell-to-cell interaction and cross-talk contributes to structural, electrical, mechanical, and metabolic properties of the functional heart.

In the human heart, dialogue between CMs, cardiac ECs, and CFs is essential to ensure proper cardiac function: on the one hand, cardiac ECs supply oxygen and free fatty acids to the CMs and release paracrine factors (NRG-1, PDGF-B, NO, ET-1) that support CM metabolism, survival and contractile function (Brutsaert, 2003; Tirziu et al., 2010); on the other hand, CFs produce

extracellular matrix, which promotes tissue and matrix stretch, and form direct gap junctions with the CMs, influencing their electrophysiological properties and providing a substrate for electrical conduction between separated CMs over extended distances (Kakkar and Lee, 2010). Thus, to develop a model that fully recapitulates the complexity of this organ, the influence of these specific features of cell-to-cell cross talk needs to be taken into account.

Miniaturized heart tissues for disease modeling: using hiPSCs to study multilineage cardiovascular disorders

It is well established that human induced pluripotent stem cell derived-cardiomyocytes (hPSC-CMs) have great potential for studying cardiac developmental processes and mechanisms underlying cardiac disorders, as they capture the genetic background of the patient from which they were originated and incorporate aspects of the normal and pathological physiology of the heart tissue. However, one of their drawbacks that they also have in common with other differentiated derivatives of hPSCs, is their developmental immaturity, as they show a much closer resemblance to first- and second trimester human embryos than to adult tissues (Pavlovic et al., 2018).

In addition, other cardiac-specific cell types such as those described earlier, are essential for studying complex multilineage cardiovascular diseases in which not only the CMs but also other cardiac-specific cell types may be affected and play a role. For many diseases of the heart, it is still unclear what the cell- or tissue of origin for the disease is. One example is arrhythmogenic cardiomyopathy (ACM), a rare genetic disease predominately associated with mutations in desmosomal genes (*PKG* and *PKP2* among others) and characterized by arrhythmias and fibro-fatty replacement of the myocardium (Lazzarini et al., 2015; Sommariva et al., 2017). The origin of ACM, particularly the fibro-fatty deposits in the heart, is still largely unknown. *In vitro* models (mouse and human; primary and hPSC-derived) investigated so far have been derived from either the CM- or the non-CM- (bucca mucosal cells, fibro-adipocytes progenitors, human embryonic kidney 293 cells, cardiac fibroblasts) compartment (Caspi et al., 2013; Cerrone et al., 2014; El-Battrawy et al., 2018; Kim et al., 2014; Sommariva et al., 2016). Mouse models have greatly enhanced our understanding of ACM and of the desmosome, such as that PKG and PKP2 deficiency causes heart rupture in cardiac development, and that PKP2 mutations are shown to cause gap junction remodeling (Awad et

al., 2008). Nevertheless, mouse models did not reveal the extensive cardiac fibro-fatty deposits typical of ACM patients (Cerrone et al., 2012; Krusche et al., 2011), thus suggesting that human (cell-based) models might be better for investigating causative pathways linked to lipid metabolism. In addition, multicellular models that incorporate both the CM- and non-CM compartment will be extremely valuable in distinguishing the "culprit" cells from their "victims" and in understanding how these compartments singularly or synergistically contribute to different aspects of a complex disease pathogenesis in conditions such as ACM.

Other examples include (rare) metabolic syndromes and mitochondrial diseases that are often associated with young-onset cardiac failure or even death, yet which cell type in the heart causes the condition is still often unclear. Given the important (metabolic) roles of CFs and cardiac ECs in the heart, their contribution needs to be taken into account.

In addition, it has been proposed that *in vitro* three-dimensional (3D) culture systems better recapitulate the complexity of natural tissues compared to two-dimensional (2D) cultures, and, specifically for the heart, better mimic the real myocardial environment rather than 2D cultures on plastic (McDonald et al., 1972; Veerman et al., 2015)·(Laschke and Menger, 2017). As an example, the development of 3D tissues from hiPSC-CMs carrying *PRKAG2* cardiomyopathy revealed key links between metabolic sensing by AMPK and CM survival, metabolism and TGFb signalling that were not observed previously in 2D cultures of hiPSC-CMs (Hinson et al., 2016).

Thus, the development of miniaturized 3D multicellular models of the heart could allow in-depth mechanistic assessments of the nature of the disease, the identification of potential drug targets, as well as tissue-level validation of the effect of novel therapeutic compounds.

The need of culture maturation systems for cardiac disease modeling

As mentioned earlier, a largely unresolved issue in using hiPSC-CMs as preclinical cardiac disease models and drug discovery platforms is their immature state and similarity to fetal- rather than adult cells (reviewed in (Veerman et al., 2015)). This presents hurdles to using these models for studying adult-onset

cardiac genetic diseases in which expression of the gene (or splice variant) of interest only occurs during postnatal heart development. Two examples are ion channel-related diseases, one caused by an imprinted gene (*KCNQ1*), the other by a postnatally expressed splice variant (*SCN5A*).

Mutations in the *KCNQ1* gene, a voltage-gated potassium channel, are associated with congenital long QT syndrome type 1 (LQT1), a cardiac disorder associated with severe cardiac arrhythmias which causes sudden death especially in young individuals (Bokil et al., 2010). The *KCNQ1* gene is initially imprinted (expressed from only one allele) but becomes bi-allelic (loss of imprinting) during postnatal heart development (Korostowski et al., 2011). However, this imprinting is not lost in fetal- and immature hiPSC-CMs.

The cardiac sodium channel Nav1.5, encoded by *SCN5A*, mediates the cardiac sodium current (I_{Na}) crucial for the rapid depolarization of CM action potential and impulse propagation in the heart (Gellens et al., 1992). Mutations in *SCN5A* have been associated with a broad spectrum of inherited cardiac rhythm disorders, such as long QT syndrome type 3 (LQT3), Brugada syndrome (BrS), and cardiac conduction disease (CCD) (reviewed in (Zimmer and Surber, 2008)). Several *SCN5A* splice variants are expressed in the heart and in various other tissues including brain, dorsal root ganglia, breast cancer cells and neuronal stem cell lines (reviewed in (Schroeter et al., 2010)). Particularly in the heart, "fetal" and "adult" splice variants have been described (Chioni et al., 2005). The "adult" isoform differs from the" fetal" *SCN5A* isoform in the alternate usage of exon 6: splicing of exon 6 occurs in a mutually exclusive manner, with inclusion of either the adult exon 6b or the fetal exon 6a (Chioni et al., 2005). The fetal splice isoform of *SCN5A* is predominantly expressed before birth and is gradually replaced by the adult isoform postnatally. As a result, "adult" splicing variants are not expressed in fetal- and immature hiPSC-CFs.

Based on these considerations, the development of a culture system for maturation in which the CMs resemble those in postnatal heart is required to reveal aspects of these and other adult-onset disease phenotypes that have not been yet characterized.

Aim and outline of the thesis

Using a building-block approach, this thesis describes stepwise progression in the complex interactions between distinct cell populations present in the human heart. The overall aim of this thesis was to induce structural, electrical, mechanical and metabolic maturation of hiPSC-CMs by 1) developing a **miniaturized 3D** cardiac model using small cell numbers and to make it cost effective in scaling production and thus amenable to screening compound libraries, and 2) developing a **multi-cell type** cardiac model using multiple cardiac cell types, to make it possible to distinguish "culprit" cells from their "victims" in complex multi-lineage cardiovascular disorders.

In **chapter 2**, we reviewed the development of CM induction from human pluripotent stem cells (hPSCs), the progress in cardiac disease modeling using hiPSC-CMs, and the challenges associated with understanding complex cardiac diseases.

In **chapter 3**, we established conditions for simultaneous differentiation of CMs and cardiac-specific ECs from common hPSC-cardiac mesoderm progenitors. We then described the development of a bi-culture system, termed "cardiac microtissue", that integrates both cell types in 3D, using just 5000 cells per tissue. Finally, we demonstrated that presence of cardiac ECs was essential to induce CM maturation in this system.

In **chapter 4,** we firstly described in depth the protocol for co-differentiation of CMs and cardiac ECs from cardiac mesoderm using both human embryonic stem cells (hESCs) and hiPSCs, and provided details for the enrichment of both cell populations from heterogeneous-differentiated cultures as well as cell maintenance, characterization, dissociation and cryopreservation. Secondly, we described the detailed bench protocol for generation of cardiac microtissues, and we provided guidelines for their culture and characterization for downstream applications.

In **chapter 5**, by adapting the protocol we developed in chapter 3 for simultaneous differentiation of CMs and cardiac ECs from common cardiac mesoderm, we found that RA and BMP4 synergistically promote the formation of epicardial cells in both hESCs and hiPSCs. As epicardial cells have the ability to undergo EMT and give rise to CFs, this work provided the foundation for chapter 6.

In **chapter 6**, we established a 3D triple-cell type model of cardiac microtissue by adding hiPSC-CFs (derived from hiPSC-epicardium) to the bi-cell type culture model developed in chapter 3. We demonstrated that inclusion of CFs was crucial for inducing (post-natal) structural, electrical, mechanical and metabolic maturation of hiPSC-CMs in microtissues containing hiPSC-ECs. We found that primary adult cardiac- but not skin fibroblasts could replace hiPSC-CFs, and that three-cell-type crosstalk between the three major cardiac cell components was essential for metabolic maturation of hiPSC-CMs. Finally, we showed that features of the arrhythmogenic cardiomyopathy (ACM) phenotype were uniquely recapitulated by inclusion of patient hiPSC-derived cardiac fibroblasts. This work provided proof of concept that tissue- and organ-specific cells differentiated from hiPSCs following developmental principles are essential to 1) mediate maturation and 2) uncover mechanistic insights in multi-lineage disease phenotypes, specifically in the heart.

In **chapter 7**, we discussed the significance of findings presented in this thesis and limitations and advantages of our model compared to existing systems developed by other groups. Scope for future work in the field is also proposed.

References

Armour, J.A., Murphy, D.A., Yuan, B.-X., MacDonald, S., Hopkins, D.A., 1997. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat. Rec. 247, 289–298. doi:10.1002/(SICI)1097-0185(199702)247:2<289::AID-AR15>3.0.CO;2-L

Awad, M.M., Calkins, H., Judge, D.P., 2008. Mechanisms of disease: molecular genetics of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Nat Clin Pract Cardiovasc Med 5, 258–267. doi:10.1038/ncpcardio1182

Bokil, N.J., Baisden, J.M., Radford, D.J., Summers, K.M., 2010. Molecular genetics of long QT syndrome. Molecular Genetics and Metabolism 101, 1–8. doi:10.1016/j.ymgme.2010.05.011

Brade, T., Pane, L.S., Moretti, A., Chien, K.R., Laugwitz, K.L., 2013. Embryonic heart progenitors and cardiogenesis. Cold Spring Harbor Perspectives in Medicine 3, a013847–a013847. doi:10.1101/cshperspect.a013847

Brutsaert, D.L., 2003. Cardiac Endothelial-Myocardial Signaling: Its Role in Cardiac Growth, Contractile Performance, and Rhythmicity. Physiological Reviews 83, 59–115. doi:10.1152/physrev.00017.2002

Caspi, O., Huber, I., Gepstein, A., Arbel, G., Maizels, L., Boulos, M., Gepstein, L., 2013. Modeling of Arrhythmogenic Right Ventricular Cardiomyopathy With Human Induced Pluripotent Stem Cells. Circulation: Cardiovascular Genetics 6, 557–568. doi:10.1161/CIRCGENETICS.113.000188

Cerrone, M., Lin, X., Zhang, M., Agullo-Pascual, E., Pfenniger, A., Chkourko Gusky, H., Novelli, V., Kim, C., Tirasawadichai, T., Judge, D.P., Rothenberg, E., Chen, H.S.V., Napolitano, C., Priori, S.G., Delmar, M., 2014. Missense Mutations in Plakophilin-2 Cause Sodium Current Deficit and Associate With a Brugada Syndrome Phenotype. Circulation 129, 1092–1103. doi:10.1161/CIRCULATIONAHA.113.003077

Cerrone, M., Noorman, M., Lin, X., Chkourko, H., Liang, F.-X., van der Nagel, R., Hund, T., Birchmeier, W., Mohler, P., van Veen, T.A., van Rijen, H.V., Delmar, M., 2012. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. Cardiovascular Research 95, 460–468. doi:10.1093/cvr/cvs218

Chioni, A.-M., Fraser, S.P., Pani, F., Foran, P., Wilkin, G.P., Diss, J.K.J., Djamgoz, M.B.A., 2005. A novel polyclonal antibody specific for the Nav1.5 voltage-gated Na+ channel "neonatal" splice form. Journal of Neuroscience Methods 147, 88–98. doi:10.1016/j.jneumeth.2005.03.010

El-Battrawy, I., Zhao, Z., Lan, H., Cyganek, L., Tombers, C., Li, X., Buljubasic, F., Lang, S., Tiburcy, M., Zimmermann, W.-H., Utikal, J., Wieland, T., Borggrefe, M., Zhou, X.-B., Akin, I., 2018. Electrical dysfunctions in human-induced pluripotent stem cell-derived cardiomyocytes from a patient with an arrhythmogenic right ventricular cardiomyopathy. EP Europace 20, f46–f56. doi:10.1093/europace/euy042

Furtado, M.B., Nim, H.T., Boyd, S.E., Rosenthal, N.A., 2016. View from the heart: cardiac fibroblasts in development, scarring and regeneration. Development 143, 387–397. doi:10.1242/dev.120576

Gellens, M.E., George, A.L., Chen, L.Q., Chahine, M., Horn, R., Barchi, R.L., Kallen, R.G., 1992. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc Natl Acad Sci USA 89, 554–558.

Harari, E., Guo, L., Smith, S.L., Braumann, R.E., Virmani, R., Finn, A.V., 2017. Heart-resident macrophages: are they involved in the rhythm of every beat? J Thorac Dis 9, 2264–2267. doi:10.21037/jtd.2017.07.43

Hinson, J.T., Chopra, A., Lowe, A., Sheng, C.C., Gupta, R.M., Kuppusamy, R., O'Sullivan, J., Rowe, G., Wakimoto, H., Gorham, J., Zhang, K., Musunuru, K., Gerszten, R.E., Wu, S.M., Chen, C.S., Seidman, J.G., Seidman, C.E., 2016. Integrative Analysis of PRKAG2 Cardiomyopathy iPS and Microtissue Models Identifies AMPK as a Regulator of Metabolism, Survival, and Fibrosis. CellReports 17, 3292–3304. doi:10.1016/j.celrep.2016.11.066

Hulsmans, M., Clauss, S., Xiao, L., Aguirre, A.D., King, K.R., Hanley, A., Hucker, W.J., Wülfers, E.M., Seemann, G., Courties, G., Iwamoto, Y., Sun, Y., Savol, A.J., Sager, H.B., Lavine, K.J., Fishbein, G.A., Capen, D.E., Da Silva, N., Miquerol, L., Wakimoto, H., Seidman, C.E., Seidman, J.G., Sadreyev, R.I., Naxerova, K., Mitchell, R.N., Brown, D., Libby, P., Weissleder, R., Swirski, F.K., Kohl, P., Vinegoni, C., Milan, D.J., Ellinor, P.T., Nahrendorf, M., 2017. Macrophages Facilitate Electrical Conduction in the Heart. Cell 169, 510–522.e20. doi:10.1016/j.cell.2017.03.050

Johnson, R.D., Camelliti, P., 2018. Role of Non-Myocyte Gap Junctions and Connexin Hemichannels in Cardiovascular Health and Disease: Novel Therapeutic Targets? IJMS 19, 866. doi:10.3390/ijms19030866

Kakkar, R., Lee, R.T., 2010. Intramyocardial Fibroblast Myocyte Communication. Circulation Research 106, 47–57. doi:10.1161/CIRCRESAHA.109.207456

Kim, C., Wong, J., Wen, J., Wang, S., Wang, C., Spiering, S., Kan, N.G., Forcales, S., Puri, P.L., Leone, T.C., Marine, J.E., Calkins, H., Kelly, D.P., Judge, D.P., Chen, H.-S.V., 2014. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. Nature 494, 105–110. doi:10.1038/nature11799

Korostowski, L., Raval, A., Breuer, G., Engel, N., 2011. Enhancer-driven chromatin interactions during development promote escape from silencing by a long non-coding RNA. Epigenetics Chromatin 4, 21. doi:10.1186/1756-8935-4-21

Krusche, C.A., Holthöfer, B., Hofe, V., van de Sandt, A.M., Eshkind, L., Bockamp, E., Merx, M.W., Kant, S., Windoffer, R., Leube, R.E., 2011. Desmoglein 2 mutant mice develop cardiac fibrosis and dilation. Basic Res Cardiol 106, 617–633. doi:10.1007/s00395-011-0175-y

Laschke, M.W., Menger, M.D., 2017. Life is 3D: Boosting Spheroid Function for Tissue Engineering. Trends Biotechnol. 35, 133–144. doi:10.1016/j.tibtech.2016.08.004

Lazzarini, E., Jongbloed, J.D.H., Pilichou, K., Thiene, G., Basso, C., Bikker, H., Charbon, B., Swertz, M., van Tintelen, J.P., van der Zwaag, P.A., 2015. The ARVD/C Genetic Variants Database: 2014 Update. Human Mutation 36, 403–410. doi:10.1002/humu.22765

McDonald, T.F., Sachs, H.G., DeHaan, R.L., 1972. Development of sensitivity to tetrodotoxin in beating chick embryo hearts, single cells, and aggregates. Science 176, 1248–1250.

Moorman, A.F.M., Christoffels, V.M., 2003. Development of the cardiac conduction system: a matter of chamber development. Novartis Found. Symp. 250, 25–34– discussion 34–43– 276–9.

Pavlovic, B.J., Blake, L.E., Roux, J., Chavarria, C., Gilad, Y., 2018. A Comparative Assessment of Human and Chimpanzee iPSC-derived Cardiomyocytes with Primary Heart Tissues. Sci Rep 8, 15312. doi:10.1038/s41598-018-33478-9

Schroeter, A., Walzik, S., Blechschmidt, S., Haufe, V., Benndorf, K., Zimmer, T., 2010. Structure and function of splice variants of the cardiac voltage-gated sodium channel Nav1.5. Journal of Molecular and Cellular Cardiology 49, 16–24. doi:10.1016/j.yjmcc.2010.04.004

Sommariva, E., Brambilla, S., Carbucicchio, C., Gambini, E., Meraviglia, V., Russo, Dello, A., Farina, F.M., Casella, M., Catto, V., Pontone, G., Chiesa, M., Stadiotti, I., Cogliati, E., Paolin, A., Ouali Alami, N., Preziuso, C., d'Amati, G., Colombo, G.I., Rossini, A., Capogrossi, M.C., Tondo, C., Pompilio, G., 2016. Cardiac mesenchymal stromal cells are a source of adipocytes in arrhythmogenic cardiomyopathy. European Heart Journal 37, 1835–1846. doi:10.1093/eurheartj/ehv579

Sommariva, E., Stadiotti, I., Perrucci, G.L., Tondo, C., Pompilio, G., 2017. Cell models of arrhythmogenic cardiomyopathy: advances and opportunities. Disease Models & Mechanisms 10, 823–835. doi:10.1242/dmm.029363

Thiriet, M., 2007. Biology and Mechanics of Blood Flows. Springer Science & Business Media, New York, NY. doi:10.1007/978-0-387-74849-8

Tirziu, D., Giordano, F.J., Simons, M., 2010. Cell Communications in the Heart. Circulation 122, 928–937. doi:10.1161/CIRCULATIONAHA.108.847731

Veerman, C.C., Kosmidis, G., Mummery, C.L., Casini, S., Verkerk, A.O., Bellin, M., 2015. Immaturity of Human Stem-Cell-Derived Cardiomyocytes in Culture: Fatal Flaw or Soluble Problem? Stem Cells and Development 24, 1035–1052. doi:10.1089/scd.2014.0533

Xin, M., Olson, E.N., Bassel-Duby, R., 2013. Mending broken hearts: cardiac development as a basis for adult heart regeneration and repair. Nat. Rev. Mol. Cell Biol. 14, 529–541. doi:10.1038/nrm3619

Zimmer, T., Surber, R., 2008. SCN5A channelopathies – An update on mutations and mechanisms. Progress in Biophysics and Molecular Biology 98, 120–136. doi:10.1016/j.pbiomolbio.2008.10.005

Zipes, D.P., Jalife, J., 2013. Cardiac Electrophysiology: from Cell to Bedside. Elsevier Health Sciences.