**Abstract:** Cardiac disease causes 33% of deaths worldwide but our knowledge of disease progression is still very limited. In vitro models utilising and combining multiple, differentiated cell types have been used to recapitulate the range of myocardial microenvironments in an effort to delineate the mechanical, humoral, and electrical interactions that modulate the cardiac contractile function in health and the pathogenesis of human disease. However, due to limitations in isolating these cell types and changes in their structure and function in vitro, the field is now focused on the development and use of stem cell-derived cell types, most notably, human-induced pluripotent stem cell-derived CMs (hiPSC-CMs), in modelling the CM function in health and patient-specific diseases, allowing us to build on the findings from studies using animal and adult human CMs. It is becoming increasingly appreciated that communications between cardiomyocytes (CMs), the contractile cell of the heart, and the non-myocyte components of the heart not only regulate cardiac development and maintenance of health and adult CM functions, including the contractile state, but they also regulate remodelling in diseases, which may cause the chronic impairment of the contractile function of the myocardium, ultimately leading to heart failure. Within the myocardium, each CM is surrounded by an intricate network of cell types including endothelial cells, fibroblasts, vascular smooth muscle cells, sympathetic neurons, and resident macrophages, and the extracellular matrix (ECM), forming complex interactions, and models utilizing hiPSC-derived cell types offer a great opportunity to investigate these interactions further. In this review, we outline the historical and current state of disease modelling, focusing on the major milestones in the development of stem cell-derived cell types, and how this technology has contributed to our knowledge about the interactions between CMs and key non-myocyte components of the heart in health and disease, in particular, heart failure. Understanding where we stand in the field will be critical for stem cell-based applications, including the modelling of diseases that have complex multicellular dysfunctions.

**Keywords:** disease modelling; patient-specific; human induced pluripotent stem cells; cardiomyocyte; personalized medicine; microenvironment; hereditary diseases; drug screening; non-myocyte

1. Introduction

Heart failure is a global pandemic affecting over 26 million people worldwide and is becoming increasingly prevalent with an ageing population [1]. Despite the significant advances in therapies and prevention, mortality and morbidity are still high, and quality of life is poor. Current treatments delay the progression of the disease, but there are still no treatments to effectively reverse the maladaptive changes that occur in remodelling. Earlier identification of patients with a predisposition to the
disease due to genetic or environmental factors or understanding key therapeutic targets in the disease progression would allow both earlier prevention and more effective treatments to be developed.

Despite our increasing knowledge about factors influencing the initiation and progression of heart failure, historical and current study designs are unable to map the intricate interactions between cardiomyocytes (CMs) and their surrounding environment in an accurate model of the disease. Major limitations when modelling heart failure include species mismatch when using CMs isolated from animals [2], in vitro human CM models lacking the native extracellular interactions with non-myocyte that modulate CM phenotype [3], and lack of patient specificity in modelling this complex condition [4]. The recent advancements in induced pluripotent stem cell (hiPSC)-derived cell types have broadened an avenue for the development of more accurate in vitro disease models. However, more needs to be done in understanding the native cell-cell and cell-matrix interactions to fully realize the potential of hiPSCs.

In this review, we discuss the disease models of the physiological and pathological composition of the myocardium, paying a particular focus on the potential that stem-cell derived cell types present in developing an accurate in vitro model of heart failure, and also to the key myocyte-non-myocyte interactions that have been delineated thus far—these findings must be considered in future models.

2. Heart Disease Models

There is clear clinical relevance in being able to accurately model human cardiac diseases in vitro. The withdrawal of drugs from the market due to unobserved toxic effects is unfortunately common. A systematic review identified that in the US, 14% of post-marketing drug withdrawals between 1953 and 2014 occurred due to cardiac toxicity [5]. Up until today, virtually all models of disease modelling and drug screening heavily rely on the use of CMs from animal models, or isolated CMs as a single cell type [6,7]. Historically, these have been grown in 2D and/or 3D cultures in an artificial environment under chemical, mechanical and electrical stimulation very different from the native environment. Among the challenges of these models are the high costs, difficult manipulations, ethics, and poor predictive capacity. Furthermore, these in vitro systems, although informative, cannot closely recapitulate the dynamics of the biological and mechanical properties of the complex, native, 3D environment, hence, lacking physiological and disease features. We summarize the benefits and limitations of currently available disease models in Table 1. These limitations must be overcome if we are to optimize a model for human cardiac failure.

Table 1. The summary of the benefits and limitations of currently available disease models.

| Model Type | Description | Benefits | Limitations | Ref. |
|------------|-------------|----------|-------------|------|
| Animal     | Animals with defined genetic background or subject to acute intervention (e.g., coronary obstruction) to mimic discrete time points. | Small animals: delineate molecular pathways in early- or late-stage heart failure, aiding the identification of biomarkers and therapeutic targets. Large animals: preclinical proof of concept for novel therapies before clinical trials. | Gene expression-silencing or drug-induced pathogenesis does not recapitulate the disease initiation in humans. Many human diseases are human-specific. Differences in physiology (circulation, etc.), cardiac output requirements, myocardial composition (vascular supply, etc.), biochemical absorption, distribution, metabolism and immunoresponses. | [8–14] |
Table 1. Cont.

| Model Type                         | Description                                                                 | Benefits                                                                 | Limitations                                      | Ref.    |
|-----------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------|---------|
| Human-specific expression in Chinese hamster ovary (CHO) and human embryonic kidney (HEK) cells | Model protein force expression, e.g., tests the off-target effects to ion channels prolonging the QT interval. | Expression of human ion channels. Avoids the expense of whole animal studies. Reproducibility in cryogenically freezing and thawing cell lines for the stable expression of the desired channels. | Single ion channel does not recapitulate diseases in humans. Does not negate species mismatch. | [15,16] |
| Adult human cardiomyocytes        | Isolated from diseased or non-diseased patients during surgery.              | Human genome so we can map the response in humans to cardiac disease.    | Limited quantities (e.g., ethical limitations). Large variability in phenotype and rapid dedifferentiation. | [17–21] |
| Organoid                          | 3D in vitro culture systems derived from self-organizing stem cells and extracellular matrix (ECM) proteins secreted from the cells. | Higher complexity compared to the 2D models, with more extensive cell-ECM interactions and possible vessel formation. | Expensive and technically challenging setup, resulting in poor reproducibility. | [22,23] |
| 3D cardiac tissue                 | 3D in vitro culture systems with natural and/or synthetic ECM structural support. | Ability to manipulate ECM components enables a greater control of the scaffold composition and more complex cell-ECM interactions. Decellularized scaffold for cell adhesion mimics the naturally occurring macro and microstructures. | Limited information on cost, reproducibility, and performance. | [24–26] |

**Stem Cells**

In our search for an accurate disease model for human cardiac failure, a huge asset has been the development of human stem cell-derived cells. Human embryonic stem cells were first isolated from human embryos in 1998 [27–29]. The major limitation to their use is that the phenotype of the human from the source of the embryo is unknown. They only carry a good predictive value when the embryo is known to carry a specific highly penetrant disease mutation. Importantly, this process also involves ethical issues surrounding cell sourcing.

A new approach for producing stem cell-derived cells is through human induced pluripotent stem cells (hiPSCs) [28,30,31]. Unlike other cells, hiPSCs reflect a person’s unique genotype because they are derived from a patient’s somatic cells. hiPSCs exhibit properties that render them uniquely qualified as model systems for studying human diseases: they are of human origin, so they carry human genomes; they are pluripotent, so they can be differentiated into any of the human body’s somatic cell types; and as stem cells, they can be an autologous source of cells for medical application. Importantly, the patient specificity of hiPSCs offers the opportunity to study cells genetically matched to individual patients, and together with genome-editing tools, they allow us to introduce or correct genetic variants.

Initial progress has been made in using hiPSCs to better understand cardiomyopathies [32], rhythm disorders [33], valvular and vascular disorders [34], and metabolic risk factors for ischemic heart disease [35]. In the last decade, CMs have been extensively derived from hiPSCs [36,37]. In disease modelling, many researchers have reprogrammed patient-specific cells or performed genetic editing to replicate diseases, as shown in Table 2. Various human pathologies, including long-QT syndrome (LQTS), Brugada syndrome, Timothy syndrome (also called LQT8), and catecholaminergic
polymorphic ventricular tachycardia (CPVT) have been modelled with hiPSC-CMs; all faithfully recapitulating the cardiac phenotypes observed in patients [38].

Table 2. The summary of the available human induced pluripotent stem cell (hiPSC)-derived cells used in disease modelling.

| Pathology                              | Cell Type Involved | Mutation                  | (Drug/Treatment) Test                          | Ref.  |
|----------------------------------------|--------------------|---------------------------|-----------------------------------------------|-------|
| **Endothelial**                         |                    |                           |                                               |       |
| Healthy                                | EC                 | N/A                       | Flow-induced disease and simvastatin          | [39]  |
| Hutchison-Gilford Progeria Syndrome    | EC                 | Patient-derived           | N/A                                           | [40]  |
| **Smooth muscle cells**                |                    |                           |                                               |       |
| Supravalvular aortic stenosis          | SMC                | Elastin (ELN)             | Elastin recombinant protein                    | [41]  |
| Marfan syndrome                        | SMC                | FBN1                      | Gene editing and drugs                         | [42]  |
| **Lymphocytes**                        |                    |                           |                                               |       |
| Healthy                                | B-cell lymphoid lineage | N/A                       | N/A                                           | [43]  |
| **Red Blood cell (RBC)**               |                    |                           |                                               |       |
| Healthy                                | CM and RBC         | N/A                       | Toxicity of RBC                               | [44]  |
| Hypoplastic left heart syndrome        | CM                 | Patient-derived (GM12601)| Isoproterenol                                 | [45]  |
| Arrhythmogenic right ventricular dysplasia | CM                | Plakoglobin, plakophilin-2| Metabolism induced onset                       | [46]  |
| Familial hypertrophic cardiomyopathy   | CM                 | MYH7 Arg66His             | Verapamil, Diltiazem, Mexiletine among 15 drugs | [47]  |
| LEBARD syndrome                        | CM and all three germ layers | PTPN11                   | N/A                                           | [48]  |
| Friedreich’s ataxia                    | Neurons and CM     | GAA triplet repeat expansion within the first intron of the frataxin gene | N/A                                           | [49]  |
| Catecholaminergic polymorphic ventricular tachycardia type 1 | CM | Ryanodine Receptor 2 (RYR2) | Isoproterenol                                 | [50,51] |
| Long QT 1, 2, 3, 5, 8, 14             | CM                 | Patient-derived           | Common drugs                                  | [52–57] |
| Barth syndrome                         | CM                 | Tafazzin (TAZ)            | Genetic rescue                                | [58]  |
| Ischemic heart damage                  | CM                 | Aldehyde dehydrogenase 2 (ALDH-2) deficiency | siRNA knockdown                              | [59]  |
| Brugada syndrome                       | CM                 | SCN5A-1795insD mutation   | N/A                                           | [33]  |

SMC = Vascular smooth muscle cell, CM = cardiomyocyte, EC = Endothelial, RBC = Red blood cell.

However, despite the benefits that hiPSCs have over other sources of CMs in disease modelling, it is important to note the immaturity in structure and electrophysiology of hiPSC-CMs, reviewed extensively by our group [60]. These, in vitro, have a phenotype akin to neonatal human CMs, and exhibit a relatively small size, a reduced electrical excitability, inefficient excitation-contraction coupling mechanisms, and an incomplete adrenergic response, as shown in Table 3. Indeed, investigators modelling arrhythmogenic right ventricular dysplasia using hiPSC-CMs described
how it was essential to induce more mature metabolic properties to accurately model adult-onset cardiac disease [35]. The immaturity of hiPSC-CMs in culture is thought to be due to, at least in part, a lack of key extracellular mediators of CM maturation. During the development of the human heart, the myocardium undergoes a complex series of structural changes that terminates in the formation of the health, adult phenotype. The maturation of CMs in vivo is regulated by a diverse library of factors, including topographical, mechanical, biochemical, electrical, and cellular interaction cues. Understanding how these cues mediate the maturation of CMs can also help us unlock the mechanisms that maintain the healthy phenotype and mediate changes in diseases such as heart failure.

Table 3. The summary of the differences between hiPSC-cardiomyocytes (CMs) and contractile CMs. Data extracted from References [58,60–94].

| Structure                | hiPSC-CM                          | Atrial                      | Ventricular                   |
|--------------------------|-----------------------------------|-----------------------------|------------------------------|
| **Shape**                | Any, not defined                  | Cylindrical                 | Cylindrical and bifurcated   |
| **Volume**               | Small                             | Large                       | Very large                   |
| **Sarcomere Organization** | Random                           | Orderly and aligned         | Orderly and aligned          |
| **Mitochondria population** | Few                              | Abundant                    | Abundant                     |
| **T-tubule organization** | Absent                           | Scarce                      | Abundant                     |
| **Glucose Metabolism**   | High                             | Low                         | Low                          |
| **Nucleus morphology**   | Mono                             | Mono, bi, multi             | Mono, bi, multi              |

**Electrophysiology**

|                      | hiPSC-CM                          | Atrial                      | Ventricular                   |
|----------------------|-----------------------------------|-----------------------------|------------------------------|
| **Spontaneous activity** | Very frequent                     | Absent                      | Absent                       |
| **Maximum diastolic potential** | −60 mV                           | −70 mV                      | −80 mV                       |
| **Maximum upstroke velocity** | 44–187 V/s                       | 200 V/s                     | 200 V/s                      |
| **Action potential amplitude** | 94–113 mV                        | 80–130 mV                   | 100 mV                       |
| * Action potential duration at 50% | 60–400 ms                       | 200 ms                      | 200–300 ms                   |
| * Action potential duration at 90% | 80–500 ms                       | 200–400 ms                  | 250–400 ms                   |
| **Force Generation**   | 100–150 Pa for a single cell      | Myocardium tensile          | Myocardium tensile           |
| **Elastic modulus**    | 466 Pa                           | 22–55 kPa                   | 22–55 kPa                    |

**Molecular Marker**

|                      | hiPSC-CM                          | Atrial                      | Ventricular                   |
|----------------------|-----------------------------------|-----------------------------|------------------------------|
| **Gap junction**     | Cx40                             | +                           | +                            |
|                      | Cx43                             | +                           | +                            |
|                      | Cx45                             | +                           | -                            |
| **Ion channel**      | KCNA5                            | +                           | +                            |
|                      | NCX1                             | +                           | +                            |
|                      | SERCA2a                          | +                           | +                            |
|                      | RYR2                             | +                           | +                            |
|                      | CaV 1.2                          | +                           | +                            |
|                      | Kr 2.1                           | +                           | +                            |
|                      | Kv 4.3                           | +                           | +                            |
|                      | KChip 2                          | +                           | +                            |
|                      | KCN2 (HERG)                      | +                           | +                            |
| **Structural protein** | TNNT2                            | +                           | +                            |
|                      | ACTN2                            | +                           | +                            |
|                      | MLC2A                            | +                           | +                            |
|                      | MLC2V                            | +                           | -                            |
|                      | MYL2                             | +                           | +                            |
|                      | MYH6                             | +                           | +                            |
| **Master gene**      | NKX2.5                           | +                           | ±                            |

* Action potential duration for hiPSC-CMs depends on seeding conditions and differentiation protocol.
3. The Composition of the Healthy Myocardium

The CMs are the contractile cells of the myocardium, but it is important to consider the adult human heart as a multicellular organism. The healthy adult human myocardium is composed of many cell types, with the most abundant cell types thought to be CMs, fibroblasts, endothelial cells, and perivascular cells. CMs occupy most of the myocardium volume; between 70–80% in the adult and constitutes 30–40% of the cells by number [95]. Although estimates for the density of non-myocyte components (fibroblasts, endothelial cells, smooth vascular muscle cells, lymphocytes and neurons) of the heart vary substantially, they are widely agreed to be vital for the normal homeostasis in health; non-myocyte cell types and the matrix provide the chemical, electrical, and mechanical stimulation for the CMs, and form the structures essential for the vascular supply required for efficient CM contraction, optimal shape and functions and long-term survival. This has been shown in numerous studies in which the isolation of human CMs leads to a rapid loss of contractile function, and prominent changes in the structure [17–20]. Grossly, there is a loss of the rod-like morphology and changes in the ultrastructure domain of the excitation-contraction coupling complexes; most notably, there is a detubulation—a loss of the transverse-tubules required for rapid, synchronous calcium-induced calcium release (CICR). Together with the change in ECM composition in the development of the myocardium, it is clear that the extracellular interactions of the CMs are vital in the maintenance of the healthy adult myocardium, and the interactions, at least in part, drive the changes that occur in diseases.

3.1. Heart Disease Remodelling and Its Consequences

Cardiac remodelling is defined as a group of molecular, cellular and interstitial changes that manifest clinically as changes in size, mass, geometry, and function of the heart after stimulation and stimuli at disease onset such as cardiac ischemia, inflammation, pressure overload, and pharmacological toxicity [96–99]. The main consequence of cardiac remodelling is contractile dysfunction, leading to left-sided heart failure [100]. There are two major types of left-sided heart failure; heart failure with a reduced ejection fraction, also called systolic failure and heart failure with a preserved ejection fraction, also called diastolic failure. Common to both is left ventricular myocardial remodelling, and progressive loss of ventricular function, asymptomatic at first, but evolves to signs and symptoms of heart failure. This results in a poor prognosis due to its association with ventricular dysfunction and malignant arrhythmias [101,102]. Remodelling involves various mechanisms associated with the pathophysiological role of different factors, including cell death, energy metabolism, oxidative stress, inflammation, extracellular matrix protein, contractile proteins, calcium transport, geometry, and the neurohormonal activation of CMs and non-myocytes [71,103,104]. Under the biomechanical overload seen in diseases, CMs and non-myocytes respond to the circulating neurohormones and cytokines in the altered environment via multiple mechanisms including the integrin-extracellular matrix interactions and renin-angiotensin-aldosterone axis activation as well as through the release of myocardial hormones and cytokines. The key non-myocytes components participate in these changes includes fibroblasts [105], endothelial cells [106,107], lymphocytes [108], and neurons [109,110]. They undergo, for example, a transition of cardiac fibroblasts into the more active myofibroblasts, resulting in an accumulation of extracellular matrix proteins [111], a shift in the actions of the endothelium toward reduced vasodilation [112,113], a proinflammatory state, and prothrombic properties and recruitment of immune cells [114] amongst others. This remodelling leads to, at a macroscopic level, an increased deposition and the alteration of the cardiac ECM, and subcellularly leads to CM hypertrophy, dysfunction, and death. These maladaptive changes participate in the pathogenesis of cardiac dysfunction.

3.2. Multicellularity

As we previously described, CMs and non-myocyte intercellular interactions are central in the initiation and progression of cardiac dysfunction. Therefore, CM-specific analyses cannot model all
cardiac diseases. Future models should use various cell types including CMs, fibroblasts, endothelial cells, vascular smooth muscle cells, lymphocytes, and neurons and the ECM (Table 4), as well as considering the cells recruited in disease. Despite the immaturity of stem cell-derived differentiated cell types, investigating how the changes occur in remodelling contributes to the pathogenesis of heart failure using models that utilize these cells, especially hiPSC-CMs, and offers huge potential in mapping this complex disease.

Table 4. The summary of the role of non-myocyte cell types in health and the disease.

| Cell.     | Healthy                                                   | Disease                                                      | Notes                                      |
|-----------| ----------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------|
| Fibroblasts| • ECM turnover, maintaining a balance between the         | • Scar formation (fibrosis)                                  | [105,111,115,116]                          |
|           | synthesis and degradation of the matrix                   | • Increase ECM protein                                       |                                            |
|           |                                                           | • Phagocytose apoptotic cells                                |                                            |
|           |                                                           | • Crosstalk to EC and macrophage for angiogenesis and matrix synthesis |                                            |
| ECM       | • Periostin, laminin, vimentin, fibronectin, and collagen  | • Increase in collagen I, III, IV, V, and VI                 |                                            |
|           | types I (90%), III, V, and VI                             | • laminin, fibronectin, thrombospondin, and tenasin          |                                            |
| Endothelial cells | • Structural support                                     | • Inflammation (hypertrophy, inotropy, apoptosis, mitosis)   |                                            |
|           | • Vasculature homeostasis                                 | • Neovascularization increase the density of peri-infarct vessels |                                            |
|           | • Biochemical factors such as nitric oxide, endothelin-1,| • Paracrine                                                  |                                            |
|           | IL-6                                                     |                                                              |                                            |
|           | • Progenitor of cardiac pericytes and vascular smooth     |                                                              |                                            |
|           | muscle cells                                             |                                                              |                                            |
| SMCs      | • Mechanical support of vasculature: contractile or       | • Loss of elasticity                                         |                                            |
|           | synthetic (proliferative) mode                           | • Reduced contractile                                        |                                            |
|           |                                                           | • Increased proliferation                                   |                                            |
| Neuronal cells | • Conduction fibre and pacemaker (AV, SA, Purkinje)      | • Block, slow down conduction                                |                                            |
|           | • Control of rhythmic beating                             | • Essential component for embryo development                |                                            |
| Lymphocytes| • Few residents                                           | • Macrophage has a role in ECM turnover/cell death, scar     |                                            |
|           | • Mast cells act as inflammatory mediator storage and     | formation, neutrophil recruitment, and vascularization       |                                            |
|           | activating the local renin-angiotensin system             | support                                                      |                                            |
|           | • Macrophage performs a janitorial homeostasis and        |                                                              |                                            |
|           | facilitates electrical conduction                         |                                                              |                                            |
3.2.1. Extracellular Matrix (ECM)

The complex tissue and organ architecture of the heart is maintained by extensive ECM networks, composed of fibrous proteins (e.g., collagen, elastin), adhesive glycoproteins (e.g., fibronectin, laminin), and proteoglycans. These guide the anisotropic alignment of CMs, form the mechanical environment for cells and contribute to the stress-strain relationships of the heart. In health, the fibres of the ECM use the energy produced in systole for the re-lengthening of CMs during diastole. It is important to note that in heart failure there is a CM hypertrophy as well as collagen deposition in the ECM [104,111,117]. Though hypertrophy is common in many adults, in particular, trained athletes, the changes in the ECM composition is key to differentiating pathological from physiological hypertrophy. The increase in collagen deposition in the ECM during cardiac remodelling, despite preserving an adequate cardiac output in the early phases of heart failure, proves to be chronically maladaptive in contributing to ventricular dysfunction and conduction abnormalities. It is widely agreed that the laying down of new ECM proteins in remodelling is regulated at a cellular level by a plethora of molecular, mechanical, and hemodynamic factors. Many researchers have studied the role of ECM on in vitro cultures of hiPSC-derived cells. For example, 3D cardiac cultures on different ECM matrices directed hiPSC-CMs to different cell fates [104,111,117]. Delineating the mediators of this would go some way to providing insight into therapeutic targets and more accurate models of human cardiac disease.

3.2.2. Fibroblasts

Cardiac fibroblasts account for 20% of the non-myocyte components of the heart [132]. The numbers fluctuate as the heart develops but they are substantially stimulated postnatally as the neonatal heart begins to function independently, likely correlated with the need for a greater mechanical and tensile strength as the nascent myocardium. In addition to their mechanical properties, they produce various proteins found in the ECM, including the major fibrillar collagens type I and III, which comprise the bulk of the ECM, as well as collagenases, fibronectin, and vitronectin [133]. The proliferative capacity of cardiac fibroblasts and the differentiation into the more active disease phenotype, cardiac myofibroblasts, has allowed us to investigate the effects these cells have on CMs in culture. Our group recently showed, using co-culture setups with hiPSC-CMs and human cardiac fibroblasts, that although distant paracrine interactions independent of contact between the two cell types causes the prolongation of hiPSC-CM calcium transients, the direct seeding of fibroblasts on CM monolayers improves the efficiency of CICR and a contribution of the sarcoplasmic reticulum to decay mechanisms, closer recapitulating the parameters of the adult healthy human CM [134]. Future studies must identify the significant mediators of this modulation.

3.2.3. Endothelial Cells

The vasculature is critical in the delivery of oxygen and nutrients to CMs and, thus, momentum has gathered in the modelling of vascularized microenvironments [113,135]. In the context of disease, the vascular endothelium is critical in the initiation of the inflammatory response, the triggering of inflammation, the regulation of vasomotor tone, and the control of the vascular permeability [112]. The importance of endothelial cells has been shown in fibrin gel co-culture constructs, where endothelial cells and patient-derived pericytes increase the stability of perfusable micro-vessels [25]. The benefit of these microfluidic models is that it requires comparably small amounts of cells and reagents, as well as being much easier for the constant monitoring and manipulation of the construct configuration, compared to large artificial tissue studies. Endothelial dysfunction plays a significant role in the development of atherosclerosis and the consequential strokes or myocardial infarctions. hiPSC-derived endothelial cells possess a repertoire of phenotypic plasticity and are amenable to cell-based assays probing endothelial contributions to inflammatory and cardiovascular diseases [136,137]. Folkman et al. produced de novo growth of capillary tubes in vitro. A key factor identified was Vascular Endothelial Growth Factor, but it is clear that multiple factors and cell types are
required to recreate the full myocardial vascularity in 3D models. The lack of evidence for functional vessel formation in models utilizing hiPSC-endothelial cells hinders investigations into the role that these cells play in the development and maintenance of the adult myocardial phenotype [138,139]. It is postulated that this is due to the immaturity in these iPSC-derived cells, similar to hiPSC-CMs, and thus, an improvement in hiPSC-endothelial cell maturation is needed before we can obtain evidence of functional vessel formation in human models of cardiac disease.

3.2.4. Vascular Smooth Muscle Cells

SMCs display plasticity in switching expression between contractile and proliferative (synthetic) phenotypes in the regulation of vessel tone and blood pressure. The challenges to studying diseases of the vasculature include the limited proliferation capacity and rapid senescence of adult human SMCs and hiPSC-SMC. Many studies have shown that the in vitro modulation of hiPSCs can direct them to become specific subtypes of SMCs; displaying a proliferative, synthetic or contractile phenotype. These SMCs closely resemble native SMCs at both the transcriptional and functional levels, with their pluripotency effectively silenced. There is promising evidence to suggest that SMCs have a significant role in the construction of the tunica media in the maintenance of vessel tension and contraction-relaxation capabilities and can be reproducibly created using SMC. However, less reliable is the evidence for the formation of functional vessels using hiPSC-ECs and SMCs in the co-culture. It is believed that the pattern of gene expression varies between in vivo and in vitro studies, as well as in the somatic tissue from which the stem cells are sourced [140].

3.2.5. Lymphocytes

The human body’s adaptive immune system is designed to protect the body from injury. The central cellular components of this immunity are T- and B-cells that arise from lymphoid progenitor cells in the bone marrow. The cellular components of innate immunity are myeloid cells, including monocytes, macrophages, dendritic cells, natural killer cells, as well as neutrophilic, basophilic, and eosinophilic granulocytes. Heart failure is a state of chronic inflammation [97], with a characteristic increase in circulating and myocardial pro-inflammatory cytokines that have been shown to promote pathological left ventricular remodelling. Though pre-clinical and early human studies suggest a therapeutic role for cytokine antagonism in heart failure, the poor characterization of the cascade of events that occur in the inflammation in heart failure is thought to be key in the failure of immunomodulation trials.

Studies in which coronary ligation in adult C57BL/6 mice has been used to mimic chronic ischemic heart failure showed that the initial injury causes a global expansion and activation of CD4+ T-lymphocytes and the expansion of memory T-cells in the spleen. The key findings are that the cardiac and splenic T-cells in heart failure are primed to induce cardiac injury and remodelling (long-term left ventricular dysfunction, fibrosis, and hypertrophy), and retain this memory upon the adoptive transfer from donor mice to naïve recipient mice [141]. There is extensive evidence to show that CD4+ T-cells contribute to the size of the infarct following a myocardial ischemia-reperfusion injury. Recently, regulatory T-cells were shown to contribute to the rosvastatin-induced cardioprotection against myocardial-reperfusion injuries [142]. The degree of recruitment, the activity of neutrophils, and the most numerous leukocyte subset in the first hours after ischemia-reperfusion critically influence the extent of the injury. Though there are limited studies into the roles of other lymphocytes in this disease and much of the evidence is from mouse myocardial infarction models, the importance of considering the lymphocytes in their individual subsets when identifying their role in the disease and as therapeutic targets is clear [128].

3.2.6. Neurons

The dynamic interconnections between the heart and the neuronal system that controls cardiac function are bidirectional; dysfunction in either system can trigger a cascade that alters the functioning
of the other. Peripheral neural networks are composed of all the necessary neural machinery for the reflex control of the heart [124]. The integrated, autonomic nervous system that has tonic control over cardiac function in the quiescent environment can efficiently respond to stressors by altering the work done by the myocardium. Crosstalk between neurons and CMs in vitro has been shown to improve the functioning of both [143]. The link between the two cell types is well documented in hyperglycemia-related neuropathy secondary to diabetes, whereby the risk of developing cardiac disease is higher than in non-diabetic patients [125,126]. Conversely, neuronal dysfunction has also been seen to follow cardiac injury; in the case of scar formation in cardiac ischemic patients, it causes a delay in the conduction velocity and/or inducing cardiac arrhythmia, which leads to contractile dysfunction and, eventually, heart failure [127]. It is therefore clear that there is a close interplay between the two cell types, and future studies must consider the temporal relationship between events that conclude with chronic heart disease.

Current disease modelling simplifies the complexity of the heart by using a simple co-culture; whereas cardiac organoids have not yet been reported [144]. Recently, Ronaldson-Bouchard et al. demonstrated a structural improvement of hiPSC-CMs co-culture with primary human fibroblasts and endothelial cells. Although this 3D cardiac tissue more closely mimics the physiological environment of the heart, a co-culture with primary cells from different sources compromises their patient specificity. Therefore, these models cannot reach the potential that we envisage of multicellular models based on autologous hiPSC cell types in the future.

Looking ahead, beyond modelling the heart as a closed system, we must also consider its place as an organ within the human circulatory system. A number of clinical and animal studies have implicated inflammation as a key contributor to myocardial remodelling. Prolonged exposure to inflammatory cytokines not only triggers signalling cascades within the heart but also other organs [145,146]. The importance of the cardio-splenic axis has been shown in animal studies, in which mice without a spleen are shown to have an attenuation of lymphocyte production and reduced cardiac dysfunction [147]. The importance of the kidneys has been shown clinically, whereby patients with chronic kidney diseases experience higher rates of mortality following myocardial infarction [148–150]. It is therefore critical that signalling from beyond the myocardial cell types must be considered in order to develop a model that fully recapitulates the heart in disease.

4. Conclusions and Future Prospects

Our knowledge of human diseases has improved enormously over the last few decades, owing to advancements in the in vitro and animal models that allow us to investigate the intercellular interactions between CMs and non-CM cell types, demonstrating their importance in simple 2D co-cultures, as well as within a 3D scaffold. Increasingly, it has become standard to use hiPSCs, especially hiPSC-CMs, in models for human diseases due to their patient’s origin. However, with many of the models of human diseases dependent on the ability of the CMs to recapitulate native adult human CM physiology, differences between CMs in vitro and in the native myocardium pose challenges to fully realizing the potential of human disease models. The advancements in stem cell biology and tissue engineering have spearheaded the development of in vitro cardiac models that can employ patient- or disease-specific CMs and non-myocytes in culture. As it becomes increasingly clear that CMs are intricately modulated by the extensive extracellular interactions in the native myocardium, more accurate future in vitro myocardial models will be more physiologically relevant with the ability to provide multiple biochemical, mechanical and electrical readouts, for real-time monitoring of disease progression and functional endpoints. The development on new biomaterials will not only provide support to patient-specific stem cell-derived cells but will also contribute to their maturation and their guiding alignment, providing the relevant stiffness and be dynamically replaced over time by ECM components secreted from the cells [31,151–155]. Additionally, the multidisciplinary approach that integrates in vitro, in vivo, and ex vivo datasets by using in silico computational methodology for analysis and prediction could potentially generate new insights in Cardiology [156].
Overall, a more solid picture of the myocyte-non-myocyte interactions that occur in the native myocardium using relevant models will enable us to develop a greater understanding of how the adult myocardium functions. This will provide the unique opportunity to study strategies for disease intervention in human in vitro disease models, spanning the gap between 2D culture and in vivo testing, thus reducing the cost, time, and ethical burden of the current approaches.

**Author Contributions:** Writing—original draft preparation, B.X.W and W.K.-A.; writing—review & editing, B.X.W, W.K.-A. and C.M.N.T.; supervision, C.M.N.T.

**Funding:** This research was funded by the BRITISH HEART FOUNDATION; B.X.W was supported by an MBPhD Fellowship (FS/16/76/32409) and W.K.A was supported by a Centre of Research Excellence grant (RE/13/4/20184).

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

- 2D: Two-dimensional
- 3D: Three-dimensional
- ALDH-2: Aldehyde dehydrogenase 2
- CICR: Calcium induced calcium release
- CM: Cardiomyocyte
- CPVT: Catecholaminergic polymorphic ventricular tachycardia
- CHO: Chinese hamster ovary
- EC: Endothelial cell
- ECM: Extracellular matrix
- ELN: Elastin
- HEK: Human embryonic kidney
- HiPSC: Human induced pluripotent stem cell
- LQTS: Long-QT syndrome
- LQT8: Timothy Syndrome
- RBC: Red blood cell
- SMC: Smooth muscle cell
- TAZ: Tafazzin

**References**

1. British Heart Foundation. Cardiovascular Disease Statistics 2015—BHF. Published 2015. Available online: https://www.bhf.org.uk/publications/statistics/cvd-stats-2015 (accessed on 15 September 2018).
2. Edwards, A.G.; Louch, W.E. Species-dependent mechanisms of cardiac arrhythmia: A cellular focus. *Clin. Med. Insights Cardiol.* 2017, 11. [CrossRef] [PubMed]
3. Kofron, C.M.; Mende, U. In vitro models of the cardiac microenvironment to study myocyte and non-myocyte crosstalk: Bioinspired approaches beyond the polystyrene dish. *J. Physiol.* 2017, 595, 3891–3905. [CrossRef] [PubMed]
4. Kim, H.S.; Bernitz, J.M.; Lee, D.-F.; Lemischka, I.R. Genomic Editing Tools to Model Human Diseases with Isogenic Pluripotent Stem Cells. *Stem Cells Dev.* 2014, 23, 2673–2686. [CrossRef] [PubMed]
5. Onakpoya, I.J.; Heneghan, C.J.; Aronson, J.K. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: A systematic review of the world literature. *BMC Med.* 2016, 14, 191. [CrossRef] [PubMed]
6. Parameswaran, S.; Kumar, S.; Verma, R.S.; Sharma, R.K. Cardiomyocyte culture—An update on the in vitro cardiovascular model and future challenges. *Can. J. Physiol. Pharmacol.* 2013, 91, 985–998. [CrossRef] [PubMed]
7. Li, D.; Wu, J.; Bai, Y.; Zhao, X.; Liu, L. Isolation and Culture of Adult Mouse Cardiomyocytes for Cell Signaling and in vitro Cardiac Hypertrophy. *J. Vis. Exp.* 2014, 2–9. [CrossRef]
8. Henrique Franco, N. Animal experiments in biomedical research: A historical perspective. *Animals* 2013, 3, 238–273. [CrossRef] [PubMed]
9. Ericsson, A.C.; Crim, M.J.; Franklin, C.L. A brief history of animal modeling. *Mo. Med.* 2008, 110, 201–205. [CrossRef] [PubMed]

10. Guénet, J.-L. Animal models of human genetic diseases: Do they need to be faithful to be useful? *Mol. Genet. Genom.* 2011, 286, 1–20. [CrossRef] [PubMed]

11. Smithies, O. Animal models of human genetic diseases. *Trends Genet.* 1993, 9, 112–116. [CrossRef]

12. Erickson, R.P. Minireview: Creating Animal Models of Genetic Disease. *Am. J. Hum. Genet.* 1988, 43, 582–586. [PubMed]

13. Whitelaw, C.B.A.; Sheets, T.P.; Lillico, S.G.; Telugu, B.P. Engineering large animal models of human disease. *Regul. Toxicol. Pharmacol.* 2000, 32, 56–67. [CrossRef] [PubMed]

14. Olson, H.; Betton, G.; Robinson, D.; Thomas, K.; Monro, A.; Kolaja, G.; Lilly, G.; Sanders, J.; Sipes, G.; Bracken, W.; et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Int. J. Mol. Sci.* 2018, 19, 3361. [CrossRef] [PubMed]

15. Jones, D.K.; Liu, F.; Vaidyanathan, R.; Eckhardt, L.L.; Trudeau, M.C.; Robertson, G.A. hERG 1b is critical for human cardiac repolarization. *Proc. Natl. Acad. Sci. USA* 2014, 111, 1–5. [CrossRef] [PubMed]

16. Physiol, C. Ion Channels in the Heart. *Compr. Physiol.* 2016, 5, 1423–1464. [CrossRef]

17. Zhang, Y.; Li, T.S.; Lee, S.T.; Wawrowsky, K.A.; Cheng, K.; Galang, G.; Malliaras, K.; Abraham, M.R.; Wang, C.; Marbán, E. Dedifferentiation and proliferation of mammalian cardiomyocytes. *PLoS ONE* 2010, 5, e12559. [CrossRef] [PubMed]

18. Wang, W.E.; Li, L.; Xia, X.; Fu, W.; Liao, Q.; Lan, C.; Yang, D.; Chen, H.; Yue, R.; Zeng, C.; et al. Dedifferentiation, proliferation, and redifferentiation of adult mammalian cardiomyocytes after ischemic injury. *Circulation* 2017, 136, 843–848. [CrossRef] [PubMed]

19. Dutta, D.; Heo, I.; Clevers, H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. *Trends Mol. Med.* 2017, 23, 393–410. [CrossRef] [PubMed]

20. Ryu, A.J.; Brougham, C.M.; Garciaena, C.D.; Kerrigan, S.W.; O’Brien, F.J. Towards 3D in vitro models for the study of cardiovascular tissues and disease. *Drug Discov. Today* 2016, 21, 1437–1445. [CrossRef] [PubMed]

21. Mathur, A.; Ma, Z.; Loskill, P.; Jeewa, S.; Healy, K.E. In vitro cardiac tissue models: Current status and future prospects. *Adv. Drug Deliv. Rev.* 2016, 96, 203–213. [CrossRef] [PubMed]

22. Thomson, J.A.; Itskovitz-Eldor, J.; Shapiro, S.S.; Waknitz, M.A.; Swiergiel, J.J.; Marshall, V.S.; Jones, J.M. Embryonic stem cell line derived from human blastocysts. *Science* 1998, 282, 1145–1147. [CrossRef] [PubMed]

23. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007, 131, 861–872. [CrossRef] [PubMed]

24. Shelton, M.; Kocharyan, A.; Liu, J.; Skerjanc, I.S.; Stanford, W.L. Robust generation and expansion of skeletal muscle progenitors and myocytes from human pluripotent stem cells. *Methods* 2016, 101, 73–84. [CrossRef] [PubMed]

25. Zwi, L.; Caspi, O.; Arbela, G.; Huber, I.; Gepstein, A.; Park, I.H.; Gepstein, L. Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation* 2009, 120, 1513–1523. [CrossRef] [PubMed]
31. Lian, X.; Hsiao, C.; Wilson, G.; Zhu, K.; Hazeltine, L.B.; Azarin, S.M.; Raval, K.K.; Zhang, J.; Kamp, T.J.; Palecek, S.P. PNAS Plus: Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc. Natl. Acad. Sci. USA* 2012, 109, E1848–E1857. [CrossRef] [PubMed]

32. Tse, H.-F.; Ho, J.C.Y.; Choi, S.-W.; Lee, Y.K.; Butler, A.W.; Ng, K.M.; Siu, C.-W.; Simpson, M.A.; Lai, W.-H.; Chan, Y.C.; et al. Patient-specific Induced Pluripotent Stem Cells Derived Cardiomyocytes Recapitulates the Pathogenic Phenotypes of Dilated Cardiomyopathy due to a Novel DES Mutation Identified by Whole Exome Sequencing. *Hum. Mol. Genet.* 2013, 22, 1395–1403. Available online: [http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23300193&retmode=ref&cmd=prlinks§id=26E30F$npapers2://publication/doi/10.1093/hmg/dds556](http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23300193&retmode=ref&cmd=prlinks§id=26E30F$npapers2://publication/doi/10.1093/hmg/dds556) (accessed on 22 September 2018).

33. Veerman, C.C.; Mengarelli, I.; Guan, K.; Stauske, M.; Barc, J.; Tan, H.L.; Wilde, A.A.M.; Verkerk, A.O.; Bezzina, C.R. HiPSC-derived cardiomyocytes from Brugada Syndrome patients without identified mutations do not exhibit clear cellular electrophysiological abnormalities. *Sci. Rep.* 2016, 6, 30967. [CrossRef] [PubMed]

34. Kehl, D.; Weber, B.; Hoerstrup, S.P. Bioengineered living cardiac and venous valve replacements: Current status and future prospects. *Cardiovasc. Pathol.* 2016, 25, 300–305. [CrossRef] [PubMed]

35. Wen, J.-Y.; Wei, C.-Y.; Shah, K.; Wong, J.; Wang, C.; Chen, H.-S.V. Maturation-Based Model of Arrhythmogenic Right Ventricular Dysplasia Using Patient-Specific Induced Pluripotent Stem Cells. *Circ. J.* 2015, 79, 1402–1408. [CrossRef] [PubMed]

36. Freund, C.; Davis, R.P.; Gkatzis, K.; Ward-van Oostwaard, D.; Mummery, C.L. The first reported generation of human induced pluripotent stem cells (iPS cells) and iPS cell-derived cardiomyocytes in The Netherlands. *Neth. Heart J.* 2010, 18, 51–54. [PubMed]

37. Zwi-Dantsis, L.; Huber, I.; Habib, M.; Winterstern, A.; Gepstein, A.; Arb, G.; Gepstein, L. Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients. *Eur. Heart J.* 2013, 34, 1575–1586. [CrossRef] [PubMed]

38. Karakikes, I.; Ameen, M.; Termglinchan, V.; Wu, J.C. Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes: Insights into Molecular, Cellular, and Functional Phenotypes. *Circ. Res.* 2015, 117, 80–88. [CrossRef] [PubMed]

39. Adams, W.J.; Zhang, Y.; Cloutier, J.; Kuchimanchi, P.; Newton, G.; Sehrawat, S.; Aird, W.C.; Mayadas, T.N.; Luscinskas, F.W.; García-Carderra, G. Functional Vascular Endothelium Derived from Human Induced Pluripotent Stem Cells. *Stem Cell Rep.* 2013, 1, 105–113. [CrossRef] [PubMed]

40. Zhang, J.; Lian, Q.; Zhu, G.; Zhou, F.; Sui, L.; Tan, C.; Mutalif, R.A.; Navasankari, R.; Zhang, Y.; Tse, H.F.; et al. A human iPS model of hutchinson gilford progeria reveals vascular smooth muscle and mesenchymal stem cell defects. *Cell Stem Cell* 2011, 8, 31–45. [CrossRef] [PubMed]

41. Ge, X.; Ren, Y.; Bartulos, O.; Lee, M.Y.; Yue, Z.; Kim, K.Y.; Li, W.; Amos, P.J.; Bozkulak, E.C.; Iyer, A.; et al. Modeling supravalvular aortic stenosis syndrome with human induced pluripotent stem cells. *Circulation* 2012, 126, 1695–1704. [CrossRef] [PubMed]

42. Granata, A.; Serrano, F.; Bernard, W.G.; McNamara, M.; Low, L.; Sastry, P.; Sinha, S. An iPSC-derived vascular model of Marfan syndrome identifies key mediators of smooth muscle cell death. *Nature* 2013, 494, 105–110. [CrossRef] [PubMed]

43. French, A.; Yang, C.-T.; Taylor, S.; Watt, S.M.; Carpenter, L. Human Induced Pluripotent Stem Cell-Derived B Lymphocytes Express slgM and Can Be Generated via a Hemogenic Endothelium Intermediate. *Stem Cells Dev.* 2015, 24, 1082–1095. [CrossRef] [PubMed]

44. Fan, F.; Yu, Y.; Sun, L.; Wang, S.; Wang, R.; Zhang, L.; Li, C.; Wang, D. Induction of Pluripotent Stem Cell-Derived Cardiomyocyte Toxicity by Supernatant of Long Term-Store Red Blood Cells in Vitro. *Cell Physiol. Biochem.* 2018, 46, 1230–1240. [CrossRef] [PubMed]

45. Jiang, Y.; Habibollah, S.; Tilgner, K.; Collin, J.; Bart, A.; Al-Aama, J.Y.; Tesaros, L.; Hussain, R.; Trafford, A.W.; Kirkwood, G.; et al. An Induced Pluripotent Stem Cell Model of Hypoplastic Left Heart Syndrome (HLHS) Reveals Multiple Expression and Functional Differences in HLHS-Derived Cardiac Myocytes. *Stem Cells Transl. Med.* 2014, 3, 416–423. [CrossRef] [PubMed]

46. Kim, C.; Wong, J.; Wen, J.; Wang, S.; Wang, C.; Spiering, S.; Kan, N.G.; Forcales, S.; Puri, P.L.; Leone, T.C.; et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 2013, 494, 105–110. [CrossRef] [PubMed]
47. Lan, F.; Lee, A.S.S.; Liang, P.; Sanchez-Freire, V.; Nguyen, P.K.K.; Wang, L.; Han, L.; Yen, M.; Wang, Y.; Sun, N.; et al. Abnormal Calcium Handling Properties Underlie Familial Hypertrophic Cardiomyopathy Pathology in Patient-Specific Induced Pluripotent Stem Cells. Cell Stem Cell 2013, 12, 101–113. [CrossRef] [PubMed]

48. Carvajal-Vergara, X.; Sevilla, A.; Dsouza, S.L.; Ang, Y.S.; Schaniel, C.; Lee, D.F.; Yang, L.; Kaplan, A.D.; Adler, E.D.; Rozov, R.; et al. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. Nature 2010, 465, 808–812. [CrossRef] [PubMed]

49. Hick, A.; Wattenhofer-Donze, M.; Chintawar, S.; Tropel, P.; Simard, J.P.; Vaucamps, N.; Gall, D.; Lambot, L.; Andre, C.; Reutenauer, L.; et al. Neurons and cardiomyocytes derived from induced pluripotent stem cells as a model for mitochondrial defects in Friedreich’s ataxia. Dis. Model Mech. 2013, 6, 608–621. [CrossRef] [PubMed]

50. Fatima, A.; Xu, G.; Shao, K.; Papadopoulos, S.; Lehmann, M.; Arnáiz-Cot, J.J.; Rosa, A.O.; Nguemo, F.; Matzkies, M.; Dittmann, S.; et al. Cellular Physiology Cellular Physiology Cellular Physiology Cellular Physiology In vitro Modeling of Ryanodine Receptor 2 Dysfunction Using Human Induced Pluripotent Stem Cells. Cell Physiol. Biochem. 2011, 28, 579–592. [CrossRef] [PubMed]

51. Novak, A.; Barad, L.; Zeevi-Levin, N.; Shick, R.; Shtrichman, R.; Lorber, A.; Itskovitz-Eldor, J.; Binah, O. Cardiomyocytes generated from CPVT D307H patients are arrhythmogenic in response to β-adrenergic stimulation. J. Cell. Mol. Med. 2012, 16, 468–482. [CrossRef] [PubMed]

52. Lahti, A.L.; Kujala, V.J.; Chapman, H.; Koivisto, A.-P.; Pekkanen-Mattila, M.; Kerkela, E.; Hyttinen, J.; Kontula, K.; Swan, H.; Conklin, B.R.; et al. Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis. Model Mech. 2012, 5, 220–230. [CrossRef] [PubMed]

53. Davis, R.P.; Casini, S.; Van Den Berg, C.W.; Hoekstra, M.; Remme, C.A.; Dambrot, C.; Salvatori, D.; Oostwaard, D.W.; Wilde, A.A.; Bezzina, C.R.; et al. Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation 2012, 125, 3079–3091. [CrossRef] [PubMed]

54. Moretti, A.; Bellin, M.; Welling, A.; Jung, C.B.; Lam, J.T.; Bott-Flügel, L.; Dorn, T.; Goedel, A.; Höhnke, C.; Hofmann, F.; et al. Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome. N. Engl. J. Med. 2010, 363, 1397–1409. [CrossRef] [PubMed]

55. Yazawa, M.; Hsueh, B.; Jia, X.; Pasca, A.M.; Bernstein, J.A.; Hallmayer, J.; Dolmetsch, R.E. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 2011, 471, 230–236. [CrossRef] [PubMed]

56. Rocchetti, M.; Sala, L.; Dreizhnter, L.; Crotti, L.; Sinnecker, D.; Mura, M.; Pane, L.S.; Altomare, C.; Torre, E.; Mostacciolo, G.; et al. Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. Cardiovasc. Res. 2017, 113, 531–541. [CrossRef] [PubMed]

57. Limpitikul, W.B.; Dick, I.E.; Tester, D.J.; Boczek, N.J.; Limphong, P.; Yang, W.; Choi, M.H.; Babich, J.; Disilvestre, D.; Kanter, R.J.; et al. A Precision Medicine Approach to the Rescue of Function on Malignant Calmodulinopathic Long-QT Syndrome. Circ. Res. 2017, 120, 39–48. [CrossRef] [PubMed]

58. Wang, G.; McCoy, M.L.; Yang, L.; He, A.; Pasqualini, F.S.; Agarwal, A.; Yuan, H.; Jiang, D.; Zhang, D.; Zangi, L.; et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat. Med. 2014, 20, 616–623. [CrossRef] [PubMed]

59. Ebert, A.D.; Kodo, K.; Liang, P.; Wu, H.; Huber, B.C.; Riegler, J.; Churko, J.; Lee, J.; de Almeida, P.; Lan, F.; et al. Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system. Sci. Transl. Med. 2014, 6, 255ra130. [CrossRef] [PubMed]

60. Kane, C.; Couch, L.; Terracciano, C.M. Excitation–contraction coupling of human induced pluripotent stem cell-derived cardiomyocytes. Front. Cell Dev. Biol. 2015, 3, 59. [CrossRef] [PubMed]

61. Watanabe, M.; Rollins, A.; Polo-Parada, L.; Ma, P.; Gu, S.; Jenkins, M. Probing the Electrophysiology of the Developing Heart. J. Cardiovasc. Dev. Dis. 2016, 3, 10. [CrossRef] [PubMed]

62. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. Nature 2007, 448, 313–317. [CrossRef] [PubMed]
63. Gwathmey, J.K.; Slawsky, M.T.; Hajjar, R.J.; Briggs, G.M.; Morgan, J.P. Role of intracellular calcium handling in force-interval relationships of human ventricular myocardium. *J. Clin. Invest.* 1990, 85, 1599–1613. [CrossRef] [PubMed]

64. Jacobson, S.L.; Piper, H.M. Cell cultures of adult cardiomyocytes as models of the myocardium. *J. Mol. Cell. Cardiol.* 1986, 18, 661–678. [CrossRef]

65. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006, 126, 663–676. [CrossRef] [PubMed]

66. Gouadon, E.; Moore-Morris, T.; Smit, N.W.; Chatenoud, L.; Coronel, R.; Harding, S.E.; Jourdon, P.; Lambert, V.; Rucker-Martin, C.; Pucéat, M. Concise Review: Pluripotent Stem Cell-Derived Cardiac Cells, A Promising Cell Source for Therapy of Heart Failure: Where Do We Stand? *Stem Cells* 2016, 34, 34–43. [CrossRef] [PubMed]

67. Beuckelmann, D.J.; Nabauer, M.; Erdmann, E. Intracellular Calcium Handling in Isolated Ventricular Myocytes from Patients with Terminal Heart Failure. *Am. Heart J.* 1992, 85, 1046–1055. [CrossRef]

68. Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Ichisaka, T.; Aoi, T.; Okita, K.; Mochiduki, Y.; Takizawa, N.; Yamanaka, S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 2008, 26, 101–106. [CrossRef] [PubMed]

69. Kraev, A.; Chumakov, I.; Carafoli, E. The organization of the human gene NCX1 encoding the sodium-calcium exchanger. *Genomics* 1996, 37, 105–112. [CrossRef] [PubMed]

70. Huangfu, D.; Osafune, K.; Maehr, R.; Guo, W.; Eijkelenboom, A.; Chen, S.; Muhlestein, W.; Melton, D.A. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat. Biotechnol.* 2008, 26, 1269–1275. [CrossRef] [PubMed]

71. Xin, M.; Olson, E.N.; Bassel-Duby, R. Mending broken hearts: Cardiac development as a basis for adult heart regeneration and repair. *Nat. Rev. Mol. Cell Biol.* 2013, 14, 529–541. [CrossRef] [PubMed]

72. Grubb, S.; Calloe, K.; Thomsen, M.B. Impact of KChIP2 on cardiac electrophysiology and the progression of heart failure. *Front. Physiol.* 2012, 3, 1–9. [CrossRef] [PubMed]

73. Deschenes, I. Regulation of Kv4.3 Current by KChIP2 Splice Variants: A Component of Native Cardiac Ito? *Circulation* 2002, 106, 423–429. [CrossRef] [PubMed]

74. Razeghi, P.; Young, M.E.; Alcorn, J.L.; Frazier, O.H.; Taegtmeyer, H. Metabolic Gene Expression in Fetal and Failing Human Heart. *Circulation* 2001, 104, 2923–2931. [CrossRef] [PubMed]

75. Bergmann, O.; Bhardwaj, R.D.; Bernard, S.; Zdunek, S.; Barnabá-Heider, F.; Walsh, S.; Zupicich, J.; Allkass, K.; Buchholz, B.A.; Druid, H.; et al. Evidence for cardiomyocyte renewal in humans. *Science* 2009, 324, 98–102. [CrossRef] [PubMed]

76. Gross, D.B.; Jongsm, H.J. Connexins in mammalian heart function. *Bioessays* 1996, 18, 719–730. [CrossRef] [PubMed]

77. Dick, E.; Rajamohan, D.; Ronksley, J.; Denning, C. Evaluating the utility of cardiomyocytes from human pluripotent stem cells for drug screening. *Biochem. Soc. Trans.* 2010, 38, 1037–1045. [CrossRef] [PubMed]

78. Gaborit, N.; Le Bouter, S.; Szuts, V.; Varro, A.; Escande, D.; Nattel, S.; Demolombe, S. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J. Physiol.* 2007, 582, 675–693. [CrossRef] [PubMed]

79. Van den Berg, C.W.; Okawa, S.; Chuva de Sousa Lopes, S.M.; van Iperen, L.; Passier, R.; Braam, S.R.; Tertoolen, L.G.; Del Sol, A.; Davis, R.P.; Mummery, C.L. Transcriptome of human foetal heart compared with cardiac myocytes from pluripotent stem cells. *Development* 2015, 142, 3231–3238. [CrossRef] [PubMed]

80. Hwang, H.S.; Kryshtał, D.O.; Feaster, T.K.; Sánchez-Freire, V.; Zhang, J.; Kamp, T.J.; Hong, C.C.; Wu, J.C.; Knollmann, B.C. Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories. *J. Mol. Cell. Cardiol.* 2015, 85, 79–88. [CrossRef] [PubMed]

81. Bizy, A.; Guerrero-Serna, G.; Hu, B.; Ponce-Balbuena, D.; Willis, B.C.; Zarzoso, M.; Ramirez, R.J.; Sener, M.F.; Mundada, L.V.; Klos, M.; et al. Myosin light chain 2-based selection of human iPSC-derived early ventricular cardiac myocytes. *Stem Cell Res.* 2013, 11, 1335–1347. [CrossRef] [PubMed]

82. Higuchi, T.; Miyagawa, S.; Pearson, J.T.; Fukushima, S.; Saito, A.; Tsuchimochi, H.; Sonobe, T.; Fujii, Y.; Yagi, N.; Astolfi, A.; et al. Functional and Electrical Integration of Induced Pluripotent Stem Cell-Derived Cardiomyocytes in a Myocardial Infarction Rat Heart. *Cell Transplant.* 2015, 24, 2479–2489. [CrossRef] [PubMed]
83. Du, D.T.M.; Hellen, N.; Kane, C.; Terracciano, C.M.N. Action potential morphology of human induced pluripotent stem cell-derived cardiomyocytes does not predict cardiac chamber specificity and is dependent on cell density. *Biophys. J.* 2015, 108, 1–4. [CrossRef] [PubMed]

84. Sallam, K.; Li, Y.; Sager, P.T.; Houser, S.R.; Wu, J.C. Finding the Rhythm of Sudden Cardiac Death: New Opportunities Using Induced Pluripotent Stem Cell-Derived Cardiomyocytes. *Circ. Res.* 2015, 116, 1989–2004. [CrossRef] [PubMed]

85. Li, S.; Cheng, H.; Tomaselli, G.F.; Li, R.A. Mechanistic basis of excitation-contraction coupling in human pluripotent stem cell-derived ventricular cardiomyocytes revealed by Ca2+ spark characteristics: Direct evidence of functional Ca2+-induced Ca2+ release. *Heart Rhythm.* 2014, 11, 133–140. [CrossRef] [PubMed]

86. Harris, K.; Aylott, M.; Cui, Y.; Louttit, J.B.; McMahon, N.C.; Sridhar, A. Comparison of Electrophysiological Data From Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes to Functional Preclinical Safety Assays. *Toxicol. Sci.* 2013, 134, 412–426. [CrossRef] [PubMed]

87. Herron, T.J.; Lee, P.; Jalife, J. Optical Imaging of Voltage and Calcium in Cardiac Cells & Tissues. *Circ. Res.* 2012, 110, 609–623. [CrossRef] [PubMed]

88. Yang, X.; Pabon, L.; Murry, C.E. Engineering Adolescence: Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Circ. Res.* 2014, 114, 511–523. [CrossRef] [PubMed]

89. Pokushalov, E.; Romanov, A.; Steinberg, J.S. Stem Cell Therapy for Electrophysiological Disorders. *Curr. Cardiol. Rep.* 2013, 15, 408. [CrossRef] [PubMed]

90. Burridge, P.W.; Holmström, A.; Wu, J.C. Chemically Defined Culture and Cardiomyocyte Differentiation of Human Pluripotent Stem Cells. In *Current Protocols in Human Genetics*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2015.

91. Liu, J.; Laksman, Z.; Backx, P.H. The electrophysiological development of cardiomyocytes. *Adv. Drug Deliv. Rev.* 2016, 96, 253–273. [CrossRef] [PubMed]

92. Mehta, A.; Verma, V.; Nandihalli, M.; Ramachandra, C.J.A.; Sequiera, G.L.; Sudibyo, Y.; Chung, Y.; Sun, W.; Shim, W. A systemic evaluation of cardiac differentiation from mRNA reprogrammed human induced pluripotent stem cells. *PLoS ONE* 2014, 9, e103485. [CrossRef] [PubMed]

93. Argenziano, M.; Lambers, E.; Hong, L.; Sridhar, A.; Zhang, M.; Chalazan, B.; Menon, A.; Savio-Galimberti, E.; Wu, J.C.; Rehan, J.; et al. Electrophysiologic Characterization of Calcium Handling in Human Induced Pluripotent Stem Cell-Derived Atrial Cardiomyocytes. *Stem Cell Rep.* 2018, 10, 1867–1878. [CrossRef] [PubMed]

94. Marcu, I.C.; Illaste, A.; Heuking, P.; Jaconi, M.E.; Ullrich, N.D. Functional characterization and comparison of intercellular communication in stem cell-derived cardiomyocytes. *Stem Cells* 2015, 33, 2208–2218. [CrossRef] [PubMed]

95. Zhou, P.; Pu, W.T. Recounting Cardiac Cellular Composition. *Circ. Res.* 2016, 118, 368–370. [CrossRef] [PubMed]

96. Vedin, O.; Lam, C.S.P.; Koh, A.S.; enson, L.; Teng, T.H.K.; Tay, W.T.; Braun, O.O.; Savarese, G.; Dahlström, U.; Lund, L.H. Significance of Ischemic Heart Disease in Patients with Heart Failure and Preserved, Midrange, and Reduced Ejection Fraction: A Nationwide Cohort Study. *Circ. Heart Fail.* 2017, 10, e003875. [CrossRef] [PubMed]

97. Shirazi, L.F.; Bissett, J.; Romeo, F.; Mehta, J.L. Role of Inflammation in Heart Failure. *Curr. Atheroscler. Rep.* 2017, 19, 27. [CrossRef] [PubMed]

98. Tuzun, E.; Bick, R.; Kadipasaoglu, C.; Conger, J.L.; Poindexter, B.J.; Gregoric, I.D.; Frazier, O.H.; Towbin, J.A.; Radovanovic, B. Modification of a volume-overload heart failure model to track myocardial remodeling and device-related reverse remodeling. *Isrn Cardiol.* 2011, 2011, 831062. [CrossRef] [PubMed]

99. Mercurio, V.; Pirozzi, F.; Lazzarini, E.; Marone, G.; Rizzo, P.; Agnelli, G.; Tocchetti, C.G.; Ghigo, A.; Ameri, P. Models of Heart Failure Based on the Cardiotoxicity of Anticancer Drugs. *J. Card. Fail.* 2016, 22, 449–458. [CrossRef] [PubMed]

100. Azevedo, P.S.; Polegato, B.F.; Minicucci, M.F.; Paiva, S.A.R.; Zornoff, L.A.M. Cardiac Remodeling: Concepts, Clinical Impact, Pathophysiological Mechanisms and Pharmacologic Treatment. *Arq. Bras. Cardiol.* 2016, 106, 62–69. [CrossRef] [PubMed]

101. Li, A.H.; Liu, P.P.; Villarreal, F.J.; Garcia, R.A. Dynamic changes in myocardial matrix and relevance to disease: Translational perspectives. *Circ. Res.* 2014, 114, 916–927. [CrossRef] [PubMed]
102. Gerdes, A.M.; Kellerman, S.E.; Moore, J.A.; Muffly, K.E.; Clark, L.C.; Reaves, P.Y.; Malec, K.B.; McKeown, P.P.; Schocken, D.D. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. *Circulation* 1992, 86, 426–430. [CrossRef] [PubMed]

103. Peter, A.K.; Bjerke, M.A.; Leinwand, L.A. Biology of the cardiac myocyte in heart disease. *Mol. Biol. Cell* 2016, 27, 2149–2160. [CrossRef] [PubMed]

104. Howard, C.M.; Baudino, T.A. Dynamic cell-cell and cell-ECM interactions in the heart. *J. Mol. Cell. Cardiol.* 2014, 70, 19–26. [CrossRef] [PubMed]

105. Doppler, S.A.; Carvalho, C.; Lahm, H.; Deutsch, M.-A.; Dreesen, M.; Puluca, N.; Lange, R.; Krane, M. Cardiac fibroblasts: More than mechanical support. *J. Thorac. Dis.* 2017, 9, S36–S51. [CrossRef] [PubMed]

106. Segers, V.F.M.; Brutsaert, D.L.; De Keulenaer, G.W. Cardiac remodeling: Endothelial cells have more to say than just NO. *Front. Physiol.* 2018, 9, 382. [CrossRef] [PubMed]

107. Talman, V.; Kivelä, R. Cardiomyocyte—Endothelial Cell Interactions in Cardiac Remodeling and Regeneration. *Front. Cardiovasc.* 2018, 5, 101. [CrossRef] [PubMed]

108. Forte, E.; Furtado, M.B.; Rosenthal, N. The interstitium in cardiac repair: Role of the immune–stromal cell interplay. *Nat. Rev. Cardiol.* 2018, 15, 601–616. [CrossRef] [PubMed]

109. Zhang, D.; Tu, H.; Wang, C.; Cao, L.; Muellerman, R.L.; Wadman, M.C.; Li, Y.L. Correlation of ventricular arrhythmogenesis with neuronal remodeling of cardiac postganglionic parasympathetic neurons in the late stage of heart failure after myocardial infarction. *Front. Neurosci.* 2017, 11, 252. [CrossRef] [PubMed]

110. Joki, Y.; Ohashi, K.; Yuasa, D.; Shibata, R.; Kataoka, Y.; Kambara, T.; Uemura, Y.; Matsuo, K.; Hayakawa, S.; Hiramatsu-Ito, M.; et al. Neuron-derived neurotrophic factor ameliorates adverse cardiac remodeling after experimental myocardial infarction. *Circ. Heart Fail.* 2015, 8, 342–351. [CrossRef] [PubMed]

111. Fan, D.; Takawale, A.; Lee, J.; Kassiri, Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenesis Tissue Repair* 2012, 5, 15. [CrossRef] [PubMed]

112. Rajendran, P.; Rengarajan, T.; Thangavel, J.; Nishigaki, Y.; Sakthisekaran, D.; Sethi, G.; Nishigaki, I. The vascular endothelium and human diseases. *Int. J. Biol. Sci.* 2013, 9, 1057–1069. [CrossRef] [PubMed]

113. Endemann, D.H.; Schiffrin, E.L. Endothelial dysfunction. *J. Am. Soc. Nephrol.* 2004, 15, 1983–1992. [CrossRef] [PubMed]

114. Epelman, S.; Liu, P.P.; Mann, D.L. Role of innate and adaptive immune mechanisms in cardiac injury and repair. *Nat. Rev. Immunol.* 2015, 15, 117–129. [CrossRef] [PubMed]

115. Cartledge, J.E.; Kane, C.; Dias, P.; Testfom, M.; Clarke, L.; Mckee, B.; Al Ayoubi, S.; Chester, A.; Yacoub, M.H.; Camelliti, P.; et al. Functional crosstalk between cardiac fibroblasts and adult cardiomyocytes by soluble mediators. *Cardiovasc. Res.* 2015, 105, 260–270. [CrossRef] [PubMed]

116. Nakaya, M.; Watari, K.; Tajima, M.; Nakaya, T.; Matsuda, S.; Ohara, H.; Nishihara, H.; Yamaguchi, H.; Hashimoto, A.; Nishida, M.; et al. Cardiac myofibroblast engulfment of dead cells facilitates recovery after myocardial infarction. *J. Clin. Investig.* 2017, 127, 383–401. [CrossRef] [PubMed]

117. Kong, P.; Christia, P.; Frangogiannis, N.G. The pathogenesis of cardiac fibrosis. *Cell. Mol. Life Sci.* 2014, 71, 549–574. [CrossRef] [PubMed]

118. Snider, P.; Standley, K.N.; Wang, J.; Azhar, M.; Doetschman, T.; Conway, S.J. Origin of Cardiac Fibroblasts and the Role of Periostin. *Circ. Res.* 2012, 105, 934–947. [CrossRef] [PubMed]

119. Jourdan-LeSaux, C.; Zhang, J.; Lindsey, M.L. Extracellular matrix roles during cardiac repair. *Life Sci.* 2010, 87, 391–400. [CrossRef] [PubMed]

120. He, L.; Huang, X.; Kanisicak, O.; Li, Y.; Wang, Y.; Li, Y.; Pu, W.; Liu, Q.; Zhang, H.; Tian, X.; et al. Preexisting endothelial cells mediate cardiac neovascularization after injury. *J. Clin. Investig.* 2017, 127, 2968–2981. [CrossRef] [PubMed]

121. Chen, Q.; Zhang, H.; Liu, Y.; Adams, S.; Elken, H.; Stehling, M.; Corada, M.; Dejana, E.; Zhou, B.; Adams, R.H. Endothelial cells are progenitors of cardiac pericytes and vascular smooth muscle cells. *Nat. Commun.* 2016, 7, 12422. [CrossRef] [PubMed]

122. Cheung, C.; Bernardo, A.S.; Trotter, M.W.B.; Pedersen, R.A.; Sinha, S. Generation of human vascular smooth muscle subtypes provides insight into embryological origin–dependent disease susceptibility. *Nat. Biotechnol.* 2012, 30, 165–173. [CrossRef] [PubMed]
123. White, C.I.; Jansen, M.A.; McGregor, K.; Mylonas, K.J.; Richardson, R.V.; Thomson, A.; Moran, C.M.; Seckl, J.R.; Walker, B.R.; Chapman, K.E.; et al. Cardiomyocyte and vascular smooth muscle-independent 11β-hydroxysteroid dehydrogenase 1 amplifies infarct expansion, hypertrophy, and the development of heart failure after myocardial infarction in male mice. *Endocrinology* 2016, 157, 346–357. [CrossRef] [PubMed]

124. Ardell, J.L.; Andresen, M.C.; Armour, J.A.; Billman, G.E.; Chen, P.S.; Foreman, R.D.; Herring, N.; O’Leary, D.S.; Sabbah, H.N.; Schultz, H.D.; et al. Translational neurocardiology: Preclinical models and cardioneurial integrative aspects. *J. Physiol.* 2016, 594, 3877–3909. [CrossRef] [PubMed]

125. Gilbert, R.E.; Connelly, K.; Kelly, D.J.; Pollock, C.A.; Krum, H. Heart failure and nephropathy: Catastrophic and interrelated complications of diabetes. *Clin. J. Am. Soc. Nephrol.* 2006, 1, 193–208. [CrossRef] [PubMed]

126. Margariti, A. Peripheral neuropathy may be a potential risk of cardiovascular disease in diabetes mellitus. *Heart* 2014, 100, 1823–1824. [CrossRef] [PubMed]

127. Akar, F.G.; Nass, R.D.; Hahn, S.; Cingolani, E.; Shah, M.; Hesketh, G.G.; DiSilvestre, D.; Tunin, R.S.; Kass, D.A.; Tomaselli, G.F. Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 2007, 293, H1223–H1230. [CrossRef] [PubMed]

128. Hofmann, U.; Frantz, S. Role of lymphocytes in myocardial injury, healing, and remodeling after myocardial infarction. *Circ. Res.* 2015, 116, 354–367. [CrossRef] [PubMed]

129. Tejada, T.; Tan, L.; Torres, R.A.; Calvert, J.W.; Lambert, J.P.; Zaidi, M.; Husain, M.; Berc, M.D.; Naib, H.; Pejler, G.; et al. IGF-1 degradation by mouse mast cell protease 4 promotes cell death and adverse cardiac remodeling days after a myocardial infarction. *Proc. Natl. Acad. Sci. USA* 2016, 113, 6949–6954. [CrossRef] [PubMed]

130. Marino, A.; Martelli, A.; Citi, V.; Fu, M.; Wang, R.; Calderone, V.; Levi, R. The novel H2S donor 4-carboxy-phenyl isothiocyanate inhibits mast cell degranulation and renin release by decreasing intracellular calcium. *Br. J. Pharmacol.* 2016, 173, 3222–3234. [CrossRef] [PubMed]

131. Hulsmans, M.; Clauss, S.; Xiao, L.; Aguirre, A.D.; King, K.R.; Hanley, A.; Hucker, W.J.; Wülfers, E.M.; Seemann, G.; Courties, G.; et al. Macrophages Facilitate Electrical Conduction in the Heart. *Cell* 2017, 169, 510–522.e20. [CrossRef] [PubMed]

132. Pinto, A.R.; Ilinykh, A.; Ivey, M.J.; Kuwabara, J.T.; Michelle, L.; Antoni, D.; Debuque, R.; Chandran, A.; Wang, L.; Arora, K. Revisiting Cardiac Cellular Composition. *Circ. Res.* 2017, 118, 400–409. [CrossRef] [PubMed]

133. Tracy, L.E.; Minasian, R.A.; Caterson, E.J. Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. *Adv. Wound Care* 2016, 5, 119–136. [CrossRef] [PubMed]

134. Kane, C.; Terracciano, C.M. Human Cardiac Fibroblasts Engage the Sarcoplasmic Reticulum in Induced Pluripotent Stem Cell-Derived Cardiomyocyte Excitation–Contraction Coupling. *J. Am. Coll. Cardiol.* 2018, 72, 1061–1063. [CrossRef] [PubMed]

135. Hsieh, P.C.H.; Davis, M.E.; Lisowski, L.K.; Lee, R.T. Endothelial-cardiomyocyte interactions in cardiac development and repair. *Annu. Rev. Physiol.* 2006, 68, 51–66. [CrossRef] [PubMed]

136. Challet Meylan, L.; Patsch, C.; Thoma, E. Endothelial cells differentiation from hPSCs. *Protein. Expr. Purify.* 2015. [CrossRef]

137. Tulloch, N.L.; Muskheli, V.; Razumova, M.V.; Korte, F.S.; Regnier, M.; Hauch, K.D.; Pabon, L.; Reinecke, H.; Murry, C.E. Growth of engineered human myocardium with mechanical loading and vascular coculture. *Circ. Res.* 2011, 109, 47–59. [CrossRef] [PubMed]

138. Clayton, Z.E.; Sadeghipour, S.; Patel, S. Generating induced pluripotent stem cell derived endothelial cells and induced endothelial cells for cardiovascular disease modelling and therapeutic angiogenesis. *Int. J. Cardiol.* 2015, 197, 116–122. [CrossRef] [PubMed]

139. Margariti, A.; Winkler, B.; Karamariti, E.; Zampetaki, A.; Tsai, T.-N.; Baban, D.; Ragoussis, J.; Huang, Y.; Han, J.-D.; Zeng, L.; et al. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. *Proc. Natl. Acad. Sci. USA* 2012, 109, 13793–13798. [CrossRef] [PubMed]

140. Dash, B.C.; Jiang, Z.; Suh, C.; Qyang, Y. Induced pluripotent stem cell-derived vascular smooth muscle cells: Methods and application. *Biochem. J.* 2015, 465, 185–194. [CrossRef] [PubMed]
141. Bansal, S.S.; Ishmahil, M.A.; Goel, M.; Patel, B.; Hamid, T.; Rokosh, G.; Prabhu, S.D. Activated T Lymphocytes are Essential Drivers of Pathological Remodeling in Ischemic Heart Failure. *Circ. Heart Fail.* 2017, 10, e003688. [CrossRef] [PubMed]

142. Ke, D.; Fang, J.; Fan, L.; Chen, Z.; Chen, L. Regulatory T cells contribute to rosuvastatin-induced cardioprotection against ischemia-reperfusion injury. *Coronary Artery Dis.* 2013, 24, 334–341. [CrossRef] [PubMed]

143. Oh, Y.; Cho, G.-S.; Li, Z.; Hong, I.; Zhu, R.; Kim, M.-J.; Kim, Y.J.; Tampakakis, E.; Tung, L.; Huganir, R.; et al. Functional Coupling with Cardiac Muscle Promotes Maturation of hPSC-Derived Sympathetic Neurons. *Cell Stem Cell* 2016, 19, 95–106. [CrossRef] [PubMed]

144. Rother, J.; Richter, C.; Turco, L.; Knoch, F.; Mey, I.; Luther, S.; Janshoff, A.; Bodenschatz, E.; Tarantola, M. Crosstalk of cardiomyocytes and fibroblasts in co-cultures. *Open Biol.* 2015, 5, 150038. [CrossRef] [PubMed]

145. Mozaffari, M.S.; Liu, J.Y.; Abebe, W.; Baban, B. Mechanisms of load dependency of myocardial ischemia reperfusion injury. *Am. J. Cardiovasc. Dis.* 2013, 3, 180–196. [PubMed]

146. Jahng, J.W.S.; Song, E.; Sweeney, G. Crosstalk between the heart and peripheral organs in heart failure. *Exp. Mol. Med.* 2016, 48, e217. [CrossRef] [PubMed]

147. Ishmahil, M.A.; Hamid, T.; Bansal, S.S.; Patel, B.; Kingery, J.R.; Prabhu, S.D. Remodeling of the Mononuclear Phagocyte Network Underlies Chronic Inflammation and Disease Progression in Heart Failure: Critical Importance of the Cardiosplenic Axis. *Circ. Res.* 2014, 114, 266–282. [CrossRef] [PubMed]

148. Smolina, K.; Wright, F.L.; Rayner, M.; Goldacre, M.J. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: Linked national database study. *BMJ* 2012, 344, d8059. [CrossRef] [PubMed]

149. Tonelli, M.; Muntner, P.; Lloyd, A.; Manns, B.J.; Klarenbach, S.; Pannu, N.; James, M.T.; Hemmelgarn, B.R. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: A population-level cohort study. *Lancet* 2012, 380, 807–814. [CrossRef]

150. Szummer, K.; Lundman, P.; Jacobson, S.H.; Schön, S.; Lindbäck, J.; Stenestrand, U.; Wallentin, L.; Jernberg, T. Influence of renal function on the effects of early revascularization in non-st-elevation myocardial infarction: Data from the Swedish web-system for enhancement and development of evidence-based care in heart disease evaluated according to recommended th. *Circulation* 2009, 120, 851–858. [CrossRef] [PubMed]

151. Burridge, P.W.; Matsa, E.; Shukla, P.; Lin, Z.C.; Churko, J.M.; Ebert, A.D.; Lan, F.; Diecke, S.; Huber, B.; Mordwinkin, N.M.; et al. Chemically defined generation of human cardiomyocytes. *Nat. Methods* 2014, 11, 855–860. [CrossRef] [PubMed]

152. Rodriguez, B.; Carusi, A.; Abi-Gerges, N.; Ariga, R.; Britton, O.; Bub, G.; Bueno-Orovio, A.; Burton, R.A.B.; Carapella, V.; Cardone-Noott, L.; et al. Human-based approaches to pharmacology and cardiology: An interdisciplinary and intersectorial workshop. *Europace* 2016, 18, 1287–1298. [CrossRef] [PubMed]

© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).