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Cardiac macrotissues-on-a-plate models for phenotypic drug screens

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ABSTRACT

Facilitated by the introduction of human induced pluripotent stem cells and protocols for their efficient directed differentiation at high quantity and quality, innovative human heart muscle models are being developed for applications in drug screens. Employed models range from the microscopic cardiomyocytes-on-a-chip scale to the cardiac macrotissues-on-a-plate scale. Whilst cardiomyocyte-on-a-chip models can be readily adapted to high-throughput primary screening, they are limited as to the deep phenotyping of contractility, and here in particular contractile force development. In lower throughput cardiac macrotissue-on-a-plate platforms, organotypic function, including anisotropic electrical spread of excitation and contractility, can be recapitulated at the macroscopic scale. This review serves as an overview of cardiac macrotissue-on-a-plate technologies with a focus on their application in the investigation of drug effects on heart muscle contractility and disease modeling.

Contents

1. Introduction

Monolayer cultures have been exploited for decades in studies of fundamental biology and early stage drug development. Primary cultures of neonatal rat and mouse cardiomyocytes as well as cell lines with muscle properties such as HL-1 [1] derived from a mouse model with transgenic overexpression of the simian virus 40 (SV40) large T antigen in atrial cardiomyocytes [2], C2C12 myoblasts derived from skeletal muscle of dystrophic mice [3], H9c2 myoblasts derived from embryonic rat heart ventricular myocardium [4] or genetically selectable cardiomyocytes derived from transgenic mouse embryonic stem cells [5] are until today widely used. With the introduction of human embryonic [6] and subsequently induced pluripotent [7] stem cells it is now possible to study patient-specific cardiomyocytes in surrogate human heart muscle platforms.

So called on-a-chip technologies, also sometimes referred to as micro-physiological systems (MPS; [8]), encompass standard culture formats such as slides or plates with confined compartments often connected to microfluidic devices for controlled perfusion with culture medium and defined soluble stimuli. Classical cardiomyocyte cultures on stiff substrates may be considered the simplest on-a-chip format and are readily compatible with high-throughput imaging applications for investigations of cardiomyocyte morphology as well as motion, voltage,

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and calcium signals [9–11]. High-throughput requires miniaturization to a level that is rarely compatible with organotypic physiology at the tissue scale. As more advanced tools, layered cardiomyocyte cultures on pliable substrates [12–18], cell aggregate cultures [19–21] or self-organizing multi-cellular tissue formats [22–33] are being developed to recapitulate human heart muscle function, i.e., anisotropic electromechanical coupling resulting in anisotropic force-generation.

The different surrogate human heart muscle formats may be categorized as mono-to-multilayer cardiomyocyte cultures on engineered substrates (referred to in the following as cardiomyocytes-on-a-chip) [12–18], scaffold-free aggregate cultures (referred to in the following as cardiac microtissues-on-a-chip) [19–21], hydrogel-assisted self-organization models (referred to in the following as cardiac macrotissues-on-a-plate) [22–33], or cultures of bona fide human heart slices [34] (Fig. 1). We suggest these distinctions to clarify the scale of the individual models from the single cell, to microscale multicellular aggregates, and finally to macroscale heart muscle. Complexity and phenotypic resemblance to the *bone fide* heart increase with scale. This comes are the expense of throughput and costs which scale directly with the number of cardiomyocytes (1 to 10 million) and culture medium (μl to ml) required for model maintenance.

All models allow for the assessment of contractile performance either by multi-electrode arrays (e.g., in [14]), video-optic recordings (e.g., in [23]), or by direct isometric force measurements (e.g., in [35]). Whilst video-optic measurements are employed increasingly in cardiomyocytes-on-a-chip and cardiac macrotissues-on-a-plate models [36], it is important to realize that video-optic motion does not readily equate to force development as classically evaluated in bona fide heart muscle preparations, unless cardiomyocytes are well connected to their cellular neighbors as functional syncytium within an extracellular matrix environment, and investigations are performed at defined load and beating frequency to compensate for potentially confounding effects related to the Frank-Starling mechanism (force-length relationship) and Bowditch phenomenon (force-frequency relationship). Until these challenges are overcome, measurements of contractile force under isometric conditions remain the gold standard for deep phenotyping of heart muscle contractility.

Available heart muscle models differ substantially in dimensions (micro- to macro-tissue; Fig. 1), thickness (20 to 500 μm [22,37]), cellularity (500 to 10×10^6 cells [26,32]), and contractile function (0.6 to 6000 μN force of contraction [14,38]). Many of the developed models are presently under scrutiny as to their application in drug screening at the academic and industry level. In the following chapters, we will introduce applications of heart-on-a-chip models in drug discovery and development with a focus on cardiac macrotissue-on-a-plate models. This is followed by an overview of existing models and a more detailed discussion of the in our view most important phenotypic parameters for advanced phenotyping, i.e., force of contraction, stiffness, rate and rhythm, as well as biomarkers.

2. Drug development with human heart muscle models

Despite a clear unmet need for novel therapeutics to address two of the most common causes of death throughout the world, namely heart failure and arrhythmia, the pharmaceutical industry has been shying away from costly campaigns for the development of novel cardiovascular drugs. This is certainly not because of a limited market potential, but rather the consequence of difficulties to translate promising preclinical candidates into clinical applications. Some of this is blamed on the lack of reproducibility of laboratory data. Another important issue is limited transferability of data obtained in animal models to humans; this is particularly the case for data obtained in rodent models. Rodents, mainly mouse and rat, in contrast to the human have a higher sympathetic tone resulting in dramatically accelerated heart rate and profound differences in contraction kinetics [39]. Accordingly, a number of molecular and metabolic adaptations to these different energetic states have been identified, e.g., high expression of fast twitch α-myosin heavy chain isoforms [39], profound differences in calcium handling [40], and differences in metabolic fluxes [41]. These differences have to be considered carefully when using rodent models in preclinical drug development. In this context, it is important to note that clinical failure is not only related to safety concerns, but mainly because of a lack of clinical efficacy [42], which is most likely the result of an incomplete understanding of specific drug activities in human subjects and as such may at least in part be interpreted as a direct result of the until today primarily animal-centric preclinical data collection.

With the more recent shift from chemistry dominated drug development to human-centric biological pharmacology and its focus on therapeutics targeting (1) human-specific protein motifs, (2) coding or non-coding RNA, or even directly (3) the human genome it can be anticipated that classical preclinical animal models will be inappropriate to predict clinical outcome. Instead, it appears foreseeable that human heart muscle models will be highly sought after. It is similarly predictable that induced pluripotent stem cell-derivatives and genome editing technologies (e.g., CRISPR/Cas) will be exploited for target validation, using for example knock-in strategies to insert disease causing mutations or knock-out strategies of a predicted drug target and tested as therapeutics in patient-specific induced pluripotent stem cell models [43–45]. Moreover, target-free approaches for drug discovery, based on the detection of phenotypic alterations upon compound exposure, are gaining momentum. For these fully unbiased approaches human models of health and disease with clearly distinguishable phenotypes must be provided to judge drug effects according to their ability to attenuate a defined disease phenotype or to prevent disease phenotypes from occurring or progressing.

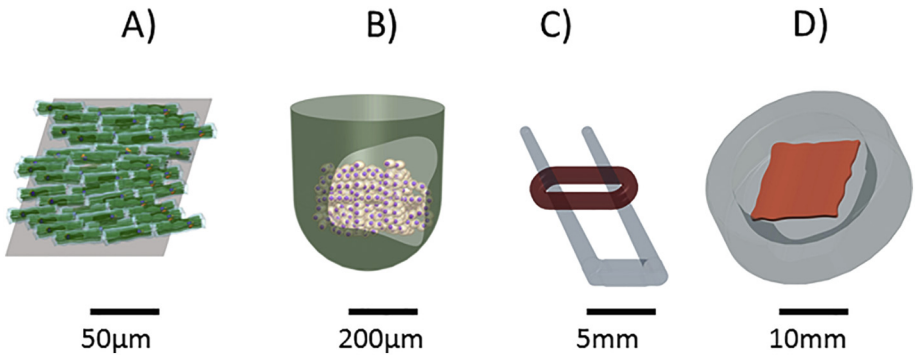

Fig. 1. Schematic overview of human heart muscle formats: (A) Mono- or multilayer-based cardiomyocytes-on-a-chip format, (B) scaffold-free cardiac microtissues-on-a-chip format, (C) hydrogel-assisted, self-organized cardiac macrotissues-on-a-plate format, and (D) bona fide heart slice models. Models differ in dimensions, cardiomyocyte content, and modes to assess contractile function, i.e., video-optic recordings in A and B versus force of contraction measurements under isometric conditions in C and D. Note that C and D may also be subjected to video-optic recordings.

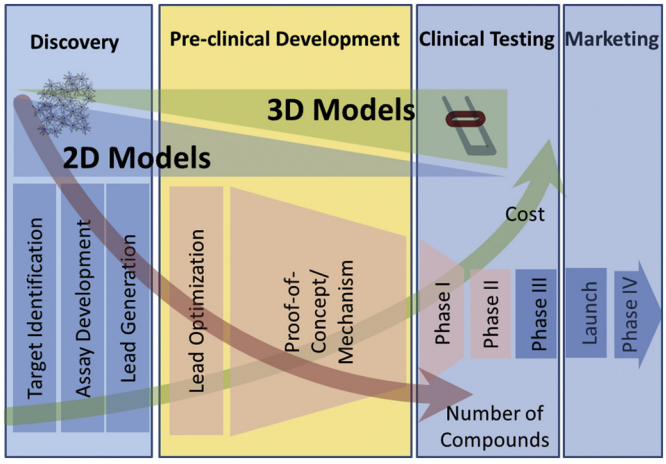

Fig. 2. Drug development pipeline with a positioning of 2D and 3D human heart muscle platforms.

There are many specific applications of human heart muscle models in drug discovery and development (Fig. 2) associated with the hope to reduce the number of false positive/negative hits and increase the number of promising therapeutic candidates brought into clinical use. Moreover, lead optimization will benefit from fast feedback from advanced human heart muscle models and even at stages of clinical development studies to further refine knowledge as to a therapeutic candidate's mode of action will be helpful to better predict outcome in late clinical trials (Phase III). It has to be emphasized, however, that despite a realistic hope for better therapies by making use of human heart muscle platforms and the anticipated opportunity for a notable reduction in animal models, it is unlikely that human models will completely replace animal experiments. ADME (absorption, distribution, metabolism, and excretion)/pharmacokinetic and toxicology studies will continue to rely on classical animal models until better models, including human liver and interconnected multi-organ-models, will become applicable.

3. Overview of human heart muscle models for drug screening applications

A number of interesting human heart muscle models have been developed with variable levels of complexity and applicability in high-throughput and high-content screening (Table 1). Which assay at what time will be advantageous depends on the stage of the drug development campaign. Hit discovery stage high-throughput screens will require simplistic and ideally automated, high-throughput cell culture models, such as 384-well 2D cultures, to narrow down the number of candidates for subsequent secondary screens in more complex cardiac macrotissue-on-plate models. Primary high-throughput screens will focus on cardiomyocyte morphology, motion, calcium or voltage alterations and will make use of genetically encoded sensors or similarly sensitive dyes [46]. These parameters considered in isolation may, however, be misleading as to their role in the regulation of contractility, i.e., in particular force development as well as contraction rate and rhythm, because activity of functionally integrated three-dimensional networks of cardiomyocytes may differ fundamentally from network activity in cardiomyocyte monolayers.

The availability of human pluripotent stem cells together with tissue engineering technologies has opened the door for the allocation of largely unlimited quantities of cardiomyocytes [47] and tools to engineer tissue dimensionality and functionality [22]. With this it is even possible to use the same cell type in monolayer screening formats in primary hit discovery and then in an engineered heart muscle macrotissue format for secondary screens. In particular in rare diseases and orphan indication, it may even become conceivable to fully personalize drug development from the patient-specific induced pluripotent stem cell-derived cardiomyocyte to engineered heart muscle and back to the patient level (graphical abstract). Limitations include immaturity of stem cell derived versus bona fide heart muscle, in case of induced pluripotent stem cells uncertainties related to epigenetic memory, and high costs compared to more classical cell models (HL-1, C2C12, H9c2).

4. Phenotypic assessments in cardiac macrotissue-on-a-plate models

A number of studies have already made use of engineered, induced pluripotent stem cell-based heart muscle models to recapitulate clinically relevant contractile phenotypes in-a-dish, this includes the use of induced pluripotent stem cells from patients with known disease-associated mutations [37,43,48–51] or from phenotypically suspicious patients without prior knowledge as to the underlying mechanisms [52]. Non-genetic disease models involve the chronic exposure to disease associated stimuli such as catecholamines, which induce a phenotype reminiscent to heart failure with reduced ejection fraction (HFrEF; [22]). Available human cardiac tissue disease models with a contractility read-out are summarized in Table 2.

The key parameter for cardiac phenotyping in all above mentioned models was force of contraction at the multicellular "tissue" scale. In addition, there is an increasing awareness for the role of myocardial stiffening and associated diastolic dysfunction in heart failure with preserved ejection fraction (HFpEF; [53]), which may also be simulated in engineered heart muscle formats. Finally, sudden cardiac death due to arrhythmia is a key concern in heart failure, but may also be drug-induced. Long-term recordings of contractile force traces allow for a straight forward identification of changes in beating rate and rhythm. Associating functional disturbances with clinically established biomarkers will be helpful to bridge in vitro findings to in-patient phenotypes. Other phenotypic analyzes, including detailed transcriptome and proteome investigations as well as studies of cell survival and metabolism, can be readily obtained using standard technologies for

Table 1
Overview of human heart muscle models for contractile phenotyping in drug screening applications. AFM: atomic force microscopy, FT: force transducer; PB: pole bending; SGW: strain gauge wires; hv: human ventricular-like; n.a.: not applicable.

Model	Format	Scaffold material	Cardiomyocytes	Max FOC	Reference
Muscular thin film (MTF)	2D	n.a.	220,000/cm^2	14 kPa (SGW)	Lind et al. [13]
BioMicroelectromechanical Systems (BioMEMS)	2D	n.a.	2500/mm^2	0.6 μN (AFM)	Stancescu et al. [14]
Micro-Heart Muscle (μHM)	3D	No matrix	2000–5000/μHM	0.3 mN (FT)	Huebsch et al. [21]
Cardiac Micro Tissue (CMT)	3D	Collagen+Fibrin	500/CMT	10 μN (PB)	Hinson et al. [37]
Biowire	3D	Collage+Fibrin+Matrigel	150.000/Biowire	33 μN (PB)	Conant et al. [28]
hvCMT	3D	Collagen+Fibrin	1000/hvCMT	10 μN (PB)	Chen et al. 2015 [31]
hvCTS (cardiac tissue strips)	3D	Collagen+Matrigel	1×10^6/hvCTS	0.7 mN (FT)`	Turnbull et al. 2013 [30]
hvCOC (cardiac organoid chambers)	3D	Collagen+Matrigel	1×10^7/hvCOC	1.26 mm H_2O	Li et al. 2018 [32]
Cardiac tissue	3D	Fibrin	2×10^6/EHT	0.9 mN (FT)	Ronaldson-Bouchard et al. 2018 [24]
Engineered Heart Tissue (EHT)	3D	Fibrin+Matrigel	1×10^6/EHT	0.15 mN (PB)	Breckwoldt et al. [29]
Engineered Heart Muscle (EHM)	3D	Collagen	1×10^6/EHM	2.5 mN (FT)	Tiburcy et al. [22]

Table 2
Overview of human heart muscle disease models with contractility readouts.

	Key phenotype	Genetric cause	Heart Muscle Model	References
Barth Syndrome	1) Reduced force of contraction	1 c.517delG (TAZ) 2 c.328T>C (TAZ)	MTF	Wang et al. 2014 [51]
Dilated Cardiomyopathy	1) Reduced force of contraction 2) Decreased muscle stiffness	1 het S635A (RBM20)	EHM	Streckfuss-Bömeke et al. 2017 [48]
Dilated Cardiomyopathy	1) Reduced force of contraction	1 pW976R+/- (TTN) 2 pA22352fs+/- (TTN) 3 pP22582fs+/- (TTN) 4 cV6382fs+/- (iTTN) 5 cN22577fs+/- (iTTN) 6 V6382fs-/- (iTTN) 7 cN22577fs-/- (iTTN) 8 cT33520fs-/- (iTTN)	CMT	Hinson et al. 2015 [37]
Duchenne Muscular Dystrophy	1) Reduced force of contraction 2) Arrhythmia	1 ΔEx8-9 (DMD) 2 ΔEx48-50 (DMD; RIKEN 51)	EHM	Kyrychenko et al. 2017 [49] Long et al. 2018 [43]
Pompe Disease	1) No contractile phenotype 2) Glycogen filled lysosomes	1 ΔEx18 (GAA) 2 1441delT/2237G>A (GAA)	EHT	Raval et al. 2015 [73]
Hypertrophic Cardiomyopathy (Cardio-facio-cutaneous syndrome)	1) Enhanced contractility 2) Shortened contraction time 3) Shortened relaxation time	1 T599R (BRAF)	CTS (ECT)	Cashman et al. 2016 [74]
Hypertrophic Cardiomyopathy	1) Reduced force of contraction 2) Reduced clinotropy	1 c.C9123T (MYH7)	EHT	Mosqueira et al. 2018 [50]
Takotsubo Syndrome	1) Reduced force of contraction 2) Higher sensitivity to catecholamines	1 no mutation identified 2 het c.1040A>G (RMB20) 5 het c.567C>G (CASQ2) 8 no mutation identified	EHM	Borchert et al. 2017 [52]
HFrEF	1) Loss of positive FFR 2) Reduced force of contraction 3) Apoptosis 4) β-adrenergic desensitization 5) Elevated NT-proBNP	None	EHM	Tiburcy et al. 2017 [22]

MTF: muscular thin film; EHM: engineered heart muscle (collagen-based); CMT: cardiac micro tissue; CTS: cardiac tissue strips (also referred to as Engineered Cardiac Tissue [ECT]); EHT: engineered heart tissue (fibrin-based); HFrEF: heart failure with reduced ejection fraction; FFR: force frequency relationship; TAZ: tafazzin; RBM20: RNA-binding motif protein 20; TTN: titin; DMD: dystrophin; GAA: acid α-glucosidase; MYH7: myosin heavy chain 7; CASQ2: calsequestrin 2; NT-proBNP: N-terminal pro b-type natriuretic peptide.

mechanistic studies. Finally, it will be highly attractive to integrate functional, structural, and molecular data obtained from individual engineered heart muscle samples for deep phenotyping and further refinement of therapeutic approaches including the identification of novel druggable targets.

In the following paragraphs we will focus on the phenotyping of contractile function in macrotissues-on-a-plate models as well as its association with clinical biomarkers:

4.1. Force of contraction

The ability to propel blood into the circulation by a squeezing action of the ventricle depends critically on the oblique organization and functional synchronization of distinct heart muscle layers [54]. The complex multilayered structure of the bona fide heart has not been recapitulated faithfully in heart-on-a-chip models and the related tissue torsion/strain is even by most advanced imaging technologies, such as echocardiography, optical coherence tomography, and magnetic resonance imaging, difficult to analyze in situ. The best accepted and already simplified surrogate for full organ scale heart muscle is the under isometric and electrically controlled conditions contracting papillary muscle [55]. Papillary muscle comprise of parallel assemblies of serial sarcomeres. This "simple" anatomy allows for precise investigations of how sarcomere impairments would affect ensemble contractility at the tissue scale under biophysically defined conditions (i.e., defined load and contraction frequency). Ventricular and atrial papillary muscle can be obtained from explanted hearts or from dissected atrial appendages. To recapitulate the highly organized papillary muscle structure, strip [23,24,37,56] or loop formats have been engineered [22], the latter recently with chamber specific functions [57] to facilitate the development of drugs targeting atrial and ventricular mechanisms.

Most iPSC-based cardiac macrotissues-on-a-plate models resemble ventricular myocardium, which should display distinct physiological functional parameters, such as an augmentation of contractile force upon increased preload (Frank-Starling mechanism), an increase in contractile force upon increasing stimulation frequency (Bowditch phenomenon), maximal inotropic responses to calcium, as well as isotropic and lusitropic responses to β-adrenoceptor stimulation with for example isoprenaline. Chronic exposure to catecholamines (e.g., norepinephrine) at levels found in patients with endstage heart failure [58], should lead to a blunting of the positive force-frequency-response, hypertrophy, and beta-adrenergic desensitization similar to what is observed in patients with HFrEF [22]. High standardization of cardiac macrotissues-on-a-plate technologies, including the use of fully defined cell compositions, matrix, and serum-free culture medium, is important for robust and highly reproducible assays, including the recently established engineered heart muscle (EHM)-HFrEF assay [22].

The analysis of force of contraction is done best under isometric conditions using force transducers. In this way, heart muscle properties and in particular muscle length can be optimally controlled and longitudinal pulling force properly measured as a function of preload (Fig. 3). Alternative modes of analysis include the deflection of flexible poles [23,37] or foils [13,14] for the assessment of auxotonic contractions [59]. Motion analysis of plated cells [17,18] or cardiomyocytes aggregates [20,21] on stiff or flexible substrates can be used to detect beating rate and rhythm as well as shear force. Software tools may help to standardize motion analysis [36]. Alternative models include engineered tissue pouches/chambers [32,60], which can be used to measure for example a surrogate for ejection fraction.

Regardless of the mode of systolic force documentation it is essential to report uncorrected force of contraction (FOC *aka* twitch tension; typically in milli-Newton [mN]). Corrections to baseline (% of baseline) or cross-sectional tissue or muscle area (mN/mm^2) are acceptable, but require a clear indication of absolute baseline FOC and tissue/muscle cross sectional area. In addition, recently introduced measures of force per input [38] or output [22] cardiomyocytes may be useful to phenotype

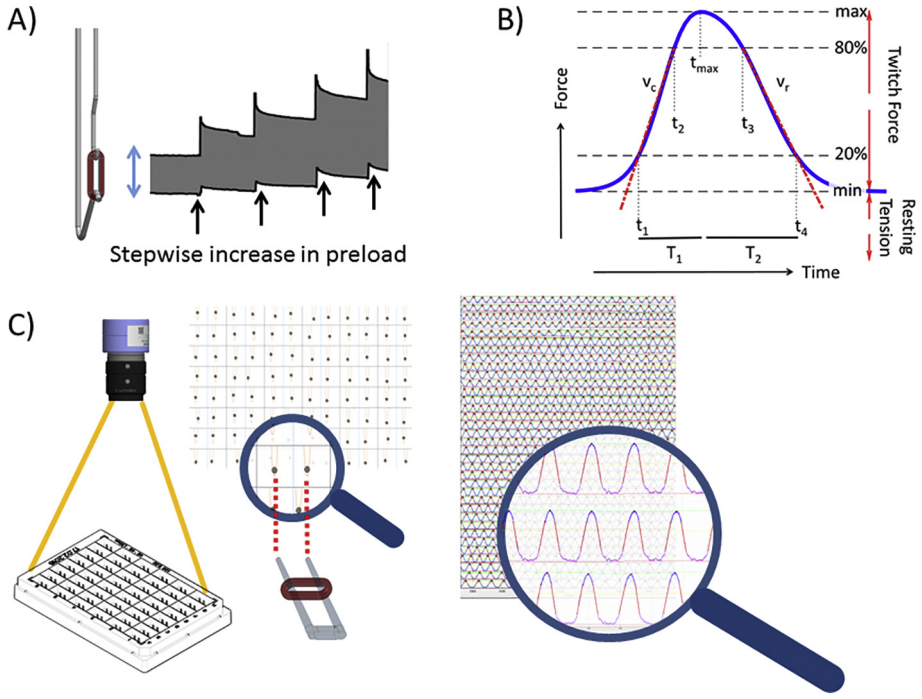

Fig. 3. Schematic overview of techniques to perform contractile force measurements in cardiac macrotissues-on-a-plate models. (A) Force transducer measurements under isometric conditions with an original data recording showing an increase in force of contraction (twitch tension – blue arrow) upon stepwise increases in preload (black arrows), in line with the Frank-Starling mechanism; schematic on the left demonstrates an isometrically suspended engineered heart muscle [EHM] on a force transducer set-up. (B) Force amplitude with classical parameters used to describe systolic force (twitch tension [TT] also referred to as force of contraction [FOC]) and diastolic tension (resting tension [RT]) as well as contraction kinetics, i.e., contraction time (T1 – time from 20% to peak contraction), relaxation time (T2 – time from peak contraction to 80% relaxation), total twitch duration (T1 + T2), contraction velocity (V_c: +dF/dt) and relaxation velocity (V_r: -dF/dt). (C) Schematic overview of a highly parallelized assessment of contractility according to pole bending theory: (left) set up for high resolution image recordings from a custom-made 48-well plate; (middle) center of gravity tracking (grey lines indicate a histogram of pole tip pixel count) for simultaneous detection of engineered heart muscle (EHM) contractility; (right) example of a parallel recording of 48 EHM-generated force traces with a magnification of an individual force traces for the determination of the contraction parameters depicted in (B).

tissue-on-plate models. We recommend contractile force per output cardiomyocytes, i.e., the cardiomyocyte count at the time of functional phenotyping, to take into consideration potential changes in cardiomyocyte content after chronic drug exposure or in case of the use of disease models with impairments that may influence cell survival and skew cell composition in favor of cardiomyocyte or non-myocyte populations. A potential caveat of this approach is that cell isolation, especially from cardiac macrotissue-on-a-plate models, is not trivial and requires highly standardized protocols to minimize bias by cell loss during the typically enzymatic isolation process.

4.2. Myocardial stiffness

Contractile performance of the heart may not only be compromised in systole, but also in diastole with impaired relaxation typically caused by a stiffening of the ventricle. Key regulators of myocardial stiffness comprise heart fibrosis and molecular alterations of the giant sarcomeric protein titin [61,62]. Increased myocardial stiffness with unaltered systolic, but impaired diastolic heart function is a characteristic clinical feature in patients with HFpEF. To date, no pharmacological therapies with a documented impact on outcome exist for affected patients. Human heart muscle models recapitulating this phenotype would be highly advantageous. Tissue stiffness is typically analyzed using dynamic stress-strain tests. The diastolic tension of heart muscle and its changes upon increasing preload, such as systematically performed during stepwise preloading to achieve a tissue length with maximal contractile performance according to the Frank-Starling mechanism (Fig. 3A), are also good indicators of myocardial stiffness. As a rule of thumb, the ratio between isolated systolic and diastolic tension, also referred to as force of contraction (FOC or and twitch tension [TT]) or resting tension (RT), respectively, is >1 (TT/RT) for healthy myocardium; disease associated stiffening results in TT/RT ratio of <1 [63]. In cardiac macrotissue-on-a-plate models FOC or TT is typically reported. Less attention is given to the quantification of RT; in EHM average RT is 0.3–0.5 mN with maximal FOC of 0.5–2.5 mN [22]. According to data obtained in human heart samples [63], TT/RT ratio <1 in cardiac marcotissue-on-a-plate models may be considered as an indication for diastolic dysfunction [26,64]. In models of pharmacologically or genetically induced HFpEF vs. HFrEF, diastolic tension (RT) should be elevated without a compromise in systolic contractile function (FOC or TT).

4.3. Heart rate and rhythm

Investigations of beating rate and rhythm can be readily performed in all spontaneously beating cardiomyocyte preparations using visual inspection and quantitative image analyses. Spontaneous beating is a feature of immature and isolated cardiomyocytes in culture. It is determined by (1) the presence of dedicated pacemaker cells, which are characterized on the molecular level by the expression of HCN4 channels, (2) cardiomyocytes with spontaneous depolarization in phase 4 of the action potential due to a lack of inward rectifying potassium channels (Ik1), or (3) spontaneous calcium release from the sarcoplasmic reticulum. In three-dimensional engineered heart muscle formulations, cardiomyocytes form a functional syncytium with spontaneous beating controlled by defined regions with pacemaker activity [22]. The spontaneous beating rate observed in cardiac macrotissue-on-a-plate platforms typically decreases with time in culture [65], whether this reflects a depressed spontaneous activity of immature cardiomyocytes or a lower spontaneous beating rate in dedicated pacemaker cells remains to be investigated. The mechanisms underlying the self-organization into pacing (nodal-like) and paced (working myocardium-like) cardiomyocytes are not fully understood, but may involve processes (i.e., FGF-, TGFβ- and Wnt-inhibition with parallel BMP and retinoic acid stimulation) recently identified to support directed

differentiation of nodal cells in pluripotent stem cell cultures [66] and induced maturation by (electro)mechanical stimulation [22,24]. In addition, there is clear evidence for the support of cardiomyocyte assembly into a functional syncytium by non-myocytes, and here in particular fibroblasts [22,27,67], which may also have a specific impact on beating rate and rhythm in heart tissue-on-plate models.

Changes in beating rate upon drug administration suggest that cells with pacemaker activity are directly affected. For the investigation of rhythm disturbances, such as atrial or ventricular fibrillation as well as ectopic activity, different mechanisms, such as QT-interval prolongation caused by for example hERG blockade or early as well as delayed after depolarization, may have to be induced in a chamber specific human heart muscle model context [57] for translational relevance. Whether cardiac macrotissue-on-a-plate models are advantageous over classical and novel cardiomyocytes-on-a-chip models is presently investigated by the CIPA consortium, with the aim to revise ICH guidelines for thorough QT assays in preclinical drug development [68]. For simple high-throughput analyses of QT-prolongation cardiomyocytes-on-a-chip multi-electrode array (MEA) platforms will likely be sufficient. For studies of more complex arrhythmia, such as reentry, larger circuit sizes are required, which according to an effective refractory period (ERP) of ~250 ms in ventricular myocardium and conduction velocities of 0.1 or 1 m/s should encompass a circuit length of >2.5 or >25 cm (effective refractory period * conduction velocity), respectively. Accordingly, upscaling to cardiac macrotissue-on-a-plate formats rather than miniaturization appears to be important for a comprehensive assessment of the arrhythmogenic potential of drugs. Conduction velocities of >0.1 m/s are typically observed in cardiac macrotissue-on-a-plate models (e.g., [25,65]).

4.4. Biomarkers

An association of functional phenotypes with clinically validated biomarkers would help greatly to classify preclinical data according to clinical observations. Commonly used markers include troponins T or I, the MB isoform of creatine kinase (CK-MB), and lactate dehydrogenase (LDH) for myocardial damage by myocardial infarction or brain natriuretic peptide (BNP) to discriminate heart and pulmonary failure as well as for the management of heart failure [69]. In a recent study, the severity of norepinephrine-induced contractile failure in engineered heart muscle (EHM) could be directly associated with NTproBNP [22]. Similarly, muscle damage in cardiac macrotissue-on-a-plate models is associated with CK-MB release into the culture medium (own unpublished observations). Further studies will be needed to establish associations between human heart muscle model phenotypes and clinically used biomarkers. This includes the validation of a pathophysiologically relevant concentration range in vitro, the determination of optimal sampling windows, and signal loss due to absorption of released factors by for example plastic culture dish materials. With a better understanding of their in vitro biomarker release properties it may be interesting to explore the use of human heart muscle platforms for the identification of disease phenotype-specific biomarkers.

5. Conclusions

Human heart muscle models have without doubt the potential to fundamentally change drug development. With the application of cardiomyocytes from induced pluripotent stem cells, the whole pipeline from discovery stage to therapeutic candidate development and optimization can be patient cohort specific or even fully personalized (graphical abstract): examples include (1) induced pluripotent stem cells from patient cohorts with cardiomyopathies caused by similar mutations; (2) induced pluripotent stem cells from patient cohorts with similar disease phenotypes or immutable characteristics such as gender, ethnicity, or age; (3) patient-specific induced pluripotent stem cells for the identification of anti-cancer drugs with minimal cardiotoxicity; (4) patient-specific induced pluripotent stem cells for screening and "dose" finding in the preclinical development of therapeutic genome editing. One challenge is to find a useful balance between high-throughput and phenotypic relevance and more pragmatically to design step-wise approaches to address the specific needs at the different stages of the drug development pipeline. Automation and parallelization by advanced image analysis will be key for higher throughput in the early stages of drug development. More advanced human heart muscle models, as to their phenotypic properties, and their deep phenotyping appear advantageous at later stages. Definitive proof for the claimed benefit of human heart muscle platforms is still outstanding and questions related to the limited maturity of stem cell based models need further attention [70]. Along these lines it is important to emphasize that (electro)mechanical stimulation at physiological rate and load in 3D culture models results in most advanced, yet still not adult heart like maturation [22,24]. Despite these caveats, the growing evidence for successful applications in phenocopying the healthy and diseased human heart provides not only support for the exploitation of human heart muscle models in drug development, but also to their exploitation in fundamental studies of disease mechanisms, which may ultimately inform future drug development. This is exemplified by the growing body of evidence as to the recapitulation of anticipated drug responses induced at clinically relevant concentrations in human heart muscle models [20,71,72]. Thus amending existing drug development pipelines to scrutinize whether human heart muscle models can indeed keep up to their promise appears to be timely. Promising human heart muscle models are developed at many scales: (1) Miniaturized cardiomyocytes-on-a-chip for high-throughput applications in primary assessments of cardiomyocyte morphology and function at the cellular level; (2) cardiac microtissues-on-a-chip with more complex multicellular assemblies lower throughput, but more pathophysiological relevance; and (3) cardiac macrotissues-on-a-plate for deep phenotyping of contractility in the most direct way, i.e., by measurements of force, frequency, and rhythm, under load and frequency controlled conditions in line with the gold standard in cardiac contractility phenotyping, i.e., force measurements of bona fide heart muscle under isometric conditions.

Acknowledgement

We have tried to present a balanced overview of human heart muscle models, but have most certainly missed excellent contributions to the field. We apologize to the investigators whose work is not considered.

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Conflict of interest

W.H.Z. is founder and together with T.M. and M.T consultant of myriamed GmbH, which is offering tissue engineered-based drug screening services. T.M., M.T., and W.H.Z. are listed as inventors on a number of assigned and filed patents with relevance to the topic of the review.

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