ESC HEART FAILURE
ESC Heart Failure 2021; **8**: 3643–3655
Published online 2 August 2021 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13504

REVIEW

Optimizing the discovery and assessment of therapeutic targets in heart failure with preserved ejection fraction

Gabrielle Fusco-Allison[1,2,3,5], Desmond K. Li[1,2,3,5], Benjamin Hunter[1,2,4,5], Dan Jackson[1,2,5,6], Paul G. Bannon[2,6], Sean Lal[1,2,4,5,7] and John F. O'Sullivan[1,2,3,5,7,8]*

[1]*Precision Cardiovascular Laboratory, The University of Sydney, Sydney, New South Wales, Australia;* [2]*Charles Perkins Centre, The University of Sydney, Sydney, New South Wales, Australia;* [3]*Heart Research Institute, Newtown, Sydney, New South Wales, Australia;* [4]*School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia;* [5]*Central Clinical School, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia;* [6]*Discipline of Surgery, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia;* [7]*Department of Cardiology, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia; and* [8]*Faculty of Medicine, TU Dresden, Dresden, Germany*

Abstract

There is an urgent need for models that faithfully replicate heart failure with preserved ejection fraction (HFpEF), now recognized as the most common form of heart failure in the world. *In vitro* approaches have several shortcomings, most notably the immature nature of stem cell-derived human cardiomyocytes [induced pluripotent stem cells (iPSC)] and the relatively short lifespan of primary cardiomyocytes. Three-dimensional 'organoids' incorporating mature iPSCs with other cell types such as endothelial cells and fibroblasts are a significant advance, but lack the complexity of true myocardium. Animal models can replicate many features of human HFpEF, and rodent models are the most common, and recent attempts to incorporate haemodynamic, metabolic, and ageing contributions are encouraging. Differences relating to species, physiology, heart rate, and heart size are major limitations for rodent models. Porcine models mitigate many of these shortcomings and approximate human physiology more closely, but cost and time considerations limit their potential for widespread use. *Ex vivo* analysis of failing hearts from animal models offer intriguing possibilities regarding cardiac substrate utilisation, but are ultimately subject to the same constrains as the animal models from which the hearts are obtained. *Ex vivo* approaches using *human myocardial biopsies* can uncover new insights into pathobiology leveraging myocardial energetics, substrate turnover, molecular changes, and systolic/diastolic function. In collaboration with a skilled cardiothoracic surgeon, left ventricular endomyocardial biopsies can be obtained at the time of valvular surgery in HFpEF patients. Critically, these tissues maintain their disease phenotype, preserving inter-relationship of myocardial cells and extracellular matrix. This review highlights a novel approach, where ultra-thin myocardial tissue slices from human HFpEF hearts can be used to assess changes in myocardial structure and function. We discuss current approaches to modelling HFpEF, describe in detail the novel tissue slice model, expand on exciting opportunities this model provides, and outline ways to improve this model further.

Keywords Heart failure with preserved ejection fraction; HFpEF; Myocardial slices; Cardiac models of HFpEF

Received: 1 December 2020; Revised: 2 June 2021; Accepted: 21 June 2021
**Correspondence to: John F. O'Sullivan, Precision Cardiovascular Laboratory, The University of Sydney, Sydney, NSW, Australia. Tel: +61 2 8627 5600; Fax: +61 2 9517 1552. Email: john.o'sullivan@hri.org.au*

Introduction

Heart failure with *preserved* ejection fraction (HF*p*EF) represents the greatest unmet need in cardiovascular medicine. There are currently no effective treatments for this type of heart failure, which now represents more than half of all heart failure cases worldwide.[1,2] Similar to the readily characterized heart failure with *reduced* ejection fraction (HF*r*EF), HFpEF has a 75% 5 year mortality and a 2.1 year median survival.[3] A fundamental understanding of key mechanisms driving disease is critical, and model systems are key.[4] Traditionally, rodent models have been used in the study of heart

failure, with *in vitro* assays used to examine molecular processes within the cardiomyocyte. Each model system has its strengths and weaknesses. Key considerations are species, cell vs. whole heart models, and etiological factors underlying the development of HFpEF. Our aim in this review was to succinctly summarize historical models that could be applied to advance understanding of this disease. We focus on a novel human myocardial tissue slice model as we believe it offers significant advantages in two critical domains: (i) human origin and (ii) preservation of the intricate network of pathological changes in the myocardium. We also discuss recent technological developments that have improved this model further, and ways in which this model can accelerate novel therapeutic discovery for HFpEF, urgently needed.

In vitro cardiomyocyte models

Induced pluripotent stem cells-derived cardiomyocytes

Human-derived *in vitro* models can overcome species differences, which is important given the poor success rate of translation from murine models to human disease.[5] However, cardiomyocytes are among the most challenging cell types with which to work and present many technical challenges related to cell viability, maturity, and functionality. Furthermore, as with all cell culture models, studying cardiomyocytes in isolation results in an experimental phenotype that is poorly representative of cardiomyocytes in the intact heart (myocardium) where the extracellular matrix plays an important role.[6]

Traditional two-dimensional (2D) 'plate' cardiomyocyte culture models generally use two approaches: differentiation from embryonic or induced pluripotent stem cells (iPSCs); and primary cardiomyocyte cell culture after dissociation from cardiac tissue. iPSC-derived cardiomyocytes (iPSC-CMs) are powerful tools for assessing patient-specific cardiomyocytes and have been used to study effects of genetic variants on morphology.[7] iPSC-CMs do not *ipso facto* represent the cardiac phenotype of patients from which they were derived, as they are typically de-differentiated from fibroblasts or monocytes, and differentiated into cardiomyocytes under different *ex vivo* epigenetic and environmental regulation. On the other hand, primary human cardiomyocytes retain certain disease characteristics but have limited longevity and functional capacity.

Additionally, a major issue with embryonic/iPSC-derived cardiomyocytes is the inability to engender a mature phenotype.[8] Various approaches can enhance maturity (e.g. metabolic substrate selection and electro-mechanical stimulation), but achieving *bone fide* cardiomyocyte maturity is extremely challenging, and in this respect, iPSC-CMs fall short of representing the human adult left ventricular cardiomyocyte.[9] It follows that cardiomyocyte cultures without intercellular connectivity to other myocardial cell types and the extracellular matrix, lack of structural maturity, and without the correct three-dimensional (3D) conformation result in major divergence between model systems and human heart failure.[10]

Primary cardiomyocytes

Primary cell culture can provide insights into pathology of the hearts from which the cardiomyocytes were obtained, although the artificial nature of cell culture diminishes this signal.[11] Furthermore, adequate tissue quantities from pre-mortem left ventricular human myocardium are difficult to obtain.[12] The cardiomyocytes are terminally differentiated and have a short lifespan. Additionally, the cells rapidly lose their phenotypic structure and undergo progressive remodelling within a relatively short period of time, precluding long-term study.[13] Digestion of the tissue samples from which they are derived not only destroys intercellular connections but also discards hallmark disease signatures outside the cardiomyocyte, which is a major lost opportunity.

Cardiac organoids

An approach to overcome limitations of 2D monolayer cultures includes cardiac 'organoids'. These consist of cardiomyocytes and other cell types seeded on matrices that promote a 3D arrangement to recapitulate intact myocardium.[14] Indeed, these models have further underscored the limitations of 2D cell culture: cardiomyocytes in 3D configuration with other cell types have vastly different transcriptional and signalling profiles.[15] Organoids are exposed to an abundance of biochemical, mechanical, electrical, and other stimuli that lead to cardiomyocyte gene expression profiles more representative of that in myocardial tissue.[15] However, these 3D models are still artificial constructs, and the resulting phenotype is quite different from intact human myocardium, highlighting the complexity of the native structure–function relationships in the heart.[6] Critically, 3D cultures lack the natural complexity of an integrated vasculature and immune system, and the cardiomyocytes present in the organoids face the same maturation limitations seen in 2D culture.[15,16]

Rodent models

Studying the intact cardiovascular system in animals offers a step up in complexity, allowing an examination of the broader cardiovascular and systemic features of HFpEF. Many extant

rodent models have been used to study HFpEF, reviewed extensively by others.[17–20]

Hypertensive models

Hypertension is the most common comorbidity associated with HFpEF, with a prevalence between 60% and 89% reported in large controlled trials, epidemiological studies, and heart failure registries.[21] Hypertension leads to left ventricular hypertrophy and cardiomyocyte remodelling,[22] faithfully replicated in numerous animal hypertension models.[17]

Transverse aortic constriction (TAC) involves placing a restrictive band around the aortic arch, causing a huge increase in afterload, with compensatory left ventricular hypertrophy, and a gradual decrease in left ventricular systolic performance.[23] Some studies report that these animals have preserved left ventricular ejection fraction (LVEF) after 14 days of banding,[23,24] but this is not consistent with other studies, which report reduced LVEF in the same timeframe.[25–27] The rapid progression from HFpEF to HFrEF, and lack of comorbidities, is not an accurate reflection of human disease.[28–30]

Dahl salt-sensitive rats[31] are an inbred Sprague–Dawley rat strain that develop hypertension, left ventricular hypertrophy, diastolic dysfunction, and insulin resistance when fed on a high-salt diet from 7 weeks of age.[32–35] Reports are inconsistent on whether these animals show preserved[36,37] or reduced LVEF,[38] which is more common when the diet is started at an older age.[32,39] In the deoxycorticosterone acetate (DOCA) infused model, DOCA is administered intraperitoneally or subcutaneously to the animal, inducing hypertension.[40] Administration with a unilateral nephrectomy and high-salt diet has been shown to cause cardiac hypertrophy and perivascular fibrosis,[41] as well as increased LVEF[42]; however, blood pressure changes are inconsistent.[43,44] Administration of aldosterone with unilateral nephrectomy and high-salt diet induces hypertension, cardiac hypertrophy, diastolic dysfunction, and fibrosis[45,46,47], exercise intolerance,[48] as well as cardiomyocyte remodelling and preserved LVEF.[49,50]

Chronic angiotensin II infusion is a reliable model of cardiac hypertrophy in both the mouse[51] and rat.[52] These animals develop hypertension, concentric hypertrophy, and diastolic dysfunction.[53–55] Angiotensin II also induces insulin resistance and a metabolic switch from glycolysis to fatty acid β-oxidation, hallmarks of type 2 diabetes.[56] However, reports vary as to whether these mice show reduced[53] or preserved LVEF,[57] and moreover, these effects seem to be strain specific.[58] Additionally, this model is dependent on supraphysiological circulating angiotensin II levels, which is not reflective of the human scenario, where there is aberrant angiotensin receptor activity.[59]

The spontaneously hypertensive (SH) rat was developed by Okamoto and Aoki in 1963 by selective breeding of a male rat with spontaneously high blood pressure of 150 to 175 mmHg persisting for more than 1 month with a female rat with blood pressures slightly above the average, 130–140 mmHg.[60] These were selected from among 68 Wister strain rats and mates to obtain F1 rats, whose offspring were found to have persistently elevated blood pressure. While the genome sequence of the SH rat has been mapped, the causative expression quantitative trait loci (eQTLs) have not been clearly identified, and the relevance to human hypertension is not clear either.

The SH heart failure rat is the result of crossbreeding of the SH rat with the SH obese rat. The SH obese rat develops an obese phenotype resulting from a single recessive trait, a nonsense mutation affecting all forms of the leptin receptor, designated fa_k.[61] The absence of hypothalamic leptin receptors leads to changes in neuropeptides that favour the development of obesity, and therefore, the metabolic syndrome that develops is consequent upon lack of leptin receptors, which is not the case in clinical metabolic syndrome. The absence of leptin signalling is a major confounder to modelling human HFpEF in the context of metabolic syndrome. Furthermore, there are several characteristics of the SH heart failure rat that are fundamentally different to human HFpEF. First, they develop dilated cardiomyopathy[62] that is more typical of HFrEF than HFpEF. Second, they develop neurohormonal perturbations in the renin–angiotensin–aldosterone system, which are more typical of HFrEF than HFpEF; in fact, this is one of the ways in distinguishing a true model of HFpEF from HFrEF.[63]

In summary, while replicative of some features, these models are different from human HFpEF in several critical ways: (i) they focus on a single dominant driver, that is, hypertension, at the expense of more representative complexity; (ii) important comorbidities such as metabolic disturbance are usually not present; (iii) timeframes are usually not representative, for example, the TAC model involves *rapid* induction of LV hypertrophy due to extreme afterload; and (iv) LVEF is frequently not preserved.

Ageing models

It is important to consider the increased prevalence of comorbidities[64,65] when trying to mechanistically understand the contribution of age to HFpEF, and there are several enabling rodent models.

The Fischer 344 ageing rat develops exercise intolerance, cardiac hypertrophy, and diastolic dysfunction[18] with preserved systolic function by 30 months of age (75 years old in humans[66]) and interstitial fibrosis with significant cardiomyocyte loss by 36 months (90 years old in humans[66]).[66,67] Female rats develop more prominent left ventricular hypertrophy and diastolic dysfunction than male rats, and may

offer an opportunity to study underlying mechanisms of this sex divergence in HFpEF.[68] However, these rats also develop eccentric hypertrophy and left ventricular dilatation, not representative of human HFpEF.[69]

Senescence-accelerated prone (SAMP) mice are an inbred model of spontaneous senescence.[70] The SAMP8 strain has been used to study cardiac ageing and has been shown to develop diastolic dysfunction and myocardial fibrosis.[71] Others have shown that hypertension, using a high-fat, high-salt diet, is required to develop this phenotype in the SAMP8 mice, which had decreased exercise tolerance, left ventricular hypertrophy, diastolic dysfunction, and preserved LVEF after 26 weeks.[72] However, these mice also failed to show other haemodynamic features of HFpEF.

A major drawback with these ageing models is the long-term nature of the studies and the added expense of long-term housing. Even the relatively shorter timeframe of the SAMP8 mouse takes 26 weeks. Additionally, there are now few biological facilities that will provide ageing mice, and those that do only do so with strict criteria, such as the National Institute on Aging (https://www.nia.nih.gov/research/dab/aged-rodent-colonies-handbook/eligibility-criteria-use-nia-aged-rodent-colonies).

Metabolic perturbation models

Overweight/obesity (body mass index $\geq$ 25 kg/m^2) is highly prevalent in HFpEF (>80%) and is increasingly recognized to drive HFpEF development.[73] Obesity has been proposed as a major driver of systemic inflammation and subsequent myocardial remodelling in HFpEF specifically.[74]

Genetic knockout mouse strains Ob/Ob[75] and Db/Db[76] mice have a deletion of the leptin gene and the leptin receptor, respectively, causing them to become hyperphagic, and then obese. These mice develop diastolic dysfunction and concentric hypertrophy,[77,78] and db/db mice have been shown to exhibit signs of HFpEF, including exercise intolerance and pulmonary hypertension.[17] However, this model is dependent on leptin deficiency, and thus is not reflective of the human scenario, where obesity is characterized by leptin resistance.[79]

The type 2 diabetic phenotype can be mimicked by combining a dietary model with low-dose drug-induced ablation of pancreatic β-cells. For example, low doses of glucosamine-nitrosourea streptozotocin (STZ) leads to the total absence of insulin secretion which, when combined with a high fat diet, mimics late-stage type 2 diabetes, then eventually cardiac hypertrophy and diastolic dysfunction.[80] However, reported LVEF values are variable.[17] Moreover, the total loss of insulin secretion is critically different to the pathogenesis of type 2 diabetes in humans, which is characterized by a prolonged phase of hyperinsulinemia followed by persistent insulin resistance.[81]

Dietary models mirroring human hypercaloric ingestion with diets rich in sucrose and fat (Western diet) can lead to several features of diastolic heart failure[82–86]; however, results are inconsistent due to use of diets with different macronutrient ratios, study duration, and mouse strain.[87] More recently, a 'two-hit' mouse model of HFpEF has emerged, whereby concomitant hypertensive and metabolic stress successfully recapitulates many of the cardiovascular and systemic features of human HFpEF.[4] A high-fat diet is used to induce obesity, and constitutive inhibition of nitric oxide synthases using Nω-nitro-L-arginine methyl ester (L-NAME) causes hypertensive stress by perturbing endothelial function. This mimics the systemic inflammation in patients with HFpEF.[88] This reproducible model only takes 5 weeks and develops cardiac hypertrophy, diastolic dysfunction, pulmonary congestion, and exercise intolerance, with preservation of LVEF.

The above animal models are often not representative, as they are dependent on supplying exogenous hypertensive, nitrosative, or metabolic stressors *via* chemical or surgical intervention. Further, due to species differences and size considerations, rodent models have not been a successful platform for clinical therapeutic development, highlighted by the high failure rate of therapeutic agents that seemed promising in rodents.[89,90]

Porcine models

Porcine models of HFpEF have also been developed, which involve similar strategies to the rodent models discussed above. Aortic-banded pigs fed on a Western diet were shown to develop numerous hallmarks of HFpEF, including pulmonary congestion, metabolic syndrome, and obesity with compensated LVH, although fell short of others such as elevated LV end-diastolic pressures.[91] Another study progressively banded pigs with a titrated cuff method, allowing for a more controlled increase in pressure compared with traditional TAC.[92] This model[92] was a hypertensive model of cardiac hypertrophy due to increased afterload using aortic cuff placed around the aortic root after left thoracotomy and pericardiotomy, maintained for 42 days. Rather than a model of HFpEF, this is more akin to a model of aortic stenosis. The authors should be congratulated for extensive assessment of the cardiac substrate changes accompanying such aggressive cardiac hypertrophy; however, such changes have been well documented before. Further, LV remodelling due to pressure-load is distinct from HFpEF, and the absence of multiorgan pathogenic effects significantly limits their clinical relevance.[93]

Other approaches involve pharmacological intervention with DOCA, along with different high-fat, high-salt, and high-sugar diets.[94,95] These pigs develop multiorgan HFpEF

characteristics, such as perturbed haemodynamic features, metabolic syndrome, and vascular disease, but there are lack other feaures such as pulmonary congestion. Moreover, while pigs are an attractive model for their biological similarity to humans, they are also expensive, complex to maintain, and without substantial resources the number of studies and interventions that can be tested are limited.[93]

Ex vivo heart failure models

Ex vivo models offer significant advantages for measurement of cardiac function in a working heart compared with *in vitro* models, but many key features are still missing such as mechanical coupling with the vascular system, cross-talk with other organs, and thoracic cavity pressure interdependence.[96,97] However, these models can use HFpEF mouse hearts to study cardiac function and myocardial fuel tracing in the Langendorff or the working/ejecting heart configuration. The Langendorff technique maintains cardiac function by retrograde perfusion through the aorta, which forces the aortic leaflets closed and the perfusate to supply the coronary system with the required nutrients for myocardial work.[98] In contrast, the working heart method represents physiological flow by supplying perfusate through the left atrium that then fills the left ventricle during diastole; during systole perfusate is ejected out of the left ventricle against a pressure afterload at the aorta, which mimics the systemic circulation, allowing perfusate to then enter the coronary system.[99] A major advantage of the working heart configuration is the facility for true measurement of ventricular pressure, volume, and assessment of diastolic function.

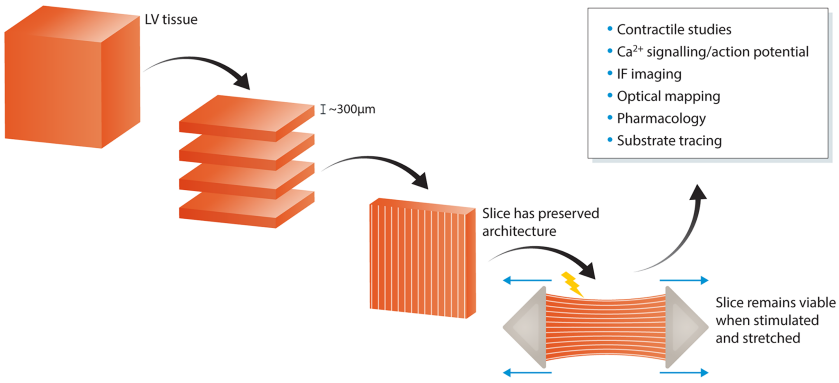
A major benefit of the *ex vivo* heart is the ability to supplement the perfusate with stable isotope substrates to allow for substrate tracing experiments. As the heart primarily utilizes fatty acids and glucose as fuel sources, supplementation of the perfusate with isotopic substrates such as [U-13C] glucose or $^{13}C_{16}$ palmitate can allow for the tracing of these substrates using targeted mass spectrometry techniques to determine the metabolic fate of these substrates.[100] Previous reports have shown a loss of metabolic flexibility of the heart in heart failure (reviewed in Karwi et al[101]), with increased glycolysis,[102,103] but there are conflicting reports on the role of β-oxidation in heart failure.[104–107] Utilization of flux experiments could help further delineate the HFpEF heart's choice of carbon source for ATP production.

Major weaknesses of *ex vivo* methods include the limited time that the heart can be maintained, lack of cross-talk with other organs,[108] and removal of ventricular-aortic coupling that is a key determinant of cardiovascular performance.[96,109] Further, perfusates used in this approach do not use fatty acids (the heart's preferred energy source) due to difficulty dissolving them in solution.

A novel approach: functional human myocardial slices

In vitro myocardial tissue slices are ultra-thin slices (150–350 μm) of ventricular myocardium, which can be prepared on a vibratome and maintained in culture under mechanical tension and electrical field stimulation (*Figure 1*). The power of this approach lies in the preservation of the 3D myocardial disease architecture, maintaining a true representation of the failing myocardium and its molecular characteristics. Keeping the slices ultra-thin allows adequate perfusion with oxygen and metabolic substrate because of reduced diffusion time. The cardiomyocytes retain their functional relationship to each other, other cell types, and the ECM in the correct proportions. Recent advances in

Figure 1 Workflow for the cardiac tissue slices, from biopsy to analysis. Cardiac slices are taken from fresh left ventricular myocardium and sliced to approximately 300 μm with a vibratome. Slices are maintained through a combination of electrical stimulation and mechanical tension, and can be used for functional, structural, and signalling assessment. Ca^{2+}, Calcium; IF, immunofluorescence.



DOI: 10.1002/ehf2.13504

technology are enabling simultaneous determination of myocardial function, energetic substrate turnover, and molecular characteristics.[110] This model could also be used to study native cardiac responses to pharmacological treatment.

Using diseased human myocardium enables unparalleled insight into structure–function relationships and substrate turnover in different cardiac pathologies, although such tissue is not easily obtained. Sufficient myocardial tissue can be excised at the time of surgery, but this requires highly skilled surgeons and a team to facilitate tissue collection and preservation. The model can equally be applied to animal myocardial tissue from model systems.

Unfortunately, there are still significant challenges when it comes to the preparation of these slices and with maintaining viability. There are conflicting reports on optimal preparatory methods in the literature, although there is consensus that appropriate fibre orientation is critical to reducing tissue damage.[111] If not maintained optimally, cardiomyocytes can undergo de-differentiation in as little as 24 h. Extending the lifespan of the tissue slices offers several advantages including the opportunity to study myocardial fuel consumption, cardiac regeneration, and drug testing.

Long-term tissue slice model

Most studies that keep the tissue for longer than a few hours place the slices into air–surface interface culture wells (*Table 1*). While this allows for adequate gas exchange, it neglects several important aspects that are required for the maintenance of cardiac tissue, namely mechanical tension and electrical stimulation.[112] There have only been a handful of studies that explore the concept of a 'biomimetic chamber' for electromechanical stimulation of slices, but their results have shown remarkable improvements in maintaining tissue slice integrity in long-term culture.

One approach uses a pacing device called a C-Pace that provides electrical stimulation to slices in air–surface interface configuration.[113] The device is set-up to provide regular (~1 Hz) bipolar pulses of electricity that stimulate the cardiac tissue to contract while in culture. Even in the absence of tension, cardiac slices showed a robust preservation of tissue architecture and metabolic gene expression profiles after 6 days of electrical stimulation compared with unstimulated tissues.[113]

Another study developed custom biomimetic cultivation chambers, as well as an electronic stimulation/recording device.[114] They kept the cardiac tissue under tension by glueing each end to steel wires that kept the slices at a constant, physiological tension. Using this approach, they were able to maintain several aspects of tissue integrity and activity for up to 4 weeks, where the tissues retained remarkable cardiomyocyte structure, function, and signalling.

Table 1 Myocardial tissue slice publications

Ref.	Species	Tissue	Human tissue source	Slice thickness	Culture dish	Mechanical stretch	Electrical pacing	Time maintained
Janssen et al[118]	Rabbit	Trabeculae	n/a	~200 μM	Custom chamber	Force transducer hooks	3–5 V, 0.5 Hz, 3-ms pulses	3 days
Bussek et al[119]	Rat, guinea pig & human	Left ventricle	Failing hearts	350 μM	n/a	n/a	n/a	2 h
Habeler et al[120]	Rat & human	Ventricles	Foetal hearts (elective abortion)	1 mm	Air–liquid interface	n/a	n/a	30–80 days
Camelliti et al[121]	Human & dog	Left ventricle	Failing hearts	250-350 μM	n/a	n/a	n/a	8 h
Brandenburger et al[112]	Human	Left ventricle	Morrow myomectomy	300 μM	Air–liquid interface	n/a	n/a	28 days
Kang et al[122]	Human	Left ventricle & right atrium	Rejected donor & failing hearts	380 μM	Air–liquid interface	n/a	n/a	24 h
Qiao et al[115]	Human	Left ventricle	Rejected donor hearts	380 μM	Heart-on-a-chip culture dish	Surgical pins	3 V, 1 Hz, 5-ms pulses	4 days
Watson et al[123]	Rat, rabbit & human	Left ventricle	Failing hearts	300 μM	Tissue stretcher in chamber	Custom stretchers	30–50 V, 0.5–1 Hz, 10-ms pulses	24 h to 5 days
Fischer et al[114]	Human	Left ventricle	Failing hearts	300 μM	Custom chamber	Custom wire apparatus	50 mA, 0.2 Hz, 1-ms pulses	35 days
Ou et al[124]	Human & pig	Left ventricle	Donor hearts	300 μM	Air–liquid interface	n/a	20 V, 1.2 Hz, 1-ms pulses	6 days

Other work demonstrated the importance of preserving sarcomere length by applying measured mechanical tension to each slice using custom-made stretching devices.[110] By measuring the sarcomeres of each slice and adjusting them to physiological length accordingly, they were able to optimize the static mechanical stretch for each individual slice. They also developed their own bespoke biomimetic chamber to house the tissue, which allowed for electrical stimulation, sustained oxygen delivery, and enabled media perfusion directly to the slices. This group kept slices viable for up to 5 days, demonstrating preserved structure and function compared with non-electromechanically stimulated slices.

Another study took a slightly different approach, developing an automated, self-contained, heart-on-a-chip system that maintains cardiac slices for up to 4 days.[115] The system supports media circulation, oxygenation, temperature control, electrical stimulation, and unmeasured static mechanical loading, using a combination of open-access electronic platforms and custom electromechanical components. This system allows for the greatest control of the culture environment compared with others and even allows for the transport of tissue between locations as it can be autonomously maintained on battery power for up to 3 days. The slices maintained in these chambers retained their electrophysiological and contractile properties. As evidenced by these studies, mechanical and electrical stimulation prolong viability and preserve function of the tissue slices.

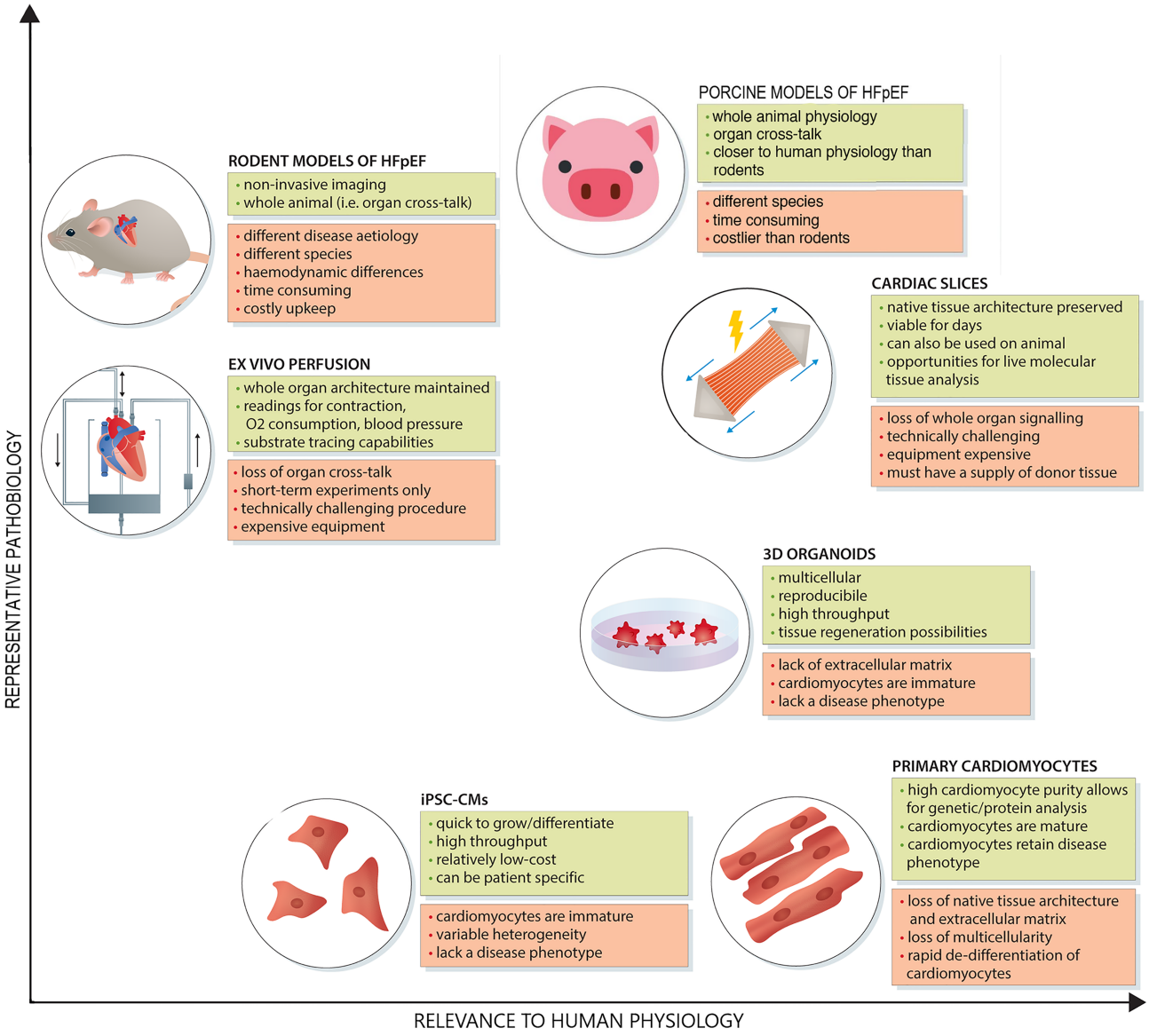
A major limitation of HFpEF models is capturing an accurate representation of clinical comorbidities that together cause the disorder. Pre-clinical models lend themselves well to a reductionist approach; for example, TAC models examining the molecular changes in a pressure-loaded left ventricle. While there are a few phenogroups within the broader HFpEF phenotype, recent state-of-the-art reviews have highlighted that the major phenogroup responsible for the increased prevalence of HFpEF is the type presenting in the context of metabolic syndrome. Human myocardial tissue slices from these patients would allow assessment of functional and molecular changes directly in their myocardium.

This model offers an opportunity to transform our understanding of myocardial energetic substrate utilization directly in human HFpEF myocardium, and numerous outputs are available (*Table 2*). Metabolic changes portend disease: for example, maintaining fatty acid oxidation in the face of increased afterload mitigates pathological hypertrophy.[116] Energetic substrate changes and metabolic inflexibility are central to HFpEF development, and supply of ketone bodies as an alternative fuel source may be particularly effective in this disease.[117] Substrate fate mapping has generally been performed *ex vivo* in rodent hearts, but species differences and inability to deliver fatty acids are serious limitations. Human HFpEF myocardial tissue slices can overcome these limitations with the potential to deliver transformative insights (*Figure 2*).

Table 2 Tissue slice experimental outputs

Analysis type	Methods	Outputs	Reference
Contractile function	Force transducer	Contractility, relaxation, β-adrenergic response, arrhythmia provocation, inotropic response, etc.	Previous studies[110–112,114,124,125]
Electrophysiological characterization	Multi-electrode Array Optical mapping Calcium signalling dyes	Map action potentials, calcium handling, mitochondrial inner member potential, etc.	Previous studies[110–112,114,115,119,121,122,124–127]
Structural analysis	Confocal microscopy (IF) Electron microscopy IHC/Histology	Intracellular organization, mitochondrial placement, sarcomeric alignment, Z-disc alignment and structure, gap junction analysis, assessment of fibrosis, etc.	Previous studies[110–112,114,119,121,122,124,125]
Drug delivery/pharmacology	Organ bath/Superfusion of pharmacological agents	Drug risk assessment, pharmacological responses, etc.	Bussek, Wettwer et al[119] and Bussek, Schmidt et al[126]
Gene expression	qPCR Transcriptomics Gene set enrichment	Gene expression analysis to further inform structural/functional/signalling outputs, etc.	Previous studies[110,112,114,124]
Metabolism	HPLC/Mass spectrometry Mitochondrial function ATP content/turnover	Assess metabolic activity, substrate usage, overall heart viability, etc.	Previous studies[121,124,125]
Disease modelling	Phenotype analysis of diseased rodent model hearts	*In vitro* analysis of rodent model diseased heart features	Hattori et al[128]

Figure 2 Key advantages and disadvantages of different cardiac models for HFpEF. Numerous cardiac models are available for the study of disease, each with pros (green box) and cons (red box). By preserving tissue pathology from human myocardium, the tissue slice model is optimal. O_2, oxygen; iPSC-CMs, induced pluripotent cardiomyocytes; HFpEF, heart failure with preserved ejection fraction.



Conclusions

A model based on human myocardium that preserves tissue characteristics of HFpEF provides unparalleled insights into pathology. Human cardiac tissue slices will help to fill critical knowledge gaps that are not currently addressed with *in vitro*, *in vivo*, or *ex vivo*-perfusion approaches. The model can act as a surrogate for an intact heart in terms of energy consumption, signalling, and function. Numerous assessments can be achieved with the model, but further outputs including metabolic flux, live-cell imaging, and high-resolution lusitropic measurements are imminently achievable. In our view, this model will accelerate mechanistic insight and therapeutic discovery for HFpEF, urgently needed.

Acknowledgements

We thank the patients and staff of Royal Prince Alfred Hospital and St Vincent's Hospital, Sydney.

Conflict of interest

None declared.

Funding

This work was supported by the Sydney Medical School Foundation [JFOS], NSW Health (JFOS DOH1003; DOH1006), and the National Heart Foundation (JFOS NHF104853).

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