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INVITED REVIEW

Myocardial slices come to age: an intermediate complexity *in vitro* cardiac model for translational research

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Abstract

Although past decades have witnessed significant reductions in mortality of heart failure together with advances in our understanding of its cellular, molecular, and whole-heart features, a lot of basic cardiac research still fails to translate into clinical practice. In this review we examine myocardial slices, a novel model in the translational arena. Myocardial slices are living ultra-thin sections of heart tissue. Slices maintain the myocardium's native function (contractility, electrophysiology) and structure (multicellularity, extracellular matrix) and can be prepared from animal and human tissue. The discussion begins with the history and current advances in the model, the different interlaboratory methods of preparation and their potential impact on results. We then contextualize slices' advantages and limitations by comparing it with other cardiac models. Recently, sophisticated methods have enabled slices to be cultured chronically *in vitro* while preserving the functional and structural phenotype. This is more timely now than ever where chronic physiologically relevant *in vitro* platforms for assessment of therapeutic strategies are urgently needed. We interrogate the technological developments that have permitted this, their limitations, and future directions. Finally, we look into the general obstacles faced by the translational field, and how implementation of research systems utilizing slices could help in resolving these.

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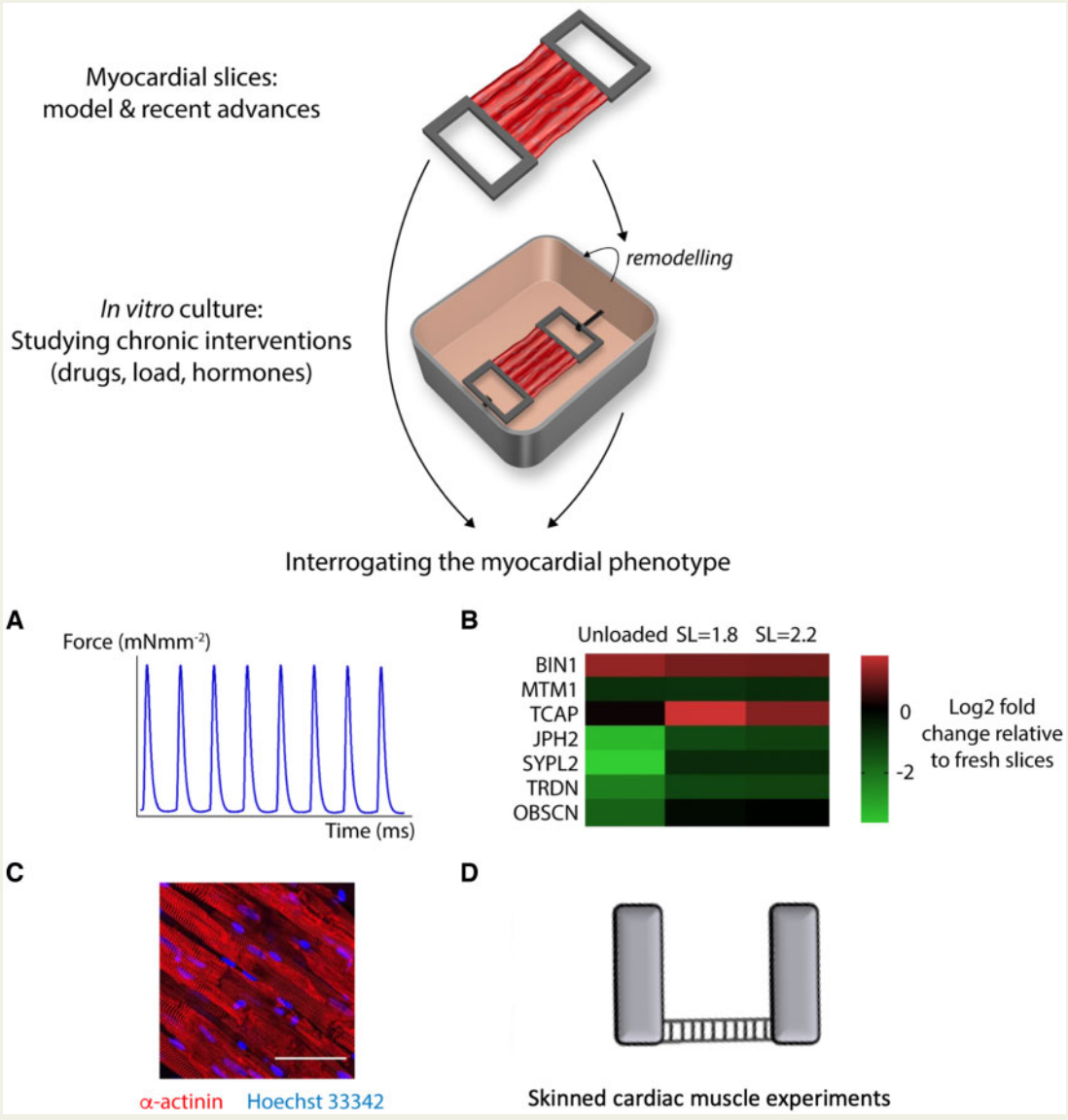
Graphical Abstract



Keywords Myocardial slices • Translational research • *In vitro* heart models • Culture • Mechanical load

1. Introduction

1.1 Conceptual framework of contemporary cardiac research

In an evidence-based research era, clinical studies are founded on the basis of information generated from the preclinical environment. Choice of experimental model is crucial, as it determines the future of a study. There are many cardiac models each with unique advantages and disadvantages. In this review, we examine myocardial slices. We focus on making practical cardiovascular progress, that is, slices are discussed within the context of the translational field.

The review is structured into four sections. In Section 1.2, we examine the slice model and recent advances in the methodology. In Section 2, we place slices in the cardiac research cosmos and compare them with other established models. Section 3 focuses on the use of slices for chronic investigations by prolonged culture experiments. Finally, in Section 4, we discuss how slices can facilitate the process of drug development and discovery.

1.2 Slicing fundamentals

Myocardial slices, also known as heart or cardiac slices, are ultra-thin (300 μm) living sections of heart tissue prepared using a high-precision microtome. Slices are 'organotypic' preparations meaning they retain the native tissue's electromechanical physiology, biochemistry, multi-, and heterocellular stoichiometry and extracellular matrix (ECM) (*Figure 1*).[1] They can be prepared from small and large mammalian hearts including

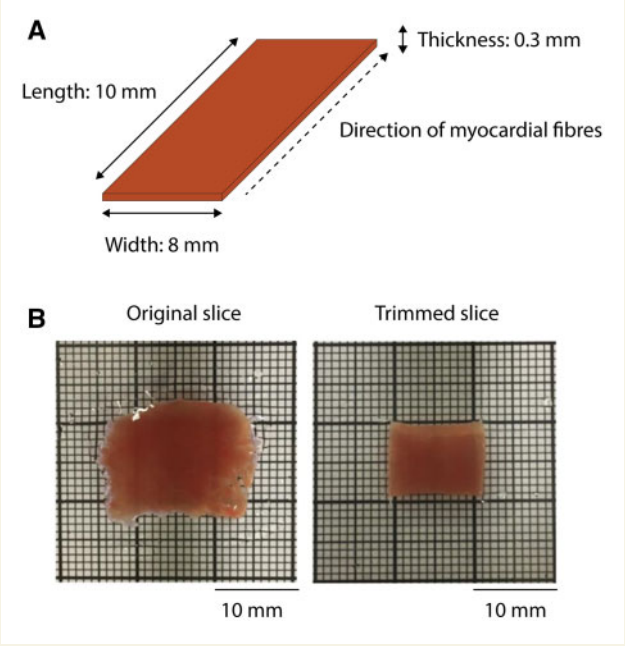


Figure 1 Cardiac slices. (*A*) Heart slice cartoon. Note that thickness is one order of magnitude less than width or length. As a result, slices have been described as pseudo-2D. (*B*) Heart slice. After the vibratome cuts through the heart tissue, a heart slice is obtained (*B*, *left*). This is subsequently trimmed using razor blades to a rectangle, typically 10 × 8 mm in dimensions, with the myofibres oriented along the long axis (*B*, *right*) of the slice. *B* adapted from Watson *et al.*[1]

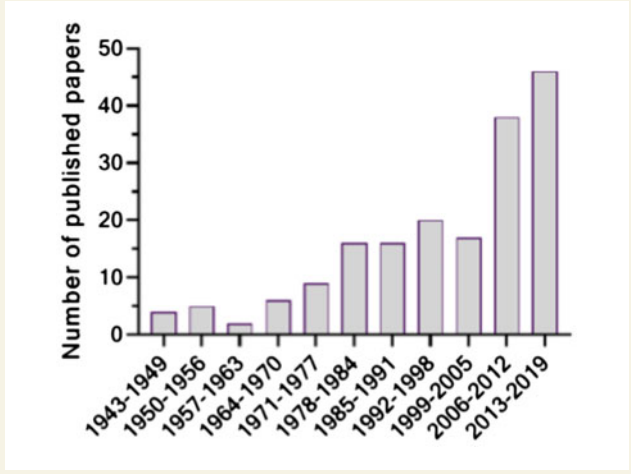


Figure 2 Number of published papers using myocardial slices between the years 1943 and 2019. One hundred and eighty-one papers were identified in a literature search.

human donors and biopsies, supporting both basic and translational research.

Slices were first described in 1946 to study metabolism of rat hearts in response to haemorrhagic shock,[2] and at the time were prepared with hand-held blades.[3,4] Since then, the technique has seen remarkable growth (*Figure* 2). Precision vibratomes capable of slicing tissue with minimal *Z*-axis error (i.e. minimizing fibre transection)[5] and protocol refinements have widened the capabilities of this model. In 1995, Parrish *et al.*[6] reported that 'heart slices do not retain [the] contractile response of cultured myocytes'. Two decades later, the contractile phenotype of human heart slices was maintained for 4 months in culture and our lab has demonstrated preserved function and structure for up to 5 days in human and 24 h in rat.[7,8]

Myocardial slice preparation has been discussed elsewhere.[1] In brief, for left ventricular (LV) studies from animal tissue, the whole-heart is explanted, the right ventricle removed, and the LV propped open by cutting across the interventricular septum. The left ventricle is then placed inside the vibratome stage and slicing begins. For human tissue, a ventricular biopsy requires minimal extra handling and can typically be placed directly on the vibratome stage.

To achieve high levels of viability, our lab slices heart tissue tangentially across the ventricle. To do that, the ventricle must (i) be placed epicardial-face down to the stage and (ii) be as flat as possible. Using this method, less than <3% of all cardiomyocytes are damaged, and this is contained to the superior and inferior layers, with the remaining layers (~10–15) intact.[1] From our experience, and knowledge of the cardiac myofibre geometry, starting as a right-handed helix and rotating to finish as a left-handed helix across the wall,[9] slicing in that way almost separates cardiac layers.[1] This is observable when slices are sequentially examined under light microscopy and the direction of myofibres is seen to rotate from the first slice (subendocardium) to the last slice (subepicardium). If the ventricle is not properly flattened or positioned endocardial face down, excess curvature can lead to myofibre damage. Recently, researchers have sliced at right angles to the long axis (perpendicular to the apex-base axis) of mice hearts.[10] Due to the twisting myofibre orientation within the ventricular wall, this type of slicing transects fibres, reduces viability, and increases variability.[1,11]

Prior to slicing several laboratories embed the ventricle in low-melting agarose.[7,12] Our lab has not typically advocated this,[1,11,13] as the extra thickness added from the agar may hamper oxygen diffusion during slicing. However, agar may confer stability to the vibratome as it cuts across the ventricle. As the vibrating blade is advancing, the ventricle is being cut but also pushed. For tissue of healthy stiffness, this is not a problem. However, if tissue homogeneity is not uniform [e.g. fibrotic and fat-rich tissues from end-stage heart failure (HF) or diseased animals], the ventricle can be disproportionately pushed at these regions and slip above or below the trajectory of the blade. This can yield irregular slices, and of uneven thickness both within the same and between different slices. Agar can maintain the blade at a single path while keeping the rest of the ventricle neatly compressed and on such occasions its use is encouraged. As the tissue is immersed in 4°C Tyrode's containing 30 mM 2,3-Butanedione Monoxime during slicing, the energetic demands of the myocardium are substantially diminished and the possibility for ischaemia minimized.

Finally, like any technical skill, preparing slices requires practice. From our experience, training with at least 10 hearts is necessary for investigators to produce highly viable quality slices.

2. Fitting slices in the cardiac research landscape

2.1 Cardiac models and the balance of complexity

In vitro cardiac models underpin basic heart research. They can be arranged across a complexity spectrum, from subcellular systems, to

isolated cells, multicellular preparations, and whole-hearts.[1,11] Ideally, an *in vitro* cardiac model should have:

a. Pathophysiological relevance
b. Resemblance of human myocardial properties
c. Ability for high-throughput experiments
d. Potential for mechanistic insight

Pathophysiological relevance means that findings made in the model are relevant to the function and structure of the heart in health and disease. The extent to which this holds true depends on how closely an *in vitro* model mirrors the *in vivo* state. For example, the mechanical operation of the heart should be echoed and orchestrated by the underlying electrophysiology with preservation of mechano-electrical feedback.[14,15] Furthermore, heterocellular cross-talk and ECM composition are known to orchestrate myocardial remodelling.[16] Thus, the heart's structure including three-dimensional anisotropy, multicellularity, heterocellularity, and ECM should be adequately reflected.[17–19]

Observations in animals do not always extrapolate to humans.[16,20–22] Ideally, the cardiac model would permit the use of human tissue from healthy donors (rejected for transplantation) and diseased hearts (e.g. myectomies from hypertrophic cardiomyopathy). In the former case, the tissue could be exposed to pathological stimuli (e.g. culturing healthy human hearts under adrenergic hyperdrive[23,24]), whereas in the latter reverse-remodelling approaches could be pursued (e.g. culturing failing hearts with pharmacological agents[25]) to uncover therapeutic pathways.

Particularly for long-term experiments (e.g. culture) and scarce tissue (e.g. human),[26] cardiac models should offer the possibility for high-throughput studies. Multiple parallel and/or combinatorial interrogations can then be performed, maximizing efficiency. For human tissue, this requires acquisition of multiple viable samples from a single specimen. Rather inconveniently, because of the complex mechanical and electrical properties of the heart, high-throughput systems are challenging to set-up. The heart is the body's largest bioelectric source[27] and is incessantly mechanically active. For physiological relevance to be preserved *in vitro*, expensive 'bioreactors' are required,[28] posing a real obstacle to upscaled platforms.

Finally, in an ideal model, the independent variable(s) could be manipulated, while the dependent variable(s) measured with minimal cross-interaction and off-target effects. For example, apelin is a potent inotrope synthesized by both cardiomyocytes and other cardiac cell populations.[29] Identifying the cardiomyocyte-specific effects of apelin may be intrinsically more challenging in a multicellular preparation, where both production of apelin by multiple cells, and its off-target effects on non-myocytes could confound results and their interpretation. Generally, identification of cause–effect relationships is easier in reductionist models; however, this is offset by difficulty in translating findings. In contrast, complex models are closer to the *in situ* environment and findings may be easier to translate; however, this is at the expense of lower experimental control and harder to obtain mechanistic insight.[28,30,31]

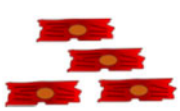
The ideal cardiac model does not exist. All models are simplifications of reality and go as far as their limitations. Choice of model thus depends on the experimental question and design. Perhaps the best strategy to adopt is that of maximizing returns and cutting losses. Where possible, they should be used to complement one another to strengthen experimental conclusions (*Table 1*).

2.2 Cardiac research models

2.2.1 Isolated cardiomyocytes

Isolated myocytes have been instrumental in studies of excitation–contraction coupling,[32,33] Ca^{2+} homeostasis,[34,35] localization and compartmentalization of cellular machinery,[36,37] and cardiomyocyte ultrastructure.[38] Their simplicity offers strong experimental control including direct[39] and indirect[40] manipulation of mechanical load, chemical milieu[41] and generally higher degrees of causality than more complex models. Additionally, development of automated systems (e.g. patch-clamp, optical mapping) has enabled high-throughput studies. However, isolation involves enzymatic digestion, leading to ECM loss and often cellular damage.[1,11] More, *in vitro* studies with isolated cardiomyocytes are typically limited to acute timepoints.[28] Although cardiomyocytes remain viable in culture, the lack of physiologically relevant conditions, and micro- and macro-environmental cues[42,43] leads to cell loss, and alterations in the electrical and contractile phenotype,[44,45] collectively referred to as cardiomyocyte de-differentiation.[46] Despite that, isolated cardiomyocytes have undoubtedly shaped most of contemporary cardiac research and our understanding of fundamental heart function is owed to this model.[47]

Table 1 Comparison of cardiac models

Features	Isolated myocytes	Papillary muscles	Whole-hearts	Engineered heart tissue	Myocardial slices
					
Proximity to *in vivo* cardiac operation	+	++	++++	++	+++
Throughput	++++	+	-	++	+++
Causality degrees	++++	++	++	++	++
Cost	+++	++	-	+	+++
Capacity for long term experiments (culture)	+	++	-	++++	+++
Personalized assays	-	-	-	+++	-

+ and - signs suggest that feature is advantageous and disadvantageous in that model relative to the other models. *Note*: there is no winner; choice of model depends on experimental question.

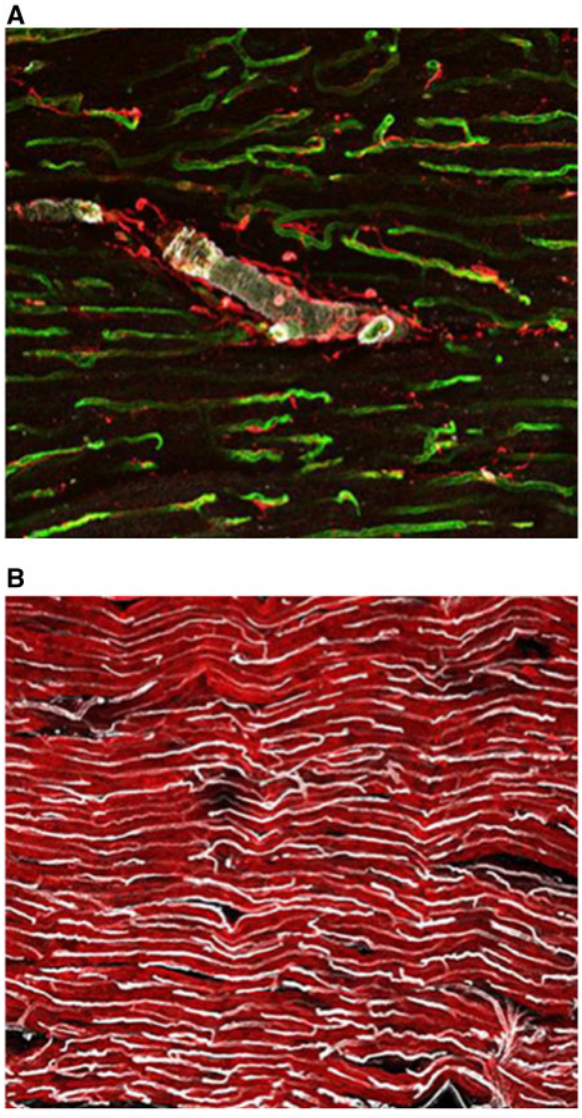


Figure 3 Canine myocardial slices. (*A*) Cardiac populations of a dog myocardial slice visualized under confocal microscopy. α-smooth muscle actin (white) was used to identify large vessels, isolectin for endothelial cells (green), and vimentin for mesenchymal cells (red). (*B*) Capillary network in a freshly prepared dog myocardial slice. Cardiomyocytes labelled with caveolin-3 (red) and endothelial cells with isolectin (white). Figure adapted from Perbellini *et al.*[18]

2.2.2 Papillary muscles, trabeculae, wedges, and whole-heart preparations

Papillary muscles, trabeculae, cardiac wedges, and whole-hearts have had a pivotal role in uncovering cardiac physiology with studies on heart mechanics,[48,49] ischaemia–reperfusion,[50] and electrophysiology.[51–53] However, cardiac wedges require dedicated *ex vivo* perfusion,[52] and Langendorff working hearts need complex and expensive set-ups.[54] Additionally, a single whole-heart, cardiac wedge, and one or two papillary muscles are typically obtained per heart.[5,26] Expensive set-ups together with low number of samples per specimen can limit throughput.[1,26,52,54] Furthermore, like isolated cells, whole-hearts and wedges are typically only studied acutely due to progressive run-down in function after more than a few hours on rig.[1,55] Although trabeculae and papillary muscles[31] have been kept in culture for days using specialized chambers,[56] hypoxic core (due to the preparation's thickness) has been reported to confound results[1,11,31] although others have reported no hypoxic damage.[31]

2.2.3 Engineered heart tissue

The field of regenerative medicine has seen great advances in development of heart tissue constructs with properties in close proximity to adult myocardium.[57–59] An exclusive advantage of engineered heart tissue (EHT) technology is the potential for precision medicine. Somatic cells can be reprogrammed to induced pluripotent stem cell-derived cardiomyocytes to make patient-specific EHTs. Disease can then be modelled and therapeutic interventions examined at a personalized level.[60]

However, it is vital that these models are sufficiently interrogated to guarantee maturity and physiological relevance.[61] Efforts to compare EHTs with established *in vitro* cardiac model counterparts have already began and results appear promising[62]; however, more validation work is needed. As more and more researchers are embarking on the vast task of mimicking *in vivo* biological cues to advance maturation of EHTs,[61] adult-like structural and functional maturation of stem-cells to cardiac tissue should be in the pipeline.

2.2.4 Heart slices

The method of isolation of cardiac slices, is unique in that it is mechanical, using a vibratome.[1,12,55] With the exception of a minority (~3%) of cells in the superior/inferior layers of a slice, the preparation remains intact and the structure and architecture of the native tissue preserved[11] (*Figure 3*). Functional reliability follows robust structure, and multiple research papers have validated slices in terms of contractility,[1,7,13,63] electrophysiology[8,26,30,55] viability,[18] as well as molecular[7,8] and metabolic signatures[8,26] (*Figure 4*).

Upwards of 30 slices can be obtained per human ventricular specimen and more than one specimen per heart.[1] Although experimental equipment is generally as expensive for slices as for other models (e.g. optical mapping), the larger number of samples per specimen allows more conditions to be tested and parallel/high-throughput studies even with scarce human tissue. Moreover, in contrast to thicker tissue models, slices are 300 μm thick, ensuring oxygen diffusion and the absence of hypoxic core (Upper O_2 diffusion limit is 200 μm.[64] O_2 can diffuse from both sides of the preparation.) without the need for coronary perfusion.

Although strictly three-dimensional, heart slices are referred to as 'pseudo-2D' (*Figure 1*) because their thickness is two to three orders of magnitude less than their length or width.[42] This is considered advantageous for electromechanical studies,[65] due to higher experimental control, spatial tracking of events, and structure–function correlations that may be compromised in thicker or more geometrically convoluted models.[66] Their size (typically 10 × 8 × 0.3 mm) also enables ease of manipulation of physical properties (e.g. stretch), without the need for microscopy, allowing the study of mechanical and electrical properties and their interaction.[30]

Multiple experiments have been conducted in freshly prepared slices,[10,26] yet slices can also be studied chronically by *in vitro* culture. In fact, the slice research arena has recently shifted towards the bigger

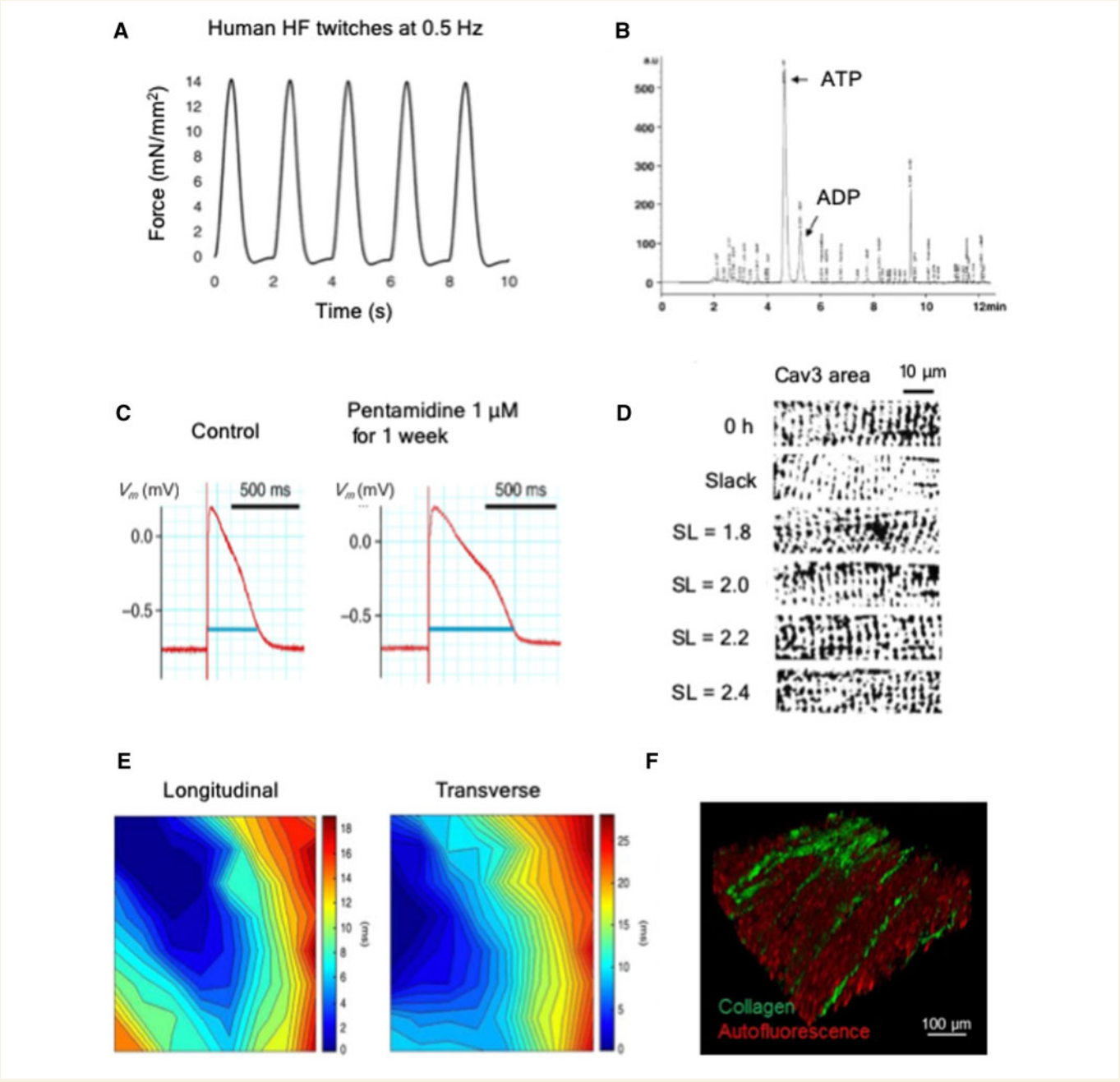


Figure 4 Experimental data acquired using myocardial slices. (*A*) Twitch force trace of human HF slices paced at 0.5 Hz. (*B*) ATP and ADP peaks from high-performance liquid chromatography for metabolic studies. (*C*) Effects of chronic pentamidine exposure on action potential in cultured human slices. (*D*) T-tubule density and regularity of cultured rat slices. (*E*) Conduction maps of freshly prepared human HF slices (left, right: longitudinal, transverse conduction velocity, respectively). (*F*) Second harmonic imaging of a slice that has been optically cleared using FASTClear. *A* and *E* adapted from Watson *et al.*[1]; *B* from Camelliti *et al.*[26]; *C* from Watson *et al.*[8]; *D* modified from Fischer *et al.*[7]; and *F* from Perbellini *et al.*[72]

reaps of preserving the adult myocardial phenotype *in vitro* for extended periods of time. Independent laboratories have been pursuing this using sophisticated culture techniques[7,8,67] (Section 3). Temporal examination of the effects of chronic interventions on slice function and structure is thus possible. Although culture remains an artificial environment, slices are sufficiently complex as a model to minimize loss of archetypical adult cardiac properties (e.g. contractility, action potential, Ca^{2+} handling). This reduces noise arising from culture-induced remodelling, and the outcomes of experimental interventions can be mapped out in a cause–effect fashion.

2.2.5 Limitations of the model

Because of the method of preparation, slices are generated transmurally from endocardium to epicardium. When designing experiments this should be accounted for (e.g. by randomization of samples from endo to epi), as differences in myocardial properties across the wall

have been documented,[68–70] and may increase variability. However, an examination of transmural cardiac properties is a research field of its own. As interrogation of isolated layers across the wall can be performed with slices, intrinsic vs. global regional differences can be identified.[10,71]

Another limitation of the model is the lack of flow across capillaries and vessels. Like trabeculae, papillary muscles, and most EHTs, slices are not perfused *in vitro*; instead, oxygen and metabolic substrate supplementation is accomplished via diffusion. As such, for researchers interested on the effects of flow on endothelium and/or myocardium, slices may not be the appropriate research platform. Moreover, even though slices are organotypic, they are an isolated system, devoid of hormonal, neuronal, and inflammatory influences and the associated feedback loops. Although there are pros and cons to this (see Section 3), care must be taken to avoid 'over-translation' of findings and interpretation should always be within the realms and context that an *in vitro* preparation can afford.

Furthermore, the geometry of slices has to be considered. When myocardial slices contract, the stress and strain vectors occur across a 2D plane, parallel to the direction of fibres. In contrast, the *in vivo* working heart undergoes constant three-dimensional (3D) pressure and volume changes. The use of geometrical models converting stress and strain to pressure and volume may ameliorate this to an extent.

Like other tissue preparations, light scattering through thick and opaque myocardium is challenging. This results in low imaging penetration and restricts acquisition to a few μm depth from the surface of the sample for most microscopy techniques. One solution is the use of FAST-clear, exemplified in our laboratory, which easily and inexpensively renders thick 3D myocardial tissue transparent, enabling full-thickness confocal or second harmonic imaging[72] (*Figure 4F*).

3. Interrogating the myocardial phenotype

3.1 The need for long-term experiments

In vitro culture allows the study of chronic responses of the myocardium to physiological, pathological, and therapeutic stimuli. The heart has a remarkable ability to adapt to changes in environmental demand.[73] This process is termed cardiac plasticity[43] and underlies physiology and progression to pathology. Cardiac plasticity is a complex process driven by mechanical load, the neurohormonal axis, and inflammatory signals among others.[74] The former two are not only complex in isolation but, particularly when studied together (e.g. *in vivo*), can interact in dynamic and multifactorial ways. For example, in a transverse aortic constriction animal model, the increased afterload induces neurohormonal changes, which introduce an array of variables with direct effects on the myocardial phenotype. In such models, the more we control for mechanical load the harder it becomes to separate its effects from the neurohormonal axis.

With reductionist culture systems the effects of mechanical load (e.g. overload or unloading), hormones (e.g. adrenergic overdrive), and inflammation on cardiac remodelling can be modelled and studied in isolation to one another. This is powerful as it allows temporal tracking of changes in the tissue's functional outputs (e.g. contractility) in response to the stimulus under investigation simultaneously with mapping of the underlying pathways responsible for the observed changes.[5,56] To do that reliably, artefacts and noise arising from the artificiality of the culture environment must be minimized. Thus, it is important to ask which factors are required for a good culture system and how we can get there.

A successful culture is one whereby the cultured myocardial phenotype reflects the functional and structural properties, and molecular and metabolic signatures of fresh myocardium. The obstacle with realizing this is the utter sophistication of the adult heart as an organ, subject to multiple extrinsic and intrinsic regulatory feedback loops,[75] and physical and biochemical signals which are ever-present and dynamically changing.[76] When such factors are absent or poorly simulated in culture the heart undergoes artificial remodelling and its archetypical structure and function is lost.[7,8,44–46] Therefore, cardiac culture systems attempt to approximate key features of the *in vivo* environment.[19,77] Such efforts are collectively termed *biomimetic culture*, and include co-culture,[78] mechanical and/or electrical stimulation,[7,8,79] and addition of hormonal agents.[80,81]

3.2 The evolution of slice culture

The organotypic nature of myocardial slices makes them an ideal candidate for culture. The minutiae of ECM, cardiac cellular stoichiometry, anisotropy, and 3D architecture of the adult heart are all reflected on a slice. In primary culture, where isolated cells are seeded on plates, only a fraction of cells survive.[82] This, known as 'culture shock',[83] and has been credited to the foreign environment (e.g. glass plates) and stress induced during the chemically and mechanically disruptive isolation procedures. This is minimal in myocardial slices where the isolation is mechanical, and the muscle remains intact.[55,84,85]

Short-term 60-min incubation protocols exploring dose-dependent cardiotoxicity of antineoplastic drugs were described as early as 1984.[86] However, the first efforts to culture slices for prolonged period of time (>24 h) came a decade later.[5] Parrish *et al.*[5] cultured adult rat ventricular myocardial slices in stainless steel cylinders, gassed with O_2 and kept in an incubator while rotating at 1 rpm. Viability assays showed that despite a small run-down attributed to the slicing procedure, the tissue remained viable for at least 24 h with membrane integrity (K^+/Ca^{2+} content), protein synthesis, ATP generation, and stabilization of LDH release.[5] These findings were corroborated by Pillekamp *et al.*,[85] who showed histological preservation of embryonic mouse heart slices for 24 h. Although slice electrophysiology had been studied before that time,[87,88] Pillekamp *et al.* were among the first to perform action potential, and multi-electrode array measurements on cultured tissue, with important implications, as it suggested that functional properties could be studied in the long-term. The next big advance was in 2009 when Habeler *et al.*[89] showed that slices from 3-day-old rats and 8- to 9.5-week-old human foetuses could be cultured for up to 80 days. The breakthrough in this was inspired by organotypic brain slices culture, which were shown to be better preserved when cultured in semi-porous membrane suspended in an air–liquid interface,[90,91] presumably due to superior oxygenation.[89] These studies laid the groundwork for culture optimization and subsequent studies on adult tissue.

Brandenburger *et al.*[12] cultured human adult slices from tissue explanted during cardiac surgery. The researchers used the semi-porous air–liquid interface and were able to keep slices viable for up to 28 days, quantified by MTT enzymatic assay.[12] Additionally, they demonstrated maintained electrophysiology and response to adrenergic stimulation of cultured tissue. However, significant structural remodelling with loss of typical rod-shaped cardiomyocyte morphology and striations together with down-regulation of sarcomeric proteins (MLC2) was observed. This was mirrored functionally by progressive decline in slice force generation and increased elasticity of the tissue.[12] Despite its limitations, the

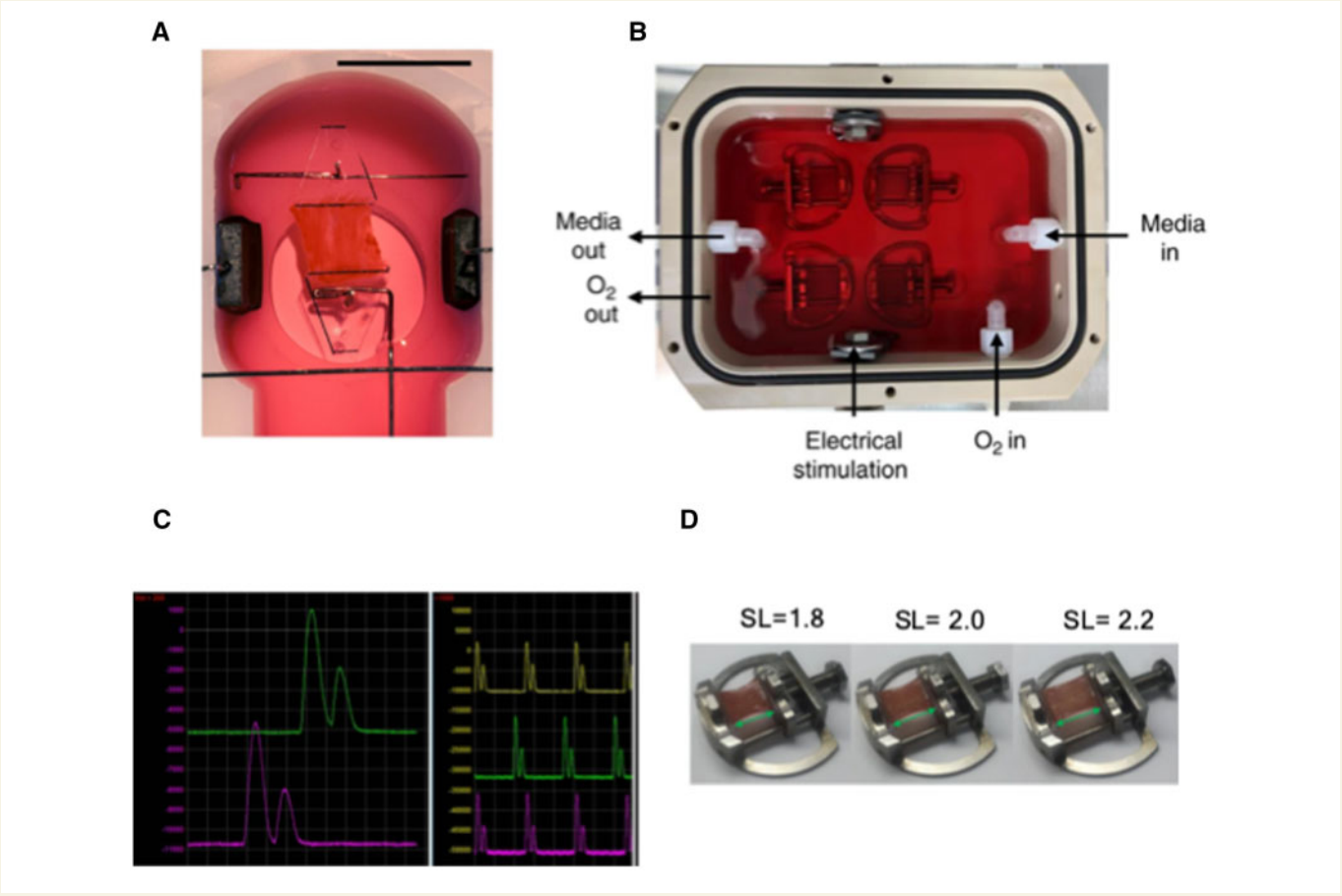


Figure 5 Culture systems developed by Fischer *et al.* (*A* and *C*), and Watson *et al.* (*B* and *D*), for prolonged maintenance of adult cardiac tissue *in vitro*. (*A*) Culture chamber developed by Fischer *et al.*, consisting of one inflexible and one flexible post allowing for auxotonic contractions, and graphite blocks for electrical stimulation. (*B*) Culture chamber developed by Watson *et al.* The chamber can accommodate up to four stainless steel stretchers and each stretcher mounts one slice. The system consists of graphite electrodes, perfusion and O_2 inlets/outlets. (*C*) This culture system allows measurements of contractility by monitoring displacement of the flexible post, quantified using a magnetic plate and the stiffness constant of the post. (*D*) Stretchers allow for manipulation of preload by changing the muscle length of the mounted slice. The stretchers posts are inflexible so that the muscle contracts isometrically. Scale bars: 10 mm. *A* and *C* adapted from Fischer *et al.*[7] and *B* and *D* from Watson *et al.*[8]

liquid–air interface was long considered a gold-standard and later studies used this or slightly modified versions[82] to culture slices with pharmacological agents, or for viral transduction studies.[55,82] With time, it became apparent that the liquid–air interface method of myocardial slice culture was in fact not *biomimetic*. The biggest drawback was the lack of mechanical load and electrical stimulation.

3.2.1 Mechanical stimulation

Mechanical load, simply described as the forces that act upon and are actuated by the heart, is a fundamental property of cardiac muscle. It drives cardiac remodelling. Its impact is so unambiguous that differential loads can induce extremely polarizing phenotypic responses. When too high (e.g. aortic stenosis) it can direct the myocardium to pathological phenotypes,[43,92] when corrected (e.g. transcatheter aortic valve replacement, mechanical assist devices)[93–96] it can revert pathological phenotypes back to healthy-like states, and when absent it can lead to myocardial atrophy.[38,43] Given the unloaded nature of culture in the air–liquid interface and the observation that many of the cultured-induced changes (e.g. loss of contractility, myofibrillar re-assembly) effectively described an atrophic myocardium, incorporation of some protocol of mechanical stimulation was deemed essential. Much of this was already highlighted by cutting edge research on EHTs, which utilized mechanical stimulation via auxotonic or isometric contractions.[97–102] As culture-induced de-differentiation of adult tissue is similar but opposite in direction to differentiation of immature cardiomyocytes, protocols that work in one system would be expected to work in the other. The natural progression of the slice field was thus assimilation of techniques seen within the EHT community. Recently, a surge of publications from our laboratory[8,13] and others[7,67] have demonstrated the latest advancements.

Our laboratory has recently highlighted a new culture technique which applies mechanical load in the form of uniaxial strain on slices.[8] Firstly, 3D printed biocompatible rings are attached to the slice. Then, the slice is mounted on the inflexible posts of a custom-made stainless-steel stretcher. The stretcher allows fine manipulation of preload by lengthening of the tissue via a rotatory screw (*Figure 5D*).[8] As the relationship between sarcomere length and % stretch from slack is approximately linear[103] and can be quantified, the experimenter can easily stretch myocardial slices to a spectrum of sarcomere lengths from unloaded (1.8 μm) to overloaded (2.4 μm). This can be done with a set of callipers by using muscle length as a surrogate to sarcomere length.

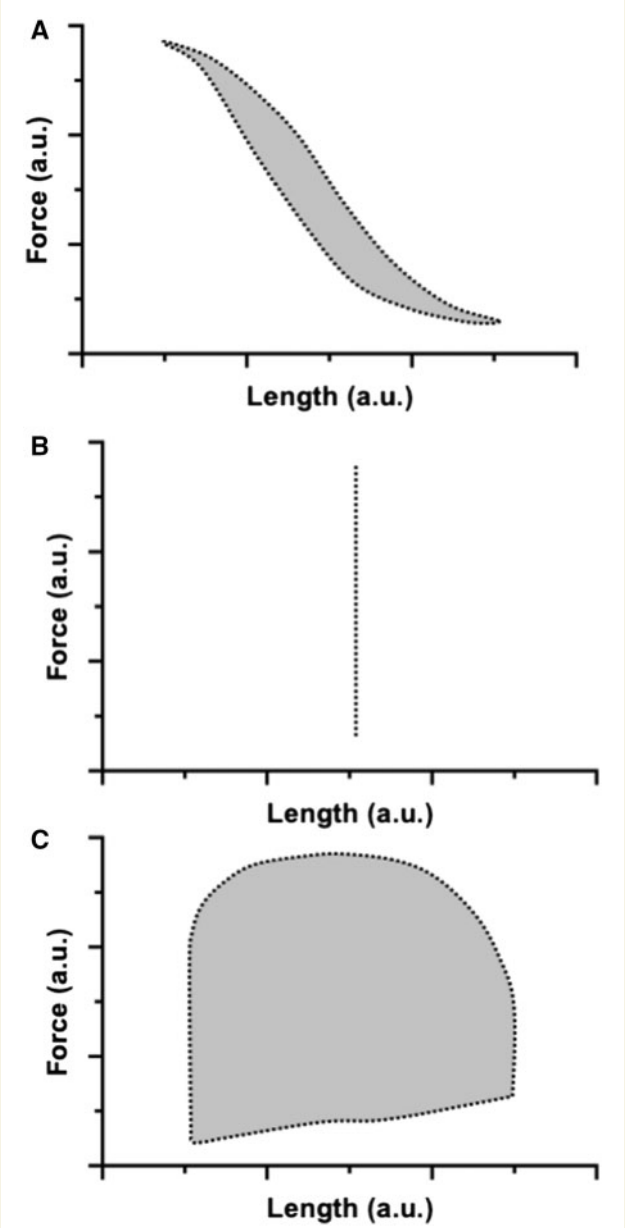


Figure 6 Computer simulations of force-length changes under the culture chambers developed by Fischer *et al.* (*A*) and Watson *et al.* (*B*) vs. the *in vivo* state (*C*). (*A*) In the system by Fischer *et al.*, the slices generate force while shortening in a linear manner. (*B*) In the system by Watson *et al.*, slices contract isometrically, that is, generate force without corresponded changes in length. (*C*) *In vivo*, the myocardium develops force while shortening in a more intricate fashion consisting of distinct phases whereby force is changing without length changes, and where both are changing simultaneously.

Thus, conditions of low- or high-preload can be investigated. The stretchers are then placed in custom-made biocompatible culture chambers. These can accommodate up to four stretchers enabling a middle-throughput system. During culture, the slices are electrically paced through graphite electrodes and the media re-circulated and directly gassed with 95% O_2 5% CO_2. Under this system of constant electromechanical stimulation, Watson *et al.*[8] showed that adult rat slices cultured for 24 h at 2.2 µm sarcomere length had significantly higher contractility, Ca^{2+} handling, energetics, as well as transcriptome profile than slices cultured in unloaded or overloaded conditions. A limitation of the study was the use of rat tissue, which complicates the translational impact. However, rat tissue de-differentiates in culture much faster than larger animals.[8,104] For example, under similar conditions of preload, rabbit tissue was preserved for up to 5 days without any drop in baseline contractility.[8] Ultimately, even though a 24 h timepoint is short within the context of chronic studies, maintenance of effectively all myocardial properties in an actively de-differentiating preparation is a remarkable improvement. A timely similar mechanical approach was also demonstrated by Qiao *et al.*,[67] who developed a heart-on-a-chip system which allowed direct manipulation of circulating media, temperature, and electrical stimulation under static mechanical load. This permitted culture of human tissue for up to 4 days.[67] Likewise, Ou *et al.*[105] developed a culture system to keep pig heart slices viable for up to 6 days. The mechanical protocol was similar to Watson *et al.* and Qiao *et al.*, but the media composition modified (to include serum, growth factors, and fatty acids), in order to support the energetic demands of the heart. Despite this, the force production of the cultured slices was one to two orders of magnitude lower than the one reported in the other studies employing similar mechanical load approaches.[8,105]

An almost identical system was developed by Fischer *et al.*[7] In this set-up, slices are mounted on a rigid post on one side, used to stretch the slice, and a spring wire on the opposite side, and field stimulated (*Figure 5A*).[7] In contrast to the other systems, this set-up allows for measurement of slice force production by quantifying the displacement of a spring of known stiffness with the use of a magnetic sensor. This is advantageous as it permits continuous monitoring of slice contractility throughout the culture. Detection of aftercontractions or changes in contractility induced by addition of pharmacological agents and/or change in load are thus possible. In their study, Fischer *et al.*[7] demonstrated that human slices could be kept beating for up to 4 months. However, the contractile phenotype was not entirely preserved as seen from divergence of Frank-Starling arms between fresh and cultured tissue, while changes in gene expression were also noted.[7]

Technically, the major differences between systems are the mode of contraction. Under the system developed by Watson *et al.* (and similarly for the others), the stretchers allow for manipulation of preload but given that stainless steel posts do not bend, the slices always contract isometrically. The twitch force is then a function of sarcomere length according to:

$$\text{Twitch force} \propto f(\text{Sarcomere length}) \quad (1)$$

Under the system developed by Fischer *et al.*, the slices contract and shorten in a linear relationship, which depends on the stiffness of the spring wire. This is an auxotonic mode of contraction and follows:

$$\text{Twitch force} \propto f(\text{Velocity}_{\text{shortening}}, \text{Sarcomere length}) \quad (2)$$

The pursuit of similar goals with comparable outcomes from independent laboratories with almost identical publication dates[7,8,67] is a demonstration of (i) the authenticity, (ii) potential impact, and (iii) robustness of myocardial slices. Such breakthroughs enable great strides in filling gaps in translational research where chronic *in vitro* experimental models are urgently needed, particularly for scarcely available human tissue.

Though these approaches have cultured the myocardium under 'biomimetic' mechanical stimulation, they are flawed in that they fail to capture the dynamic sequence of mechanical events of the *in vivo* cardiac

cycle. This can be readily appreciated in the force-length plane (*Figure 6*). *In vivo*, systole consists of distinct phases of isovolumetric contraction and ejection, and diastole consists of consists of isovolumetric relaxation, and diastolic refilling.[15] Each phase is characterized by distinct changes in pressure and volume (*Figure 6*),[15] which are mirrored at the myofibre level by length changes that occur in sync with force generation; isometric is followed by isotonic contraction, followed by isometric relaxation and diastolic re-stretching. Correspondingly, in the stretcher system, the inflexible stainless-steel posts simulate a condition of 'infinite' afterload, as the slices cannot shorten. In contrast, the spring wire simulates a condition of non-physiological shortening. Ultimately, neither isometric nor auxotonic contractions ever occur *in vivo*. More physiological-based cultured platforms are needed to adequately simulate the mechanical events of the cardiac cycle.

3.2.2 Electrical stimulation

The importance of electrical stimulation on myocardial phenotype has been largely appreciated by EHTs which mature superiorly when paced.[77,106] In the aforementioned slice systems electrodes were used to field stimulate myocardial slices, which may have contributed to the preservation of electrical properties, although not explicitly studied. Two limitations regarding the nature of the electrical stimulation should be highlighted. The first concerns the nature of electrical impulse—that is, field stimulation. *In vivo*, spread of action potential ensures that cardiomyocytes are sequentially depolarized; direct current field stimulation does not model that with uncertain consequences on the cell's electrical machinery. For example, Fischer *et al.*[7] reported a negative force-frequency response in cultured human slices suggesting electrophysiological remodelling. Likewise, the Ca^{2+} transient of the slices cultured in the optimal preload condition in Watson *et al.*[8] was much larger than that of freshly prepared slices. The second limitation concerns the sub-physiological stimulation rates used. Watson *et al.* cultured rat heart tissue at 1 Hz, whereas the typical heart rate of rat is in the order of 300–400 bpm. Likewise, Fischer *et al.* cultured adult human tissue at 0.2 Hz which is at least a five-fold reduction from the human heart rate. It is unclear how these limitations have impacted the results; however, much like improvements in mechanical load advancements in electrical stimulation could be of benefit. One way to do this could be by point/line stimulation of the cardiac tissue via fine electrodes or use of optogenetic technology.[51,67] The latter may be more welcomed as introduction of electrodes with direct physical contact to a beating (and moving) heart slice is technically difficult.

4. Translational research with slices

4.1 Target the heart

In the past decades, treatment of HF has centred around the use of neurohormonal modulating agents,[107] which may have undue haemodynamic consequences.[108] On the basis of cardiac plasticity and ability for long-term slice culture, the chronic effects of mechanical, hormonal, and/or drug protocols could be scrutinized at the *in vitro* level and the concurrent in/activation of mediating pathways mapped out. For example, slices from HF patients could be unloaded to study pathways of reverse remodelling.[109] This would permit discovery of novel therapeutic mediators and development of compounds that target the heart while being devoid of peripheral effects.

4.2 Human preclinical drug testing

The flowchart for drug development begins with preclinical testing, followed by large animal experiments, and phase 0-IV trials. Despite recognized benefits of human tissue for drug testing,[110] its preclinical use has been limited.[111] Human slices could be used in sync with current assays to complement preclinical drug assessment. Chronic incubation of slices with therapeutic agents would permit temporal analysis of their effects on myocardial phenotype. Positive hits could be promoted while negative hits deprioritized or re-evaluated. With sufficient institutional organization, human tissue could be classified into discrete categories based on patient's HF aetiology, biomarkers, clinical profile, drug background, and even symptomatology. The acquired tissue need not be limited to end-stage HF, as slices can be prepared from donor hearts,[8] myectomies,[7] and biopsies obtained during implantation and explanation of assist devices.[26] This method would homogenize patient populations, and reduce the variability associated with human samples, similarly to performing highly controlled *in vivo* experiments. The relationship between patient class, *in vitro* drug response, and drug concentrations could then be scrutinized. As multiple slices are obtained per specimen, and many assays can be conducted on a single slice, data mining techniques could be employed to uncover intricate relationships. Although this would not replace current methodologies or eliminate the need for *in vivo* animal studies, it would have a two-fold benefit. Firstly, patient populations and subpopulations likely to benefit from a given drug would be identified. Secondly, mechanistic insight directly applicable to human pathophysiology would be gained.

4.3 Detecting unfriendly cardiac compounds

Cardiac safety remains the leading cause of drug discontinuation.[112] Torsade-de-Point (TdP) is a life-threatening arrhythmia caused by QT-prolongation due to delayed repolarization as a result of hERG-channel (I_{Kr} – K^+ channel) inhibition.[113,114] Although many drugs block hERG, channel blockade does not guarantee arrhythmia.[115–117] This in part because disturbances in heart rhythm and cardiac conduction are multicellular phenomena.[118] QT-prolonging agents, change the field potential duration of human HF slices (an *in vitro* measure of QT-interval) similar to other established muscle preparations.[26] Use of human tissue is advantageous here, as elemental interspecies electrophysiology differences,[119,120] prohibit reliable cardiac safety experiments on small mammals (rats and mice) according to S7B European Medicine Agency.

Chronic cardiotoxic agents have also been studied by culturing slices for 24 h in increasing concentrations of doxorubicin or allylamine.[84] The time- and dose-dependent biochemical consequences of the compounds were detected as reduced ATP content, and protein synthesis, and increased creatinine kinase release.[6,84] Prolonged incubation protocols are vital as chronic and acute drug effects may be different and even opposite to each other.[55,117,121,122] Dofetilide, a known I_{Kr} blocker, was until recently assumed to have no other electrophysiological action.[117] When acutely exposed to adult mice cardiomyocytes, which lack I_{Kr}, no effects are seen on action potential duration (APD).[117] However, prolonged exposure (>5 h) causes APD prolongation, and increases the rate of early- and after depolarizations.[117] Likewise, in human HF slices, acute phenylephrine exposure causes APD prolongation, whereas chronic (24 h) exposure results in APD abbreviation.[55]

An inherent limitation of any cardiac model isolated from the remainder of the organism is the absence of relevant pharmacokinetic and bioavailability effects. Generation of drug metabolites and derivatives

occurs in the liver, and may confound *in vitro* findings particularly when attempting to approximate therapeutic dosages.[120] Co-culture of different tissues may resolve that. Efforts to develop multiorganoid platforms for drug screening are already underway.[123] As slices can be prepared from brain, liver, kidney, and heart, a multiorgan slice culture could be used to mimic the integrated responses of the whole-body and to study metabolite-mediated effects. This may sound creative yet rabbit myocardial slices have already been co-cultured with liver slices more than two decades ago.[124] In that system, the cardiotoxic effects of allyl alcohol, metabolized to toxic acrolein by alcohol dehydrogenase (found in the liver) were only observed in co-culture, and allyl alcohol had no effects when cultured on myocardial slices alone.[124]

5. Concluding remarks

Myocardial slices have come to age. From the days of manual slicing using hand-held blades to the use of precision cutting vibratomes slices have walked through basic and translational research avenues alike. For basic research the model's intermediate complexity permits experimental control within the physiological context of an intact preparation, bridging the cell-*in vivo* gap. For translational research, high-throughput even from scarce human samples, and capacity for prolonged culture opens avenues for novel experiments to study cardiac remodelling and pharmacological assays.

In May 2019, our laboratory held a slice theory and hands-on workshop for beginners to learn the essentials of slicing. All attendees could produce beating slices from the first day. Adoption of slices by multiple laboratories is necessary if the model is to make a dent in the field. Slices have passed the criticality phase, where a model is tested and either abandoned or further pursued and are now entering an accelerated uptake phase where multiple publications will highlight its potential.

We conclude by saying that slices are an exciting novel cardiac model; they are easy to prepare and have unique features that can propel them to transform the present cardiac research scenery.

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