METHODS PAPER

Profiling the Biomechanical Responses to Workload on the Human Myocyte to Explore the Concept of Myocardial Fatigue and Reversibility: Rationale and Design of the POWER Heart Failure Study

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Abstract

It remains unclear why some patients develop heart failure without evidence of structural damage. One theory relates to impaired myocardial energetics and ventricular-arterial decoupling as the heart works against adverse mechanical load. In this original study, we propose the novel concept of myocardial fatigue to capture this phenomenon and aim to investigate this using human cardiomyocytes subjected to a modern work-loop contractility model that closely mimics in vivo cardiac cycles. This proof-of-concept study (NCT04899635) will use human myocardial tissue samples from patients undergoing cardiac surgery to develop a reproducible protocol to isolate robust calcium-tolerant cardiomyocytes. Thereafter, work-loop contractility experiments will be performed over a range of preload, afterload and cycle frequency as a function of time to elicit any reversible reduction in contractile performance (i.e. fatigue). This will provide novel insight into mechanisms behind heart failure and myocardial recovery and serve as a valuable research platform in translational cardiovascular research.

Keywords Myocardial fatigue · Cardiomyocytes · Work-loops · Contractility · Afterload · Preload

Abbreviations

CABG	Coronary artery bypass grafting
EDFLR	End-diastolic force-length relation
ESFLR	End-systolic force-length relation
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
LV	Left ventricle
MF	Myocardial fatigue
SL	Sarcomeric length
TTP	Time to from stimulus to peak force
UHCW	University Hospitals Coventry and Warwickshire NHS Trust

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Introduction

Equipoise of Research in Heart Failure

Our understanding of heart failure (HF) pathogenesis and its treatment have plateaued in recent years, relative to the accelerating incidence of HF with over 64 million people already affected worldwide [1, 2]. A fresh perspective on HF is therefore needed. Setting the premise for this is the modern definition of HF, which finally acknowledges both structural (e.g. myocardial infarction) and functional causes [3]. The paucity of studies on functional HF may explain why the aetiology of cardiomyopathies remains unknown in > 50% of

cases, even after cardiac magnetic resonance imaging [4]. It may also explain the sizeable portion of non-responders to disease-modifying therapies, including sacubitril/valsartan, for HF with reduced ejection fraction (HFrEF) where the cause may not be driven by neurohormonal overactivation, but perhaps, a functional imbalance in ventricular-arterial coupling [1, 5–7]. More recently, the unexpected cardioprotective benefits of sodium-glucose cotransporter-2 inhibitors (SGLT2i) in both HFrEF and HF with preserved ejection fraction (HFpEF) [8, 9], through its pleomorphic action on cardiac energetics, vascular compliance and haemodynamic unloading [10], are not only a testament to our incomplete understanding of HF but also, to the possibility that this heterogeneous syndrome, particularly HFpEF, may also be a disease of the vasculature that provokes a transiently energy-depleted overworked myocardium [11–14]. One potential hypothesis is that an incessantly high vascular load may drive the myocardium into a state of chronic fatigue.

Concept of Myocardial Fatigue in Heart Failure

An in-depth narrative review and clinical case series on myocardial fatigue (MF) can be found in our previous publications [15, 16]. Briefly, MF is a mechano-energetic concept that draws on the basic tenets of muscle physiology, offering a novel functional mechanism in HF. A normal heart consumes up to 30 kg of ATP per day to contract and relax under physiological workload [17]. Like any striated muscle, the metabolic cost of force production increases with the intensity of contractions and opposing resistance [18]. Hence, imposing a greater haemodynamic load on a stressed myocardium with a limited mitochondrial oxidative capacity and fuel supply can disrupt this balance between energy consumption and delivery [19]. One example is the use of positive inotropes (e.g. noradrenaline) which may boost contractility at the expense of diminishing the cardiac energy reserve [20]. This will consequently impair ATP-dependent processes of sarcoplasmic reticulum calcium handling, myofibrillar calcium sensitivity, cross-bridge cycling and ultimately, myocyte shortening and relaxation: the fundamental traits of HFrEF and HFpEF [21, 22]. One hypothesis suggests that the primary driver of myocardial fatigue in functional HF is a decoupling of mitochondrial oxidative phosphorylation and ATP synthesis in response to the opposing mechanical load on the myocardium, resulting in the cascade of downstream events, including impaired calcium handling and cross-bridge cycling [16, 19]. This mechanism is similar to that seen in skeletal muscle fatigue [23, 24].

Moreover, since cardiomyocytes share the same contractile protein isoforms as slow-twitch skeletal muscle (both of which are considered fatigue-resistant), it is reasonable to assume that cardiomyocytes may also reach a state of fatigue when its energetic buffers become overwhelmed by excessive workload [16, 23–25]. However, unlike skeletal muscle where the mechanical activity can be consciously halted to allow recovery, the ventricle will continually receive preload and contract against varying degrees of afterload, even when severely impaired. Assuming it enters a state of chronic fatigue (e.g. from severe aortic stenosis or hypertension), myocardial recovery may be possible if the pathological load is promptly removed before irreversible damage occurs [15, 26, 27]. One way to investigate this hypothesis is to examine the in vitro behaviour of cardiomyocytes in response to a range of load, without the confounding influences of autonomic tone in vivo and extracellular tissue and fibrosis in multi-cellular preparations [16, 28].

Use of Human Cardiomyocytes from Surgical Tissue Specimens

Cardiomyocytes isolated from animals have been indispensable in furthering our understanding of human cardiac disease, but a more physiologically relevant human cell model is preferred [29]. Small and large animal models that use genetic engineering, aortic clamping or ischaemic injury to induce HF are not truly reflective of the human pathophysiology of HF [30]. Hence, the external validity of any animal-based findings will invariably be questioned. Furthermore, with substantial reductions in an already scarce supply of donor hearts since the coronavirus pandemic [31], renewed efforts have been made to develop a reproducible method of isolating cardiomyocytes from surgical tissue samples. Coupled with a global initiative to move away from one-dimensional unloaded shortenings and isometric twitches to a more physiological work-loop cycle akin to the cardiac cycle in vivo [32–34], myocytes must also be able to endure protracted contractility experiments. This would serve as a valuable platform to explore our hypothesis of MF. To date, most protocols have either relied on frozen whole hearts and large tissue wedges for coronary perfusion-based isolation [35, 36] or obtained human myocytes only for brief patch-clamp electrophysiology or unloaded contractility experiments [37–39]. Hence, there is a need to develop a means to isolate human ventricular myocytes from surgical specimens capable of sustaining prolonged mechanically loaded force-length cycles. Apart from the hypothetical syllogism of MF, the foundation of this study is also built upon previous work by our research institution on using work-loop assays to investigate the effects of fatigue on skeletal muscle performance [40–42] and in assessing drug effects on human and animal hearts [43, 44].

Aims and Objectives

This study aims to deliver two major novel outcomes: (1) by investigating the concept of cardiac fatigue in vitro as a potential mechanism of HF, and (2) by developing a

reproducible method to isolate calcium-tolerant ventricular myocytes from small surgical tissue specimens that can sustain mechanically loaded contractility experiments. If successful, this will provide a unique research platform with wide implications in cardiovascular translational science.

To achieve this, the first objective is to test various experimental conditions (e.g. enzyme concentration and chemical composition of solutions) to optimise an isolation protocol that can yield a stable population of robust, calcium-tolerant myocytes. These cells must then be able to undergo cyclical force-length changes to mimic the in vivo cardiac cycle (explained further in the methodology). Once this is achieved, the second objective is to profile the biomechanical responses of cardiomyocytes to a range of afterload at different cell lengths, stimulation frequency and drug-induced inotropy to assess for any reversible reduction in cardiac performance over time, using cardiac tissue from patients undergoing coronary artery bypass grafting (CABG) and/or valve replacement with either normal or impaired left ventricular (LV) systolic function.

Methods/Design

Study Design and Setting

This is a single-centre prospective proof-of-concept study conducted between University Hospitals Coventry & Warwickshire (UHCW) and Coventry University. UHCW is a tertiary cardiac centre with a regular volume of elective and urgent cardiac surgeries, namely coronary artery bypass grafting (CABG) and aortic or mitral valve surgery. Four cardiac surgeons have been enlisted to help obtain research tissue samples in the form of left ventricular biopsies and atrial appendages. Informed written consent will be received from patients before the day of their surgery. All procedures will be performed in accordance with the Declaration of Helsinki. The isolation of cardiomyocytes and subsequent experiments on these will be performed in the laboratory at Coventry University.

Eligibility Criteria

Given the nature of this proof-of-concept study and finite availability of myocardial tissue specimens, the inclusion criterion is intentionally kept broad and exclusion criteria to a minimum. Hence, patients aged 18 years or above, who are able to provide informed consent and listed for open-heart, on-pump cardiac surgery at UHCW will be recruited. This includes patients with significant coronary artery disease, aortic or mitral valve pathology (stenotic or incompetent valves) with or without evidence of LV systolic dysfunction. This will provide a wider insight into the hypothesis of

MF by assessing cardiomyocytes from various disease states (e.g. ischaemic heart disease with impaired versus normal LV systolic function, or aortic stenosis with and without LV hypertrophy). Patients will be excluded if they lack mental capacity to consent. For the safety of researchers, patients with active blood-borne viral infections, e.g. human immunodeficiency virus or COVID-19 within 10 days of testing positive will also be excluded. UHCW tissue bank has a licence to procure donor hearts for research if deemed non-transplantable. Hence, once consent from a legal representative has been confirmed by the local NHS organ donation team, donor hearts can also be recruited as a possible control group, if available.

Study Protocol

Baseline Data Collection

All patients will have a pre-operative electrocardiogram, transthoracic echocardiogram and for patients undergoing CABG, a coronary angiogram. From these investigations, the following will be recorded in addition to patient demographics: degree of coronary artery disease, sinus rhythm versus atrial fibrillation, LV wall thickness, atrial size, LV ejection fraction (LVEF), presence of regional wall motion abnormalities and any significant valvular heart disease. Baseline blood biochemistry including renal function, troponin in patients presenting with acute coronary syndrome and B-type natriuretic peptide in those with underlying heart failure, cardiovascular drugs and intra-operative use of any vasopressors or inotropic drugs will also be documented. This clinical data will serve three purposes. Firstly, it can help optimise the cell isolation procedure by tailoring the concentration and duration of enzyme digestion to individual patient factors, e.g. age, comorbidities and signs of cardiac remodelling (e.g. ventricular hypertrophy). Secondly, it allows for a comparison of force-frequency relationship and contractile parameters (described below in the study outcome section) between patients with preserved and reduced LVEF during isometric and work-loop contractions. Finally, if myocyte fatigue is demonstrated, the rate at which fatigue develops and degree of recovery can be correlated with the above cardiac biomarkers (e.g. BNP, troponin) to provide insight into the susceptibility and reversibility of myocardial fatigue.

Collecting Myocardial Tissue

Preliminary experiments will be performed on atrial appendages first to optimise the isolation protocol before being applied to LV biopsy tissues because of its greater tissue availability to enable more optimisation experiments to be carried out from a single atrial sample. Structural

and functional differences in atrial and ventricular myocytes can also be compared. All tissue sampling will be performed by the cardiac surgeon responsible for the surgery. After placing a purse-string suture for cannulation of the right atrium, the tip of the atrial appendage will be excised. This tip is routinely tied off after cannulation; hence, no additional risk is expected beyond that inherent to the surgery. For LV tissues, up to 3 biopsies will be taken using a 14G Tru-cut™-biopsy needle, avoiding any areas of infarction, fibrosis or significant regional wall motion abnormalities on visual inspection. The maximum size of each biopsy is limited to the 1.6 mm internal diameter of the needle with a 2-mm-long specimen notch. This estimates a maximum tissue volume of 40 mm^3 which converts to approximately 450 mg per biopsy, assuming 1.112 g/cm^3 density for fresh human tissue. Biopsies will be performed after aortic cross-clamping and cardioplegic arrest, which is usually induced by the cardioplegic solution ULTH Formula A (Huddersfield Pharmacy Special, UK). Immediately after resection, the specimen will be placed in a transport buffer at 4 °C, and pseudo-anonymised by the UHCW tissue bank before transport to

the laboratory within 30 ± 5 min. A flow-chart summary of the sequence of events is illustrated in Fig. 1.

Isolation of Cardiomyocytes

On arrival to the laboratory, the tissue sample will be immediately processed by removing any fibrous and adipose tissue and cut into approximately 1 mm^3 pieces. To isolate cardiomyocytes, a chunk digestion using proteolytic enzymes will be performed as previously described [38, 39]. Several digestion cycles at different concentrations will be assayed to attain an optimal cell yield. This process will be repeated until the tissue is fully digested. Cell viability will be determined morphologically by the following criteria: (1) intact rod-shaped appearance with clear sarcomeric striations, (2) absence of membrane blebs and spontaneous contractions and (3) intact ends of the myocyte (i.e. intercalated discs). The last criterion is unique to this study. As the intended outcome is to perform consistent work-loop contractions on suspended myocytes, cell end integrity is vital for stable cell attachment. From preliminary rat myocyte experiments, we have observed that myocytes with intact striations but

Fig. 1 Flow-chart summary of methodology
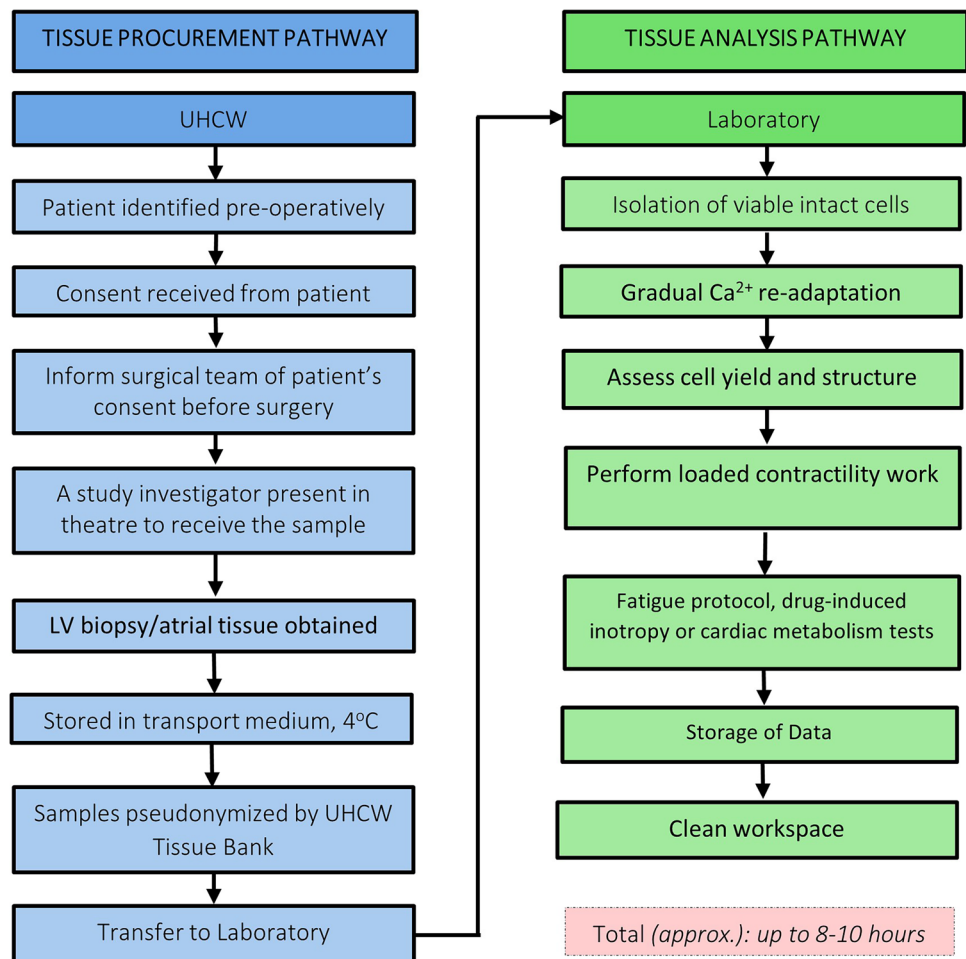
damaged ends fail to attach firmly to the MyoTak™ biological adhesive and display a tendency to become electrically and mechanically unstable during work-loop experiments. To optimise viability and yield, different chemical compositions of the transport and digestion solutions will be examined [45]. Our goal is to attain a minimum of 20% yield of viable myocytes before concluding the optimisation process.

Experimental Setup

A single calcium-tolerant myocyte will be mounted on an in-house built contractility rig inside a horizontal bath with an inverted microscope and platinum electrodes for electrical field stimulation (Fig. 2). Before attaching the cell, the unloaded myocyte should display stable contractions that are synchronous to cycle frequencies of 1–2 Hz (equivalent to 60–120 beats per minute) and have a resting sarcomere length (SL) > 1.7 µm. Below this threshold of resting SL is considered hypercontracted [46]. SL and cell length are measured optically at a sampling frequency of 240 Hz in accordance with the IonOptix manufacturer's manual. As described within this manual, a box is placed across a series of sarcomeres where a fast Fourier transformation (Ion-Wizard, IonOptix) is calculated to find the average power spectrum to estimate the average SL (Fig. 3). Once both conditions are met, the myocyte will be suspended by two microscopic glass needles using a biocompatible cellular adhesive, MyoTak™ (IonOptix, Milton, MA, USA). Each end is attached to either a high-speed length controller or highly sensitive cantilever force transducer, described in detail elsewhere [34, 47]. By attaching the MyoTak-coated rods to each end of the myocyte, the cell can then be lifted off the glass coverslip, thereby eliminating surface friction and allowing precise adjustments of preload and afterload. This provides a more three-dimensional approach, compared to traditional methods where contracting cells are attached to a surface area. At resting SL, the myocyte will be stimulated to undergo isometric contractions until a steady beat-to-beat state is reached. Any myocytes displaying > 20% variation in developed force during the stabilization period reflects cell instability or glue slippage and thus will be excluded. The suspended myocyte will be immersed in a modified Krebs-Henseleit solution (containing physiological levels of glucose and calcium chloride) oxygenated at 37 ± 0.1 °C. While fatty acid metabolism generates more ATP than glucose, it typically requires the addition of bovine serum albumin (BSA) to enhance solubility and stability. However, adding BSA can lead to air bubbles in oxygenated solutions, interfering with laser interferometry measurement of sarcomeric length. Instead, the superfusate will be drained and replaced regularly throughout the experiment.

Force-Length and Frequency Relation

With force production stabilised, a stepwise stretch will be performed under isometric conditions to elicit a force-length relationship from physiological low to high preload levels (i.e. SL 1.8 to 2.1 µm) [35]. Unlike the manipulable properties of myocardial slices, stretching a single myocyte beyond 2.1–2.2 µm to mimic a volume-overloaded state may result in glue slippage and cellular damage, particularly at higher pacing frequencies [34, 47]. Therefore, the ceiling of SL will be kept at around 2.1 µm. A random order of cycle frequencies (e.g. 1 Hz, 1.5 Hz, 2 Hz) can be applied to assess the
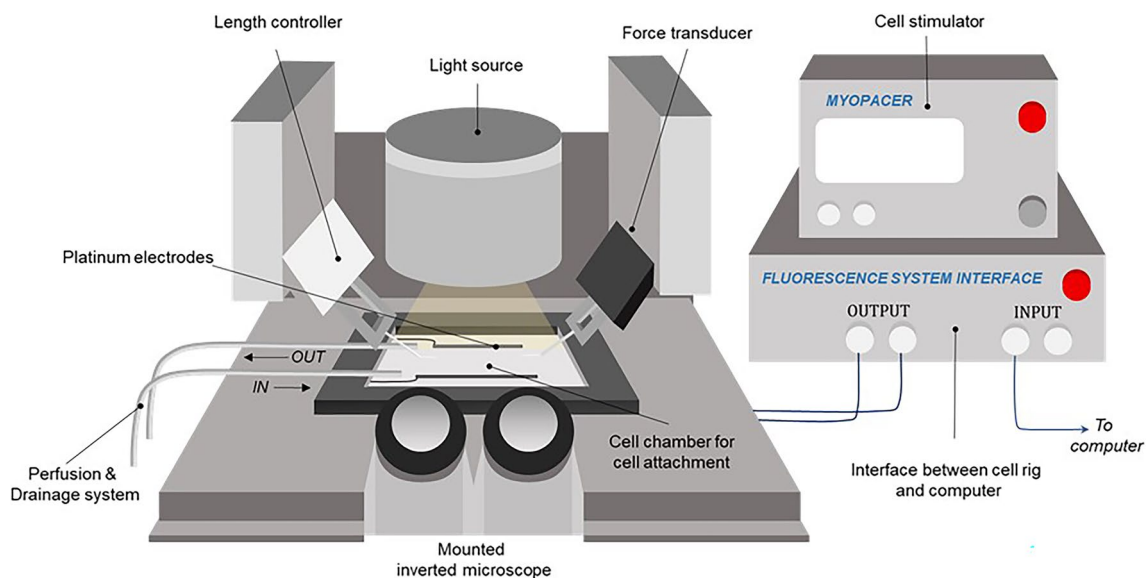

Fig. 2 Clipart of the in-house contractility rig. Illustrated by author, Patrick Tran
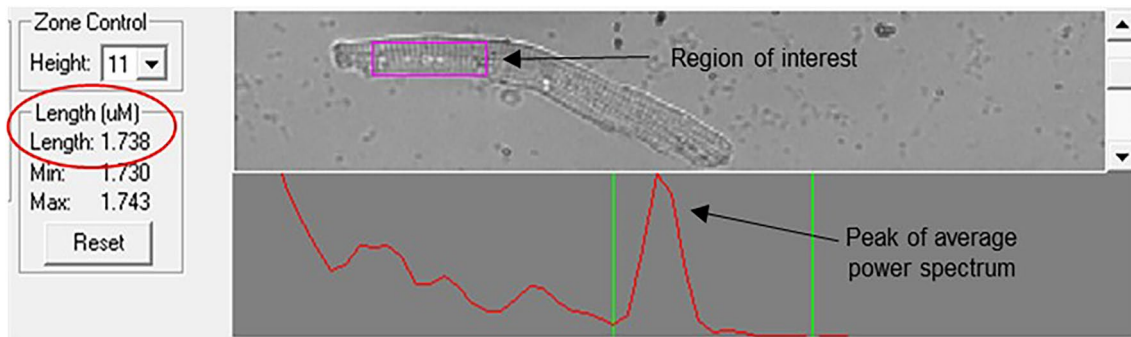
Fig. 3 Magenta box represents the region of interest from which an average peak power spectrum is calculated to estimate an average sarcomere length. Here, it is 1.738 μm as highlighted by red circular line. Undesirable frequencies are excluded by user-defined upper and lower bound boundaries (green lines)

force-frequency relationship at this optimal SL. The subsequent experiments described below is summarised in Fig. 4.

Work-Loop Stabilisation Protocol

Following a further stabilisation period, a series of work-loop contractions will be performed from low to high afterload, which is set as a percentage of the average developed isometric force. The feedback-control system to generate a mechanical loop that mimics the phasic behaviour of a pressure-volume loop in vivo is described in detail elsewhere [16, 33, 34, 47] and illustrated in Fig. 4 based on work-loops experiments with rat cardiomyocytes. Briefly, once a myocyte reaches a predefined afterload force under isometric contraction (phase 1), the controllers transition to an isotonic shortening mode and uses a feedback-control mechanism to maintain the prescribed force (phase 2). When the cell is no longer able to sustain this force, isometric relaxation occurs (phase 3), after which the cell re-lengthens to maintain its original preload value (phase 4) until the next electrical stimulus. In vitro, preload refers to the baseline tension exerted by the stretch at diastole, while afterload is the force opposing myocyte shortening.

Fatigue Protocol

Important parameters of cardiac performance including power output, end-systolic force-length relation (ESFLR), contraction/relaxation amplitude and kinetics (explained in 'Study Outcome') will be elicited from these experiments. From this data, a control work-loop and fatigue-inducing work-loop will be determined. A control work-loop at 1 Hz cycle frequency will allow monitoring of the cell's contractile function before and after the fatigue protocol. In line with our MF hypothesis, the fatigue work-loop should be similar to an in vivo pressure-volume loop at high afterload, e.g. hypertension and aortic stenosis where

the area of the loop becomes narrower (i.e. reduced stroke work) and shifts rightwards on the end-diastolic pressure-volume curve. A number of repetitive fatigue work-loops will be subjected to the myocyte; the number of cycles and level of cycle frequency (e.g. 1.0–3 Hz) that ensures stable work-loops and without causing structural damage to the cell will be determined from preliminary experiments. Changes in contractile parameters (e.g. normalised power output) will be plotted as a percentage decline relative to the peak levels from the outset of the fatigue run. Following the fatigue protocol, the afterload and cycle frequency will be set back to the control level and subsequent recovery will be examined at regular intervals (e.g. every 10–15 min). Recovery of contractile performance will be expressed as a percentage relative to the pre-fatigue control work-loop. The work-loop stabilisation protocol, where a low to high afterload ramp is performed, can be repeated to assess for any changes in ESFLR compared with the initial set of stabilisation work-loops.

Anticipated Duration and Cells Analysed per Sample

The duration of a successful experiment is estimated to be approximately 120 min. In reference to Fig. 4, the stabilisation period (step 1) will last for 10 min for myocyte acclimatisation. This will be followed by a gradual increase in sarcomeric length to 2.1 μm over 10 min, which will include increasing stimulation frequency (step 2). Step 3 will last up to 10 min. Step 4, the fatigue protocol, should continue for at least 30 min or until a reduction in force is observed, whichever is longer. The recovery phase will be assessed over 60 min. Due to the lengthy isolation procedure and the need to recoat the rods and change MyoTak™ after each experiment, it is anticipated that a maximum of two cells per sample will be analysed.
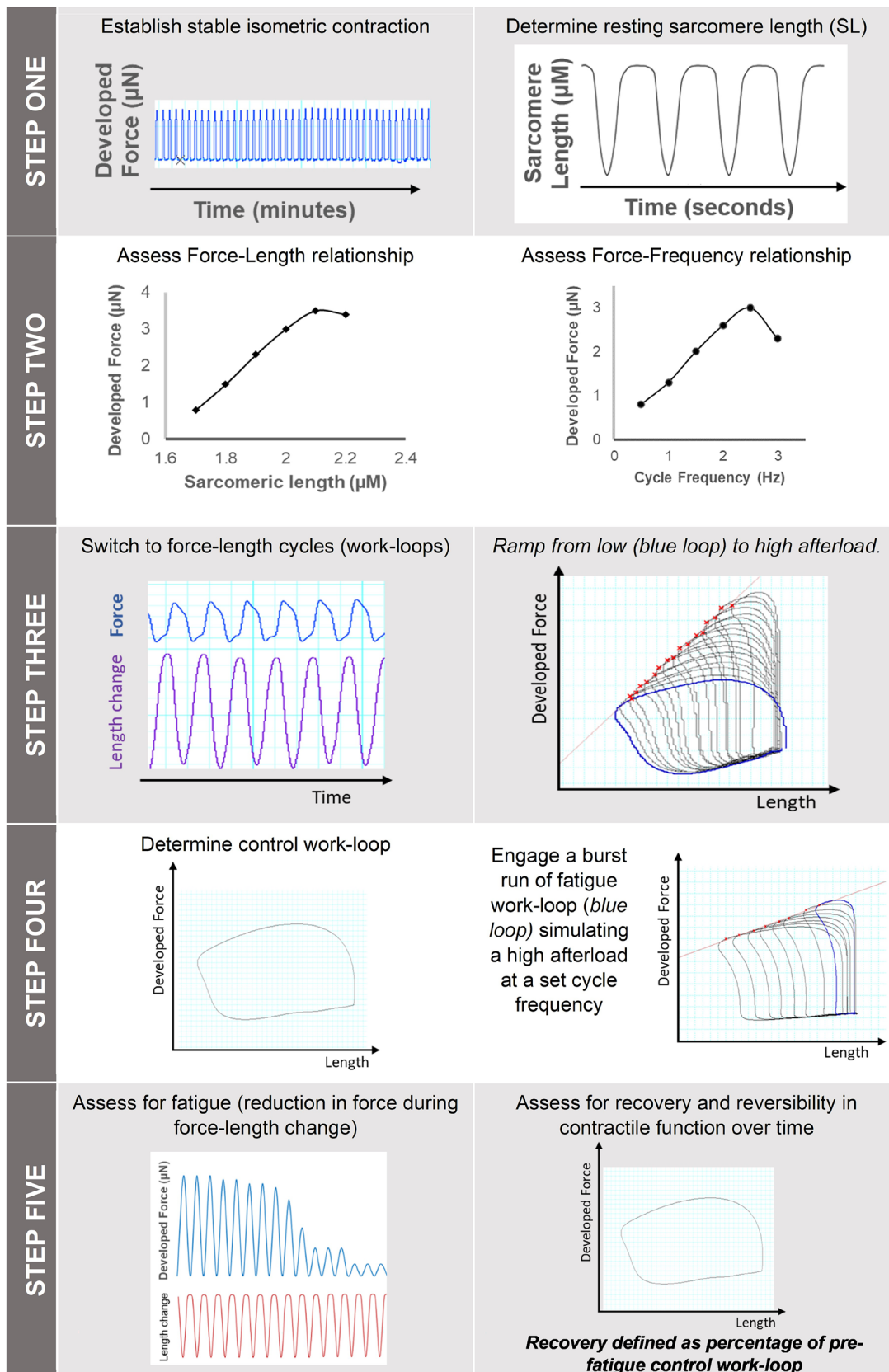
STEP ONE

Establish stable isometric contraction

Determine resting sarcomere length (SL)

STEP TWO

Assess Force-Length relationship

Assess Force-Frequency relationship

STEP THREE

Switch to force-length cycles (work-loops)

Ramp from low (blue loop) to high afterload.

STEP FOUR

Determine control work-loop

Engage a burst run of fatigue work-loop (*blue loop)* simulating a high afterload at a set cycle frequency

STEP FIVE

Assess for fatigue (reduction in force during force-length change)

Assess for recovery and reversibility in contractile function over time

Recovery defined as percentage of pre-fatigue control work-loop

Fig. 4 Summary of experiments with human cardiomyocyte

Responses to Positive Inotropic Drugs

To investigate the dose and time-dependent responses on contractile performance of fatigued myocytes, a secondary experiment will be conducted in which positive inotropic drugs will be added to the bathing solution after the fatigue protocol. The experiment aims to include at least five different human biopsy samples per inotropic drug. This additional experiment will only be performed once we have achieved the minimum number of samples required for the primary aim of the study set out in Fig. 4. These additional myocytes will not undergo the force-length and force-frequency experiments. Commonly tested drugs include dobutamine (β-1 adrenoreceptor agonist), noradrenaline (mixed α1-β1 receptor agonist), milrinone (phosphodiesterase-3 inhibitor), omecamtiv mecarbil (selective myosin activator) and levosimendan (troponin-C calcium sensitizer). This approach will be adapted and modified from previous in-house studies on work-loop assays to assess for drug-induced inotropic effects, which have been extensively tested using rat myocytes [43, 44, 48]. Our study seeks to expand on these findings by evaluating their effects in human myocytes as a secondary outcome.

Study Outcomes

By investigating the hypothesis of MF using a combination of isometric and work-loop models, a multiparametric mechanical profile of the human cardiomyocytes can be assessed in response to workload. From this, the primary outcome is the evaluation of changes in contractile function of myocytes before and after the fatigue protocol, while the secondary outcome is the assessment of changes in contractile function after the introduction of positive inotrope drugs. Other outcomes include the force-length relationship and force-frequency relationship under isometric conditions.

Measures of Fatigue

To yield myocyte stress ($\mu N.\mu m^{-2}$), the developed force (i.e. difference between active and passive force) will be normalised to the cross-sectional area of the cell based on the assumption that cell width-to-depth ratio is approximately 3:1 during slack length [47]. Using developed force as the only fatigue variable will not capture the multifaceted behaviour of muscle fatigue. Hence, similar to assessing skeletal muscle fatigue [24, 40], fatigue analysis will also include changes in sarcomeric shortening (μm), velocities of shortening (V_{max}), time to peak force (TTP) and time taken to 50% relaxation (RT50) as illustrated in Fig. 5.

Work-loops provide more comprehensive measures of cardiac contractility that interrelate with whole heart pathophysiology. Firstly, the contractile work output can be calculated by integrating the area enclosed by the force-length cycle (Fig. 5). This refers to the product of applied force on the sarcomere and length the sarcomere moves, similar to in vivo stroke work which refers to the distance of blood ejected as a product of stroke volume and mean arterial pressure. Secondly, the work per unit time represents the ventricular power output. At a cellular level, power output equates to work multiplied by the pacing frequency. Thirdly, by performing a series of work-loops over a range of afterload or preload, the end-systolic elastance can be derived (i.e. change of force per unit change in length). This is expressed by the ESFLR slope fitted by linear regression of the end-systolic points (right top corner of loop) and is widely accepted as a fairly load-independent quantification of contractility [16, 49]. That is, within a physiological range, ESFLR will be similar at a state of high or low preload/afterload. A higher contractility will shift the ESFLR up and leftwards (Fig. 5). End-diastolic force-length relation (EDFLR) is an index of stiffness.

Study Power

In the absence of previous data on fatigue experiments with cardiomyocytes, a priori power calculation was not possible. Studies on skeletal muscle fatigue have largely reported a moderate effect size in the reduction of contractile parameters at various time-points during fatigue [24, 40]. We thus opted for a conservative moderate effect size of 0.25 to be considered as clinically significant using Cohen's F effect size for repeated measures ANOVA (small $d = 0.1$, medium $d = 0.25$, large $d = 0.4$) [50]. Assuming an α-probability $= 0.05$ and power $= 0.80$ (beta $= 0.20$), a power analysis on G*Power 3.1.9 for ANOVA repeated measures over 2 measurements within factors of a single group estimates a required sample size of $n = 34$. We acknowledge that power will be lower when testing smaller interaction effects, and if required, the power analysis can be refined based on data from initial feasibility experiments.

Statistical Analysis

Data will be presented as mean ± standard error in tables and figures, unless otherwise indicated. Cardiomyocytes isolated from one patient may vary slightly in structure and function but are more closely related to each other than myocytes from another patient. Hence, it cannot be assumed that all data points of myocytes within one cluster are independent of each other [51]. To assess the similarity of clustered data, an intraclass correlation coefficient (ICC) test will be performed: a high $ICC > 0.75$ suggests that data of myocytes from a single biopsy should be treated as a single homogeneous cluster, while a low $ICC < 0.40$ suggest that myocytes within the same isolation are independent. For random effects of multiple myocyte measurements from each biopsy with a high clustering effect, a repeated measures mixed effects

model will be used to assess outcome contractile metrics (e.g. work-output) over time. Cell number will be treated as a random factor, whilst SL, mechanical load and time will be treated as fixed effects to assess the myocyte's ability to recover its contractile function. Models will include random slopes and intercepts to account for individualised differences in baseline force production and subsequent trajectories. Within-subject factors will also apply to the cells' response to different positive inotropic drugs at different timepoints. Post hoc comparisons will be performed with the Tukey test. Analysis will be conducted on commercial software (Prism 7, GraphPad, La Jolla, CA; SPSS v28, IBM, IL, USA).

Patient and Public Involvement

Patients and the public were involved in the design, conduct of the consent process, and reviewed the participant information sheet and consent form to ensure clarity and candour. They were also involved in the discussion of dissemination plans of this research.

Discussion

The suitability of tissue preparation depends on the study question. To test the hypothesis of MF, cardiomyocytes, the fundamental contractile unit of the heart, would be an appropriate research platform because we can be certain that an observed change in cardiac performance in response to an intervention is directly related to the cardiomyocyte and not from external influences. In contrast, although myocardial slices have greater viscoelasticity to withstand prolonged work-loop contractions, experimental noise from non-contractile cells and fibrosis can obscure the fatigue signal from myocytes. Nevertheless, the high internal validity of using single myocytes comes at a practical cost. The loss of extracellular matrix and viscoelasticity after enzymatic digestion can predispose the cell to irreversible damage from large force-length changes, as seen in our preliminary studies with rat myocytes. It follows that selecting myocytes that can withstand these experiments based on structural integrity and functional stability will introduce selection bias and exclude cell
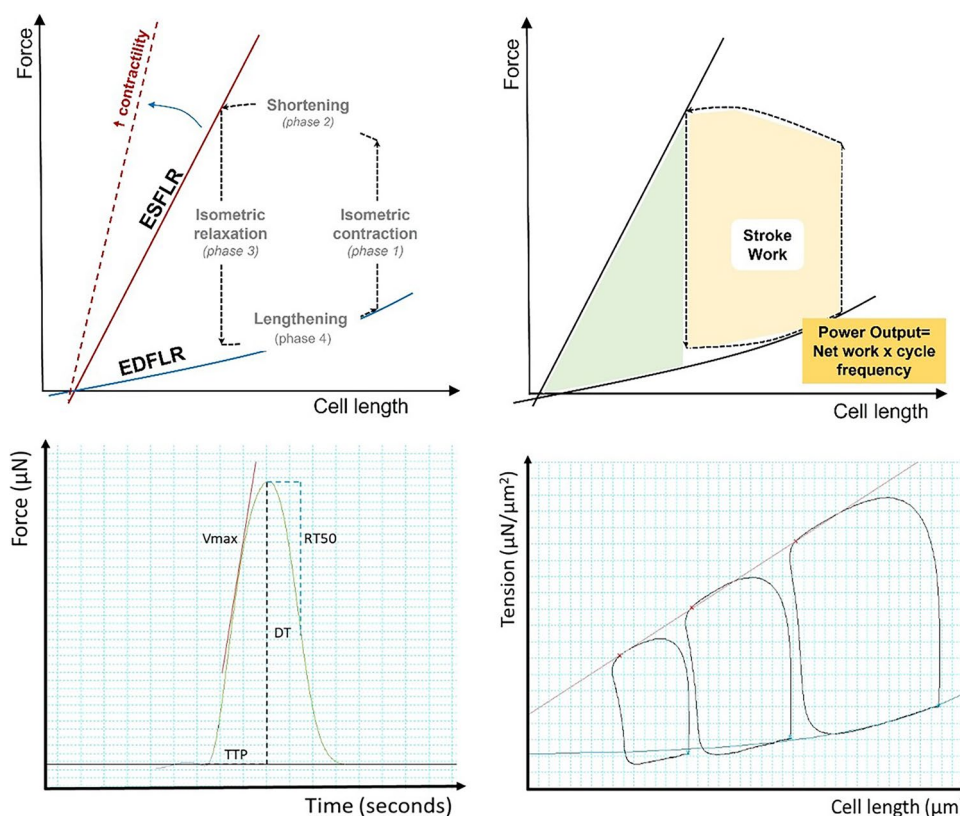

Fig. 5 (Top) Example of a work-loop cycle where the three-dimensional parameters of an in vivo pressure-volume loop have been substituted with force and length in vitro, circumscribed by the end-systolic (ESFLR) and end-diastolic force-length relation (EDFLR) and rotates anti-clockwise through four distinct phases that mimic the cardiac cycle. Area within the loop represents stroke work from shortening minus work from re-lengthening, and power output refers to the work over a number of cycles per second. (Bottom) Representative isometric twitch of a rat cardiomyocyte characterised by maximum upstroke velocity (Vmax), time from stimulus to peak force (TTP), time from peak force to 50% relaxation (RT50) and developed tension (peak force–passive force). From the same myocyte, three work-loop cycles are imposed at increasing cell lengths and circumscribed by ESFLR and EDFLR curves

populations with reduced force-generating capacity. A strength of using tissue biopsies from surgical patients is the ability to better capture non-failing to failing hearts at all stages of the HF spectrum, unlike the use of tissue from whole hearts which are usually limited to transplant recipients with terminal HF. Eliciting MF in myocytes that have deteriorated to end-stage HF with diminished contractile reserve is unlikely to be plausible to answer our study question. Finally, the major challenge with tissue availability has been compounded by the ongoing and enduring effects of the coronavirus pandemic. Cancellations and last-minute changes to the theatre list will hinder patient recruitment; hence, maintaining consistent communication with the surgical team is key. This proof-of-concept study will require subsequent validation in large tissue preparations.

Future Research Directions

Correlating contractile performance data with myocardial energetics is a critical component of investigating the hypothesis of myocardial fatigue. However, the limitation of using a single-cell assay precludes paired energetic experiments, e.g. single-cell RNA sequencing, as subsequent detachment of the myocyte from the MyoTak-coated glass rods inevitably leads to cell death. This proof-of-concept study adheres to the ethical approval of using small biopsy samples. To establish a foundation for future experiment using larger tissue samples where myocardial living slices can be produced, we plan to first demonstrate the mechanical aspect of myocardial fatigue. As stem cell-derived multicellular preparations may not represent adult failing hearts, obtaining tissue samples from patients undergoing ventricular assist device implantation or explanted hearts may be suitable for this purpose. To explore the hypothesis of mitochondrial decoupling in myocardial fatigue, live cell metabolic analysis can be performed on the tissue slice after the fatigue protocol. Our institution has experience using the Seahorse XFe96 analyser to evaluate mitochondrial function, including basal oxygen consumption rate, proton leak and ATP-coupled respiration [52].

Declarations

Human Subjects/Informed Consent Statement Health Research Authority and Health and Care Research Wales (HCRW) (REC reference: 21/WA/0138) provided ethical approval. Signed informed consent will be received from all participants before obtaining cardiac tissue samples. The study will be conducted in accordance with Good Clinical Practice, Declaration of Helsinki and Data Protection regulations.

Competing Interests The authors declare no competing interests.

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