Mechanical Considerations of Myocardial Tissue and Cardiac Regeneration

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1 Current Regenerative Strategies Fail to Restore the Myocardial Mechanical Environment

According to the World Health Organization, one-third of worldwide deaths are caused by cardiovascular diseases, with an increasing trend in the recent years [1]. Specifically, heart failure (HF) following myocardial infarction (MI) is the most fatal cardiovascular disease with a 5-year survival rate of <50% [2]. At the tissue level, HF as a result of MI is characterised by a gradual loss of cardiomyocytes and, therefore, contractile tissue. The biological healing cascades following injury trigger the fibroblasts to change their phenotype and increase extracellular matrix (ECM) production, ultimately leading to the formation of hypo-contractile tissue scar [3]. At the same time, the remaining cardiomyocytes try to compensate for the loss of contractile tissue, initiating pathological cellular responses and further cardiac remodelling. Ultimately, tissue remodelling leads to an increased myocardial wall thickening, followed by dilation and eventually diminished cardiac function [4].

Nowadays, there is no doubt that cells actively respond to their mechanical environment and this interaction is essential in load-bearing and continuously contracting tissues such as the myocardium [5]. For instance, cellular contractility and electrical stability are highly dependent on mechanical environmental cues such as stiffness or cyclic strain [6–9]; and a more physiological, 'healthy' environment – or niche – will elicit beneficial cell behaviour in contrast to the environment present after injury. Still, this insight has been largely ignored in the design of strategies to

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repair or regenerate the myocardial tissue. For instance, cell-based therapies aim to restore tissue contractility by injecting new (contractile) cells in the injured area [10]. Despite promising results in in vitro settings, long-term preclinical and clinical studies have shown that these strategies fail to regenerate myocardial tissue and overall cardiac function [11–14]. One of the main reasons is that these studies mainly focus on cell phenotype and function before implantation, disregarding the effects of the highly injured ECM niche with altered mechanical properties that will be found upon transplantation. In an attempt to induce cell adaptation to the diseased environment prior to implantation, some studies exposed cells in vitro to mechanical or biological environmental cues before in vivo injection [15–17]. However, the toxic environment still caused low cell retention, high cell death and low mechanical stability upon injection in the diseased area.

Hence, it is difficult for the cells alone to overcome the highly destabilised mechanical tissue present after cardiac injury. Several studies have shown that cells upon implantation start synthesising and remodelling their own (healthy) ECM [18, 19]. This principle is copied by scientists in the context of in vitro cardiac tissue engineering (cTE) [20]. In vitro cTE seeks to create living myocardial tissue by combining cardiac cells with a temporary 'healthy' niche prior to transplantation, either as a patch or as an injectable gel replacing the damaged area [21]. Multiple biomaterials, from either natural or synthetic sources, have been developed in the form of hydrogels or scaffolds to provide for such niches [22–26]. The use of natural source biomaterials, such as collagen or other ECM components, seems attractive, as they can offer some of the biochemical cues present in the native tissue. Decellularised cardiac tissue – e.g. from animals – can provide scaffolds with appropriate mechanical, topological and biochemical properties [27–29]. On the other hand, synthetic polymers can provide scaffolds with better tuneable mechanical properties compared to the natural processed ECM but offer poor biochemical signals with fewer cell recognition motifs if applied in pristine form. Bioactive modification of such materials is pursued for the design of life-like materials that can mimic both mechanical and biochemical environmental cues [30–33]. Overall, these approaches fulfil the aim to provide new cardiac cells with a relevant micromechanical environment.

cTE constructs, based either on natural or on synthetic biomaterials, are usually implanted on the epicardium near the infarcted region of the myocardium and serve to constrain and mechanically reinforce the ventricular wall in order to prevent or halt pathological tissue remodelling [34]. However, mechanical consequences of patch transplantation or hydrogel injection are still largely overlooked. Proper mechanical integration of the delivered cTE constructs across length scales from local cell-cell and cell-ECM interactions to global tissue contraction is necessary for the success of these strategies. Mechanical mismatch will cause complications, including heart failure and diastolic dysfunction.

More recently, in situ cTE has emerged as a promising alternative to traditional in vitro cTE to achieve tissue regeneration directly at the functional site. In situ cTE is built on the notion that the injection of a biomimetic acellular scaffold into the injured myocardium stimulates endogenous repair processes [35]. However, in situ

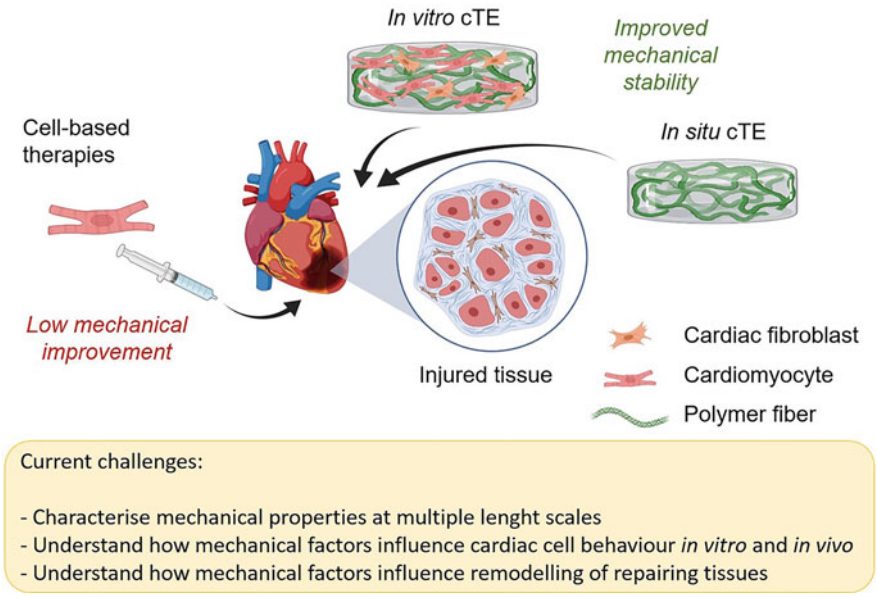

Fig. 1 Cell-based therapies cannot restore the mechanical properties of the injured myocardium. In vitro and in situ tissue engineering strategies using hydrogels and scaffolds reinforce injured myocardium with moderate success. The main challenges to improve the mechanical stability of current cardiac regenerative strategies are highlighted. Partly created with BioRender.com

cTE approaches are still in their infancy, and the governing processes of regeneration are still largely unknown. These processes range from the activated immune response in response to the biomaterial inside the body to the mechanical factors governing de novo ECM formation and organisation. In situ *c*TE has major benefits over in vitro cTE in terms of costs, availability and regulatory complexity, as it uses an acellular scaffold to harness the regenerative potential of the native tissue. In addition, in situ *c*TE does not need to account for the mechanical integration of exogenous cells in the damaged tissue. Nevertheless, the mechanical and electrical coupling and stabilisation at the cellular, tissue and organ level upon biomaterial delivery need to be addressed.

In summary, and with an eye to clinical translation, the design of successful cardiac regenerative strategies should address several aspects regarding the role of biomechanics in myocardial repair or regeneration (Fig. 1). These include questions such as: What are the mechanical properties of native and diseased tissues at the various length scales of the myocardium that will affect therapy outcomes? How will these properties influence cell and tissue behaviour in vitro and upon transplantation in vivo? How do biomechanical factors influence the remodelling of the regenerating myocardium in damaged and remote areas? In this chapter, we first describe the complex cardiac mechanical environment across length scales from macro to micro level and the techniques used for characterising mechanical behaviour at these length scales. Next, we review in vitro and in silico cardiac models to understand the impact

of the cardiac mechanical environment on cellular behaviour and tissue regeneration. Finally, we provide an outlook on the requisites to design the next-generation engineering strategies for cardiac regeneration.

2 Understanding the Multiscale Biomechanical Properties of the Myocardium

Cardiac tissue, and especially the myocardium, is a complex tissue with multiple interconnected length scales (Fig. 2a–c) organised into a highly structural and functional hierarchy, ranging from whole heart biomechanics to the functional unit of contraction (the sarcomere) inside the cardiomyocytes [40].

At the macroscale, in particular in the left ventricular (LV) myocardial wall, tissue fibres form a right-handed helical structure close to the inner part of the wall (the endocardium), left-handed towards the outer part of the wall (the epicardium) and a circumferential structure in between [41, 42] (Fig. 2a). The changes in fibre orientation are smooth throughout the LV wall. During systole, due to this helical organisation, the myocardium rotates relative to the base to ensure complete blood ejection from the left ventricle [43]. Nonetheless, this description of LV wall organisation is a simplification because it omits other anatomical structures, including the extensive vascular network across the whole myocardium [44]. At the mesoscale, each of the ventricular fibres consists of an anisotropic array of cardiac cells (cardiomyocytes and fibroblasts) in parallel alignment with the ECM fibres (mainly collagen fibres) to ensure the coordinated contraction of the LV (Fig. 2b). At the microscale, cardiac cells, specifically the cardiomyocytes, form aligned, interconnected bundles that attach to ECM fibres to transduce the cellular contractile forces throughout all myocardium and generate coordinated contraction (Fig. 2c) [36].

The mechanical properties of the myocardium differ across length scales. However, basic mechanical concepts can be identified independently of these scales. These include the deformation and forces present in the tissue (strain, ε, and stress, σ), the elastic stiffness (Young's modulus, E) and the complex G* modulus accounting for the viscoelastic properties (Table 1). Although they express the same physical concept, the interpretation of the values has to be correlated with tissue physiology and structure at each of the scales addressed.

In clinical practice, there are already established techniques to measure LV macroscopic strain based on ultrasound (echocardiography) and magnetic resonance imaging (MRI) [45–47]. One of the advantages of these techniques is that they are non-invasive, allowing the assessment of cardiac mechanical properties in patients. Echocardiography imaging is established as a gold standard technique, and it is based on analysing the LV wall motion by tracking speckles (natural acoustic markers) in the ultrasonic image. These acoustic markers appear physiologically in the myocardium and can be tracked frame to frame. By post-processing software, the

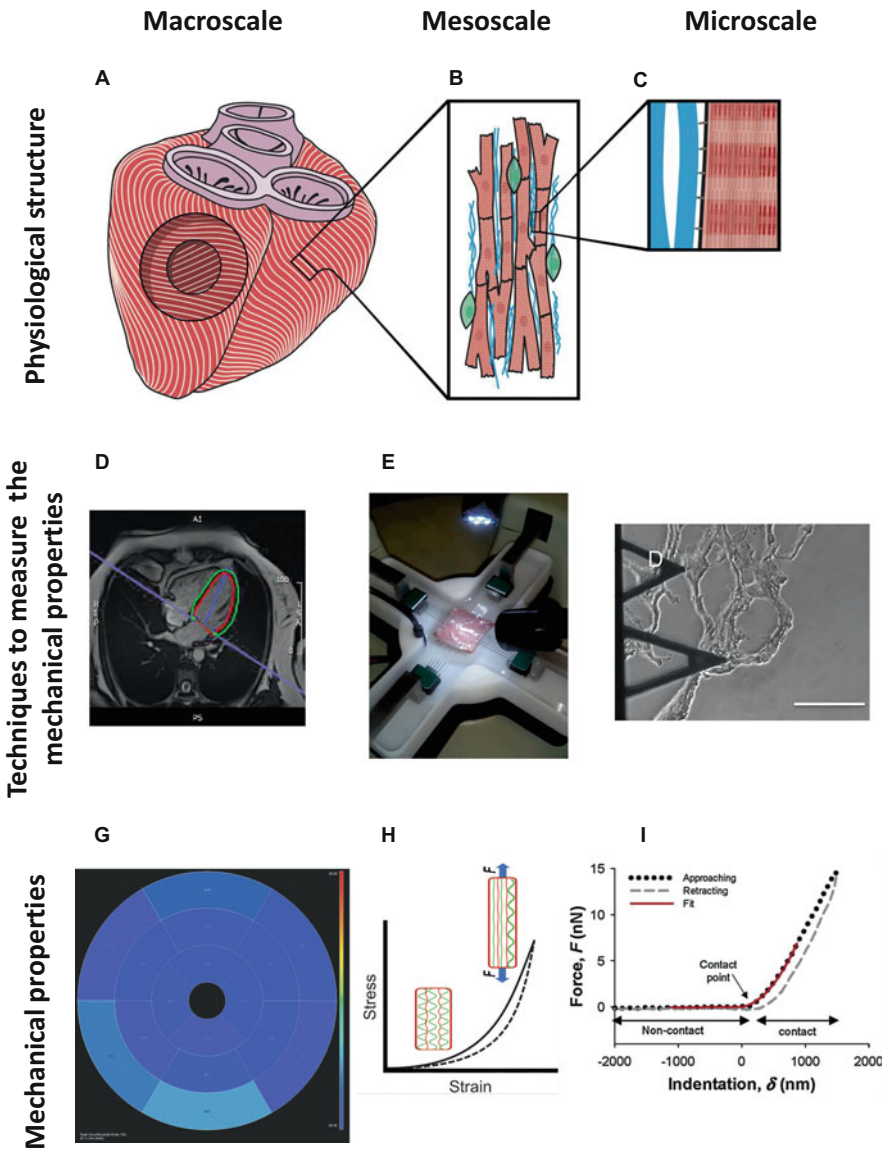

Fig. 2 Physiological structure, techniques to measure mechanical properties of cardiac tissue and mechanical properties at the three relevant scales. (**a**) Differential layer organisation of muscle fibres in the myocardium. (**b**) Cardiac cells (cardiomyocytes, red; fibroblasts, green) are anisotropically oriented along ECM fibres (blue). (**c**) Cardiomyocytes transmit the contraction force to the ECM by cell-ECM attachments (grey). (**d**) MR image of a human thorax. Red and green lines delineate the inner and outer parts of the myocardial wall necessary to compute myocardial strain. (**e**) Biaxial tensile tester setup. A porcine myocardial tissue strip is attached to four lever arms. (**f**) Microscopy phase-contrast images of 12 μm thick decellularised LV mouse heart probed with AFM. (**g**) Circumferential strain bull eye computed by MRI. Values are negative, depicting the contraction of the tissue. Regions closer to the centre correspond to myocardial regions near to the apex of the heart. (**h**) Representative strain-stress (σ-ε) curve measured by tensile testing. Non-linear visco-elastic behaviour is a characteristic of the passive mechanical properties of cardiac tissue. The

geometric shift of each speckle can be calculated to assess tissue movement and, as such, tissue strain can be computed [48]. Similarly, MRI techniques are based on tracking features in the image over time to compute the displacement of the myocardium and, therefore, the strain present in the tissue (Fig. 2d, g) [49]. The information that can be extracted from these 4D images are the longitudinal, circumferential and radial strains. Usually, the strain values are represented by negative values as the reference deformation is set at the end of the diastole [50]. The strain present in the in vivo myocardium accounts for the tissue distensibility and gives an estimation of the overall function. After MI, strain at the affected area diminishes compared to the healthy area [51]. However, detailed knowledge of myocardial wall *stress*, particularly in humans, remains elusive. This lack of knowledge is primarily because forces or stresses cannot be directly measured in the intact myocardial wall [52]. To this end, several indirect methods, including analytical and finite-element modelling, have been used to estimate the stress present in the tissue [53–56]. Based on that, stress-strain relationships can be built, and the E value calculated.

Besides the active tissue contraction present in vivo, the passive mechanical properties of the myocardium significantly contribute to overall cardiac function [57, 58]. Throughout the literature, the E for soft biological tissues has physiological values in the range of 1–100 kPa [5]. The E gives an estimation of the elastic mechanical properties of the tissue. However, just an E value is not enough to fully understand the mechanical behaviour of soft biological tissues. Additionally, it is necessary to mention at which scale and with which technique the value has been measured as this includes the information of physiological structures that are being measured.

At the mesoscale, the mechanical properties of myocardial tissue are characterised from small ex vivo tissue strips of a few millimetres, subjected to uniaxial, biaxial or even triaxial tensile tests (Fig. 2e). The strips are attached to a lever arm controlled by an electromechanical or hydraulic servo-controlled displacement actuator that deforms the strip while it measures the force applied. Therefore, the σ-ε curve can be computed by calculating the relative deformation of the strip and the force applied per unit cross-sectional strip area (Table 1; Fig. 2h). This process is done to normalise deformation and force for the sample size and, hence, to allow a comparison of experimental results from strips with different sizes. The E

Fig. 2 (continued) hysteresis between mechanical loading (solid line) and unloading (dashed line) indicates energy loss. The progressive recruitment of collagen fibres explains the non-linear behaviour. (**i**) Representative force (stress) versus indentation (strain) curve acquired with AFM on a decellularised cardiac tissue slice. Mechanical properties at the microscale are also viscoelastic (the difference between approaching and retracting curves). The appropriate contact model depending on the AFM tip geometry is used to fit the experimental data. (**a**–**c**, **h**) Adapted from [36] and reprinted under Creative Commons (CC BY) license. (**e**) Adapted from [37] and reprinted under Creative Commons Attribution 4.0 International License. (**f**) Adapted from [38] and reprinted with permission from Elsevier. (**i**) Adapted from [39] and reprinted with permission from Elsevier

Table 1 Basic mechanical concepts of viscoelastic biological tissue characterisation

Mechanical parameter	Symbol	Definition
Engineering strain	ε	Ratio of total deformation to the initial dimension of the tissue
Stress	σ	Normalised force applied per unit area of the tissue
Young's modulus	E	Relationship between σ and ε in the linear region of the tissue
Complex shear modulus	G*	Ratio of σ to ε under vibratory conditions to account for viscoelastic tissue properties Complex G* is decomposed as $G^*(f) = G' + iG''$ being i the imaginary unit. The real part, G', is the elastic modulus that accounts for the elastic features of the sample, and the imaginary part, G'', is the loss modulus which characterises viscous dissipation
Loss tangent	G''/G'	Ratio of the viscous modulus to elastic modulus in a viscoelastic material. This ratio is an index of solid- or liquid-like behaviour of the sample. For a pure elastic material $G''/G' = 0$. Conversely, a pure viscous material has $G''/G' = \infty$

value of the strip can easily be derived by computing the derivative of the σ-ε curve. However, the σ-ε curve exhibits a more complex mechanical behaviour than just elastic. The hysteresis of the curve (difference between loading and unloading curve) depicts energy loss behaviour, typical of viscoelastic materials. The energy loss results from frictional processes, such as tissue fluid movement, and is commonly observed in soft biological tissues [59]. Moreover, the σ-ε behaviour of living soft tissues is highly non-linear. This behaviour can be mainly explained by the state of the ECM fibres inside the tissue, including the structural organisation of the collagen fibres. At the relaxed state of the tissue (low strains), the collagen fibres are wavy. By increasing the strain present in the tissue, the fibres start to unfold, collagen fibre recruitment increases and the σ-ε curve becomes non-linear (Fig. 2h) [60]. This strain stiffening effect is advantageous for a tissue as it becomes increasingly resistant to extension in order to prevent excessive deformations and tissue damage [61].

The mammalian healthy and diseased mechanical properties of the myocardium at the mesoscale have been the subject of extensive investigation in the past two decades [62–64]. Healthy myocardium shows anisotropic behaviour (e.g. different mechanical properties depending on the measured direction). E of healthy myocardium ranges from 1 to 10 kPa at a physiological tissue strain [65]. On the other hand, the post-MI myocardium shows a stiffer and isotropic behaviour, correlating with the disorganised distribution of collagen fibres found in the post-MI fibrotic scar [66, 67]. These findings support the notion that the ECM fibre organisation dominates the mechanical properties of the scar. In order to measure only the contribution of the ECM in the fibrotic myocardial scar and exclude the cellular effect, some studies have decellularised the samples before testing [68, 69]. Knowledge of the

mechanics of decellularised ECM will support the design of novel biomaterials for cardiac tissue engineering.

At the microscale, the mechanical properties are determined by individual ECM fibres and cells. The most suitable technique to measure mechanics at this scale is atomic force microscopy (AFM) [39]. AFM probes micromechanical properties of a thin tissue slice (or a single cell for that matter) by indenting its surface with a microfabricated cantilever ended with a pyramidal or spherical tip (Fig. 2f) [70]. This technique allows the measurement of tip displacement with nanometre resolution and simultaneous measurement of the applied force (Fig. 2i). AFM measurements provide essential mechanical information at the scale at which cells probe their own microenvironment. Micromechanical properties of heart tissue have been studied utilising AFM on fresh (ECM and cells) and decellularised cardiac slices of different species [38, 71–74]. Post-MI tissue mechanical properties showed a dramatic increased E compared to the healthy tissues (approximately three- to fourfold increase). Moreover, the loss tangent of G* (G”/G’, see Table 1) showed that the viscous tissue contribution of cardiac tissue at the microscale is around ten times lower than the elastic contribution, indicating that tissues show a solid-like behaviour [38].

Another major contributor to tissue mechanics are the cardiac cells. Characterisation of cardiomyocyte single-cell beating forces, frequency and cellular viscoelasticity has been studied using AFM [75]. A major advantage of AFM to measure cell mechanical parameters over other techniques, such as traction force microscopy or optical tweezers, is that AFM directly measures force and deformation without complex data processing. Different cell types largely used in cTE, such as human embryonic stem cells (hESC)- and induced pluripotent stem cells (iPSC)-derived cardiomyocytes, show different mechanical phenotype [76]. hESC-derived cardiomyocytes showed a beating force twofold lower than iPSC-derived cardiomyocytes, both showing single-cell forces in the range of nanonewton. Moreover, the most interesting fact is that cells showed an increased beating force when cultured in clusters showing that cell-cell connectivity plays a role in overall tissue force [76]. Additionally, cell viscoelasticity has been linked to several diseases [77, 78]. Laminopathies are a family of genetic diseases affecting the cardiomyocyte normal function and are caused by a mutation of the intracellular proteins called lamins. This mutation causes a loss of structural cell integrity, showed by a decreased E, with a lower cell-ECM adhesion affecting force generation and transmission towards surrounding cells and tissues [77]. Also, using primary cardiomyocytes from young and old rats, it was demonstrated that age correlates with a decreased cell shortening, increased relaxation time and increased E. These results indicate that cardiomyocytes from old animals are less deformable and contractile and suggest that cardiomyocyte mechanical changes per se can contribute to age-related diastolic LV dysfunction [79]. Overall, the data demonstrate that active and passive mechanical properties of cardiomyocytes also contribute to the overall tissue mechanics.

Obviously, mechanical data need to be handled with caution when designing novel strategies and biomaterials to mimic the healthy and diseased cardiac cellular

niche. Additionally, there is still a lack of knowledge on the relation between cardiac mechanical properties and cardiac mechanical function. Fundamental insights into structure-function properties at all length scales from cell to organ are required and should be integrated to predict the consequences of mechanical changes at the microscale for cardiac function at the macroscale and vice versa. In the following sections, we will review the models used to understand the impact of cardiac mechanical properties on cell and tissue function.

3 Mechanics-Based In Vitro Models to Understand Cardiac Behaviour at the Micro- and Mesoscale

cTE strategies are also designed to study the effect of the mechanical cardiac stimuli on cardiac cells. This section reviews in vitro cTE platforms mimicking the cardiac mechanical properties at the microscale (cells) and mesoscale (microtissues).

3.1 Cardiac ECM Organisation

The healthy myocardium at the micro- and mesoscale shows highly organised, anisotropic ECM fibres in contrast to the highly disorganised, isotropic ECM structure found after injury. Several in vitro methods have been described to mimic this organisation of healthy and diseased myocardium. First, a commonly used approach is microcontact printing that serves to pattern cell culture substrates with various ECM proteins, including fibronectin, laminin, collagen, Matrigel and gelatin [80–87]. Microcontact printing allows recreating the microscale two-dimensional environment by patterning the substrate with a high resolution of just a few micrometres. By changing pattern geometry, cells are forced to adopt specific (dis)organised alignment. A logical consequence of reproducing the highly organised healthy myocardium is that the cardiomyocytes become elongated with an increased contraction force compared to cells on a disorganised pattern [81, 84, 86].

At the mesoscale, the cTE gold standard technique to study cardiac in vitro conditions is the engineered heart tissue (EHT) pioneered at the end of the last century [88]. EHTs are cardiac microtissues composed of cell-laden and ECM mimicking biomaterial, moulded between constraints or stretching posts. Classical EHTs are composed of two stretching posts creating a unidirectional microtissue mimicking ECM anisotropy of the myocardium. More recently, to mimic the chaotic tissue organisation and force distribution after injury, EHTs were fabricated either with uniaxial and biaxial post distribution. To this end, the organisation and mechanical forces have been manipulated from organised to disorganised, respectively [89, 90] (Fig. 3a). This mesoscale model will enable the understanding of the

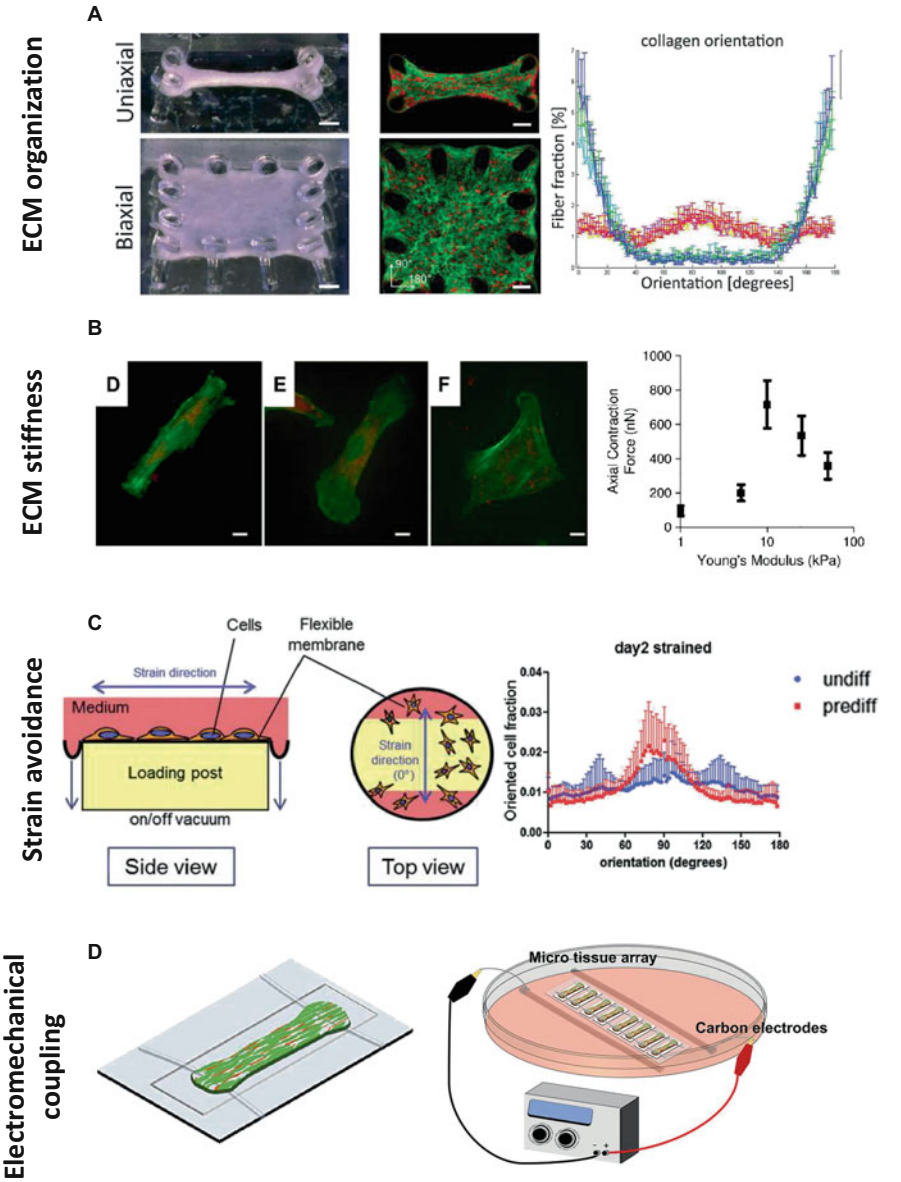

Fig. 3 Mechanical-based microscale and mesoscale in vitro models to understand cardiac cell behaviour. (**a**) Mesoscale microtissue with PDMS uniaxial or biaxial constraints to manipulate tissue organisation. The cells (red) seeded inside the collagen (green)-based hydrogels compacted around the posts. Collagen fibres' orientation shows an (an)isotropic distribution depending on the uniaxially or biaxially constrained tissues. Adapted from [89] and reprinted with permission from Oxford University Press. (**b**) Neonatal rat ventricular cardiomyocytes cultured on top of 1, 10 and 50 kPa PAA gels (left to right). Actin fibres (green) and contraction forces are maximised at physiological ECM stiffness of 10 kPa. Adapted from [91] and reprinted with permission from Elsevier. (**c**) Device based on a flexible membrane with cells seeded on top. The vacuum applied underneath the membrane stretches the flexible membrane which is supported by a loading post.

principles of ECM remodelling after injury and how it can be restored towards an organised ECM.

3.2 ECM Stiffness

A myriad of biomaterials has been developed to simulate the stiffness of healthy and diseased myocardium. Hydrogels from natural (non-cardiac ECM) and synthetic sources such as gelatin methacryloyl, polyethylene glycol (PEG), alginate, polyacrylamide (PAA) and polydimethylsiloxane (PDMS) are regularly used due to tuneability of E [84, 94–100]. It has been demonstrated that culturing cardiomyocytes on substrates with a physiological E maximises their contractility and the number of intracellular actin stress fibres, whereas increased or decreased E disrupts their cytoskeletal structure and reduces their contractile force [84, 91] (Fig. 3b). On the other hand, hydrogels from reconstituted natural cardiac ECM, such as decellularised ECM, induce better biochemical activity and increased remodelling capacity of the encapsulated cells while being able to control their mechanical properties via chemical crosslinking of the gel [29, 101, 102].

Additionally, the viscoelastic properties of some of these materials can be controlled. A recent study developed a nanostructured alginate-based hydrogel allowing control over stress-relaxation properties without changing the E. Using this material, the authors showed that stress relaxation affects cardiomyocyte intracellular contraction [103]. Another study indicated that varying the monomer and crosslinker concentration of PAA hydrogels allows to control the viscoelastic properties [104].

Overall, the described hydrogels can mimic the mechanical properties of healthy and diseased cardiac tissue but cannot capture the dynamics of cardiac remodelling after injury due to the covalent crosslinking between structural polymeric fibres in the gels. A new class of hydrogels offers control over crosslinking dynamics and consequent manipulation of hydrogel dynamic mechanical properties. By incorporating reversible crosslink methods, the properties of such hydrogels can be changed instantly by applying an external trigger, such as temperature, light or a chemical agent [105, 106]. For example, PEG was modified with a photo-sensible and reversible crosslinker that allowed to dynamically tune the viscoelastic properties by the use of blue light. Importantly, these changes were even possible when culturing cells inside the hydrogel [107]. Another recent example addresses a

Fig. 3 (continued) Cardiomyocyte progenitor cells show strain avoidance behaviour depending on their differentiation state. Adapted from [92] and reprinted with permission from Elsevier. (**d**) Schematic representation of the Biowire II platform. Cell-seeded collagen-based hydrogels are attached to uniaxially constrained wires. The system can be electrically paced with carbon electrodes and using an external electrical source. Adapted from [93] and reprinted under ACS AuthorChoice License

pH-sensitive hydrogel that enables changing the crosslinking degree by dynamic pH changes [108].

3.3 Strain on Cells

Cardiac tissue is constantly subjected to static (pre-stress present in the tissue) and cyclic strain (beating), and these strains may change with disease progression. Therefore, it is of utmost importance to investigate the mechanoresponse of cardiac cells to the experienced strain under conditions of health and disease. Most studies investigating strain responses make use of two-dimensional systems to apply (cyclic) uniaxial or equibiaxial strain [109]. Commonly, the cells are seeded on top of a flexible membrane that is stretched, transmitting the deformation of the membrane to the cells [92] (Fig. 3c). Because study designs differ in strain magnitude and frequencies used, comparison of study outcomes is cumbersome [110–112]. However, a common phenomenon has been observed in cardiac fibroblasts in response to cyclic strain. This phenomenon is called *strain avoidance* and refers to the re-orientation of cells perpendicular to the direction of applied cyclic strains [113, 114]. The physiological interpretation of this behaviour is that cells turn away from the stretch direction to experience minimal deformation on their cell body and nucleus. In fibroblasts, strain avoidance has been observed in two-dimensional and three-dimensional in vitro models [115–117]. However, strain avoidance is less clear in cardiomyocytes with several studies indicating that cardiomyocyte strain avoidance depends on the differentiation state of the cell, the strain rate and strain duration [92]. More recently, it was demonstrated that cardiomyocytes derived from a pluripotent stem cell source do not show strain avoidance. However, when co-cultured with cardiac fibroblasts (with a strain avoidance response), the cardiomyocytes did show strain avoidance and rotated along with the fibroblasts [118]. Hence, the effect of strain on cardiac cell behaviour is also influenced by the interplay between different cell types present in the tissue.

Strain generated on cardiac cells also has a significant impact on their phenotype via mechanotransduction pathways. One of the main current challenges regarding cell models in cTE is to obtain highly mature cardiomyocytes from pluripotent stem cell sources resembling adult cardiomyocytes found in vivo. Mechanical factors and, in particular, tissue strain have been shown to play a critical role in maturation process [119]. Numerous studies have been conducted to understand how cyclic mechanical strain affects cardiomyocyte maturation at single-cell and tissue levels [120–124]. Most of these studies showed that cyclic strain increases sarcomere formation, cardiac ion channel expression and contraction force and frequency of cells and tissue. Importantly, the strain magnitude and frequency applied to the tissues are essential to achieve better maturation [125, 126]. Cyclic strains around 10% showed to induce increased cardiomyocyte maturation compared to lower strains of 5% [127]. On the other hand, large strains (mimicking increased afterload)

have shown to cause pathological hypertrophy in vitro with larger cardiomyocytes but with decreased contractile function [128, 129].

3.4 Mechanoelectrical Feedback

The highly interconnected cardiomyocyte network controls the coordinated contraction of the myocardium and whole heart rhythm. Cardiomyocytes, at the microscale, depolarise their cellular membrane in the presence of an electrical stimulus. This depolarisation triggers the release of intracellular calcium ions responsible for activating the cell's contractile machinery. In an in vitro setting, generally, the mesoscale tissues containing mature adult cardiomyocytes start beating due to their autorhythmic properties [89]. However, in pathophysiological conditions these tissues often beat non-synchronously due to a lack of proper cardiomyocyte and fibroblast organisation and cell-cell contact inside the hydrogel [130]. To overcome the appearance of arrythmia in cTE tissues, these are generally paced by applying an external electric field during long-term tissue culture. To this end, several approaches have been implemented with notable success [109, 131]. As an example, the Biowire and Biowire II platforms (Fig. 3d) have been demonstrated to improve intracellular calcium handling, contraction force and synchronicity of beating [93, 132].

3.5 Developing Integrated Models for Mechanical Consideration In Vitro

In the context of designing strategies for cardiac regeneration, it is not only necessary to understand how mechanical stimuli influence cell and tissue behaviour, but it is also fundamental to integrate such stimuli across length scales to better recapitulate the in vivo situation. For this purpose, the development and use of bioreactors are pursued. A bioreactor is typically defined as a device that provides tight control of the environmental conditions and external stimuli (biochemical and biomechanical) that influence cell and tissue culture processes [133].

Oxygen tension is of paramount importance in affecting cardiac cellular and tissue behaviour [134, 135]. After MI, there is a loss of perfusion in the scar region that leads to a decreased oxygen level or hypoxia. It has been previously shown that hypoxia enhances the migration and differentiation capacity of pluripotent stem cells derived to cardiomyocytes [136, 137]. Moreover, low oxygen tensions stimulate the ECM-producing phenotype of cardiac fibroblast, maintaining the presence of fibrotic tissue after injury, having a direct impact on cardiac tissue mechanics [138]. Therefore, the use of bioreactors capable to mimic (patho)physiological oxygen tensions is

critical to further understand the mechanical implications on the behaviour of cTE strategies.

Biomechanically, the main goal for cTE is to synchronise contraction with appropriately timed mechanical or electrical stimulation to mimic ventricular filling. Independent control over individual input signals further allows for manipulating disease progression, e.g. via changes in the stretch (haemodynamics) and electrical signal patterns (e.g. arrythmias). Various bioreactors have been designed for applying mechanical and electrical stimuli simultaneously [139–141]. For example, an electromechanical bioreactor platform was able to provide static stress to microtissues using a pneumatically driven stretch device. It consisted of a tissue culture chamber where several tissue constructs (20 mm × 20 mm × 3 mm) were subjected to frequencies and amplitudes of cyclic stretches and electrical pulses matching the native tissue [142]. Moreover, it is also important to track over time the changes in mechanical properties of the tissues inside bioreactors as a parameter to understand tissue growth and remodelling. A recent study developed a bioreactor to test cardiac tissues under dynamic loading together with an ultrasound system to trace non-invasively the mechanical properties of the tissue over time [143].

4 In Silico Models to Study the Mechanics of Myocardial Remodelling and Regeneration

The rapid development of digital technologies has enabled the development and application of computational models in many fields nowadays. Computational models in bioengineering, commonly referred to as in silico models, enhance the knowledge of various biological tissues' behaviour at different scales. Moreover, a multiscale approach can integrate knowledge from different scales into an overall simulation of the tissue behaviour and thus become more relevant for analysis and predictions.

The use of in silico models for simulating cardiac tissue function has rapidly expanded in the last years, especially for simulating drug testing and considering chemical coupling within the tissue. The computational platforms for testing novel/existing drugs have achieved widespread approval on different aspects – ethical, toxicological and economic [144]. Besides the platforms for drug testing, the major interest of in silico models lies in capturing cardiac systolic/diastolic functioning and electrical coupling, from micro- to macroscale [145]. These models usually neglect the biomechanics of the heart and/or miss to include the mechanical environment for the cells [146]. Moreover, there is a gap in linking electro-mechanical coupling of the heart to the contraction of the tissue at different length scales. Improvement of in silico models in this area is suggested to result in models that can predict the change of tissue mechanical function in response to tissue remodelling under conditions of health, degeneration and regeneration. A such, these models also have to translate basic research findings on cardiac regeneration into tissue engineering or other

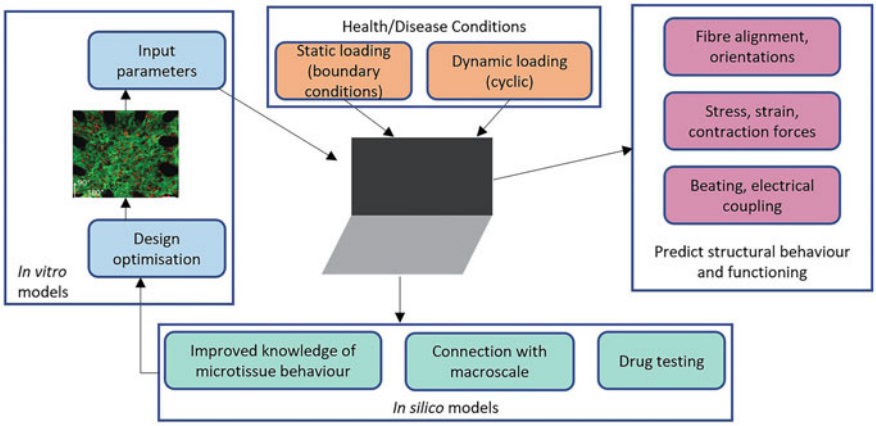

Fig. 4 Scheme of in silico approach in myocardial mesoscale modelling

regenerative strategies under pathological conditions, including their altered hemodynamic, electroconductive and tissue mechanical features [147].

Current regenerative strategies fail to properly restore the cellular microenvironment and aligned structural organisation after cardiac injury, relevant for coordinated contraction and tissue mechanical homeostasis, and this is where in silico models can be very useful in answering the questions about (changing) mechanical impact on cardiac tissue regeneration [148]. They can be developed to replicate certain experimental observations, e.g. at cell and tissue level, and be further extended to in vivo-like conditions at the organ scale. By employing a multiscale approach, they can be also used to investigate the effect of microscale modifications on macroscale function, much easier than experiments can do [149].

The starting point in the development of in silico models is the connection of simulated biological processes with data from in vitro or in vivo experiment(s), or those from the literature, since each computational model needs meaningful input data for robust predictions (Fig. 4). In this first phase of development, model outputs are compared with – or validated against – additional experimental findings so that initially applied boundary conditions of the proposed in silico model are in line with real-life data. As there are now various in silico models in cardiac research, there is a tendency to standardise in silico cardiac models in terms of verification, validation and uncertainty quantification of scientific software [150].

In the scheme depicted in Fig. 4, the input from an in vitro microtissue model is used to feed the computational model at the mesoscale (tissue level). The outputs from the in silico simulations can include, for instance, mechanical properties of the tissue, structural organisation and mechanical contraction in response to the experimental starting conditions. Once validated, the in silico model can be used to understand and predict outputs in response to new boundary/loading conditions, e.g. to mimic healthy/diseased states of the microtissue. The added value of such mesoscale in silico models can be found in the enhanced understanding and

prediction of microtissue behaviour and the reduction and optimisation of further in vitro models. An additional benefit is the possibility to integrate and translate insights from the mesoscale level to the macroscale level using multiscale in silico models [149], which is particularly useful for predicting the outcome of cardiac regenerative strategies. The final advantage is the usage of in silico models as a platform for existing/novel drug testing – as has been mentioned before. This section aims to present current in silico models that mimic the myocardium at different length scales and with a special reference to mechanical consideration for regenerative strategies. Our opinion on future directions in this area will be discussed in the concluding section.

In the context of cardiac regeneration, in silico models have been employed to predict the mechanical consequences and optimise the design or placement of next-generation cardiac patches in terms of structural organisation and mechanical properties of the myocardium and to help define the mechanical constraints invoked by such patches that would lead to reversion or inhibition of adverse cardiac remodelling.

Urdeitx and Doweidar developed a mesoscale *finite element* model to simulate cardiac cellular behaviour such as proliferation, migration, maturation and cell-matrix adhesion in response to mechanical and electrical cues from the environment [151]. Cell behaviour was described as a function of cell deformation due to changes in ECM stiffness and/or electrical stimulation. The model predicted that on soft ECM, cell alignment and migration improved with increasing (directional) electrical stimulation. On stiffer ECM, cells showed enhanced maturation and proliferation. However, the mechanical impact considered in the model is limited as it only considers changes in ECM stiffness, without incorporating the cause of ECM stiffness changes or hemodynamic loading.

To better understand the potential of cardiac regeneration, tissue (ECM) production and organisation by resident or newly delivered cells have been studied in vitro. When cultured in 3D environments, such as collagen hydrogels enriched with Matrigel, cardiac cells (both cardiac fibroblasts and cardiomyocytes) contribute to the production and maintenance of the ECM by ECM synthesis, degradation and cell-matrix interactions, including cell traction forces [89]. They also respond to environmental strains by (re)orienting their cell body as well as the ECM fibres around them (see Sect. 3.3). Mesoscale in silico models of the cardiac tissues have been developed to successfully describe the underlying biological phenomena of these processes of healthy tissue remodelling, where fibroblasts align their internal cytoskeleton (stress fibres) and ECM to form an anisotropic tissue [152–156]. In response to cardiac injury, for instance, due to ischemia, the heart's primary response is to create a scar-like tissue and protect the damaged tissue from rupturing by ongoing remodelling. Under these conditions the original cell and tissue anisotropy is lost and fibroblasts differentiate into myofibroblasts, which show a higher production of ECM proteins (mainly collagen), leading to reduced compliance and stiffening of the scar [157]. In silico models that can predict structural (re)-organisation of the cells and collagen can thus predict disease development of the tissue but are also instrumental for testing the effects of mechanical conditions

(e.g. constraints by tissue patches) that would allow the transition from an isotropic to an anisotropic organisation. To this end, the models should describe how cardiac fibroblasts remodel the collagenous matrix and incorporate cell-mediated traction forces [158] under both physiological and pathophysiological conditions [154–156]. When successfully validated, the output from an in silico model that describes tissue structural remodelling can also be used to design engineered tissues with optimised and load-bearing collagen organisations [159, 160].

When modelling the mechano-response of cardiomyocytes, in vitro and in silico models have focused on describing cardiomyocyte organisation and alignment at the mesoscale, either or not in the presence of fibroblasts. These models show that cardiomyocytes align with the stress direction and alongside collagen fibres in uniaxially constrained microtissues, but show no alignment in biaxially constrained microtissues [89, 161]. The aligned structure is essential for coordinated and synchronised contraction and proper propagation of electrical signals. Cross-sectional compaction of uniaxial constrained tissues further contributes to alignment and increases with the percentage of fibroblasts present in the tissue. Forceful contraction requires a high percentage of aligned cardiomyocytes and fibroblasts to establish conduction and synchronised beating. Hence, next to structural remodelling, cell ratios and alignment at the mesoscale should be incorporated when mimicking regenerative strategies to reverse or halt remodelling of cardiac scar tissue at the macroscale. Vice versa, the influence of hemodynamic loading conditions as well as active and passive mechanical behaviour at the macroscale [162] will influence cell alignment at the meso- and microscale.

Macroscopically, the myocardium is organised in differently orientated two-dimensional anisotropic sheets that follow a helicoidal shape from the epicardium to the endocardium. Hence, the local coordinate system is represented by three axes: fibre orientation, sheet orientation and normal to the sheet orientation to capture anisotropy of the tissue. There are two approaches to computationally include myocardial organisation within the cardiac geometry at the macroscale: patient-specific and numerically approximated. The inclusion of patient-specific organisation is the ultimate goal of all predictive in silico models, since it takes into account the real structure of the tissue, obtained by image reconstruction of patient-specific MR images. The fibre directions can be measured from MR images leading to the generation of patient-specific structure and geometry, which is a great improvement in numerical modelling despite their time-consuming feature [163]. Sometimes it can be more convenient to avoid image reconstruction for each patient separately. By mapping fibre orientations from the geometry of one patient to the cardiac geometry of another patient, time, money and effort can be reduced, but this approach is less accurate [164]. To simulate generic behaviour of the myocardium at the macroscale, including the influence of tissue organisation, numerical approximations can be used, where myofibril helix angle changes linearly from $-60^\circ \pm 10^\circ$ at the epicardium to $60^\circ \pm 10^\circ$ at the endocardium side [165]. Which approach will be selected depends on the purpose of the in silico model.

Cardiac in silico models describing macroscopic mechanical behaviour commonly model left ventricle (LV) behaviour. The LV, representing the largest shape and volume of the four-chambered heart, experiences the highest stresses and strains, and its function is most often and most severely affected by disease. The most valuable output from these LV models is the quantification of myocardial mechanical parameters under in vivo-like hemodynamic loading, in particular LV wall stress, which cannot be measured experimentally but is important in disease prediction since it is indicative of cardiac wall (dis)function. By changing the mechanical properties of the LV wall at certain locations, different pathological conditions can be simulated. For instance, when incorporating differential mechanical properties for scarred tissue, border zone and healthy remote tissue based on patient-specific MR images, these models can be used to predict the severity and functional consequence of myocardial infarction [166]. Yet, the LV models generally do not account for tissue organisation and remodelling at lower length scales, so they cannot simulate the progression of fibrosis and functional consequences at later stages after infarction. A second disadvantage of these models is a simplified representation of electrical conduction and electro-mechanical coupling.

The final goal of in silico models is the integration of insights obtained across length scales (cell, tissue and organ) into multiscale models that can provide more detailed information on cardiac behaviour, predict disease progression and improve existing or establish novel regenerative strategies. So far, multiscale in silico models have mainly focused on describing the effects of pharmacological agents and electrical conduction from cell to organ, while multiscale mechanical interactions have remained largely unexplored. Future directions in the development of in silico myocardial models to support the improvement of existing or creation of novel regenerative strategies lie in predicting the effects of mechanical loading/unloading on tissue remodelling and mechanical functioning at different length scales.

Knowledge obtained from experiments and simulations at cell and tissue scale should be translated into the in vivo situation of the whole heart to ultimately understand how cardiac tissue will remodel under whole-organ hemodynamic loading conditions and depending on (changing/heterogeneous) cardiac wall mechanical properties. The SIMULIA Living Heart Project[1] could offer the next step in the development of more complex multiscale electromechanical cardiac models. This project gathers some of the most prominent researchers in cardiovascular area from different branches, with the purpose to create a multidisciplinary vision in the development of cardiac computational models for clinical use. The project integrates various features relevant for cardiac function, such as the detailed geometry of the whole heart, electrophysiology, active and passive mechanical properties of the cardiac wall, blood flow and the feedback of the circulatory system on cardiac function. Using the available computational methods to model myocardial infarction and tissue remodelling at the mesoscale, the macro model could, for instance, predict

[1] Living Heart Project | SIMULIA™ - Dassault Systèmes® (3ds.com)| SIMULIA™ - Dassault Systèmes® (3ds.com).

how tissue-engineered epicardial patches or biomaterial injection in the cardiac wall would influence tissue remodelling in the infarct area and subsequently how cardiac function as a whole could be improved.

5 Conclusion

Even though the progress in the development of cardiac regenerative strategies can be observed, long-term clinical benefits are still to be expected. Current strategies largely omit the mechanical consequences after implantation of cells or tissue-engineered constructs, suggesting that more attention should be paid to mechanical considerations in the processes of tissue formation and remodelling. The use of in vitro and in silico models, together with a thorough mechanical characterisation of myocardial tissue at different length scales, is required to understand and predict the effects of the mechanical cues (e.g. loading and ECM stiffness) on cells and tissues. In this context, it should be noted that mechanical properties of cardiac tissue are influenced by the scale at which they are measured, highlighting the need to use the appropriate technique at each scale. Microscale and mesoscale in vitro models need to integrate these mechanical properties to better understand the effect on cell and tissue behaviour. Moreover, in silico models can provide significant assistance in simulating a multiscale cardiac response upon improvement of existent or development of novel models. Regarding in silico models that describe mechanical behaviour, the literature mainly reports on models at the meso- and macroscales but does not provide multiscale models that can simulate cardiac tissue remodelling or regeneration based on such processes at cellular or tissue level. The development of multiscale predictive models could illuminate native-like mechanical conditions that can provoke remodelling of fibrotic tissue (e.g. change of mechanical loading and change of stiffness), but more importantly translation of these conditions into the effect of regenerative strategies, such as patches with regionally different stiffness.

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Ethical Approval: This chapter does not contain any studies with animals performed by any of the authors. For Fig. 2d, g informed consent was obtained from the patients.

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