Chapter

Extracellular Matrix in Cardiac Tissue Mechanics and Physiology: Role of Collagen Accumulation

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Abstract

The extracellular matrix (ECM) forms a mesh surrounding tissue, made up of fibrous and non-fibrous proteins that contribute to the cellular function, mechanical properties of the tissue and physiological function of the organ. The cardiac ECM remodels in response to mechanical alterations (e.g., pressure overload, volume overload) or injuries (e.g., myocardial infarction, bacterial infection), which further leads to mechanical and functional changes of the heart. Collagen, the most prevalent ECM protein in the body, contributes significantly to the mechanical behavior of myocardium during disease progression. Alterations in collagen fiber morphology and alignment, isoform, and cross-linking occur during the progression of various cardiac diseases. Acute or compensatory remodeling of cardiac ECM maintains normal cardiac function. However, chronic or decompensatory remodeling eventually results in heart failure, and the exact mechanism of transition into maladaptation remains unclear. This review aims to summarize the primary role of collagen accumulation (fibrosis) in heart failure progression, with a focus on its effects on myocardial tissue mechanical properties and cellular and organ functions.

Keywords: collagen deposition, fibrosis, myocardial stiffening, left and right ventricle, mechanobiology

1. Introduction

The extracellular matrix (ECM) is a network of proteins, fibrous and non-fibrous, which form a supporting architecture for the cells in cardiac tissues. Cardiomyocytes, fibroblasts, vascular cells, and inflammatory cells that are responsible for the synthesis and degradation of ECM proteins exist in and around the cardiac ECM. A unique hallmark of the cardiovascular (CV) system is that the tissue is subject to dynamic mechanical load from the pulsatile blood pressure and flow. A perturbation of the mechanical load will be transduced to and sensed by the cells via the ECM to trigger acute or chronic remodeling of the tissue, resulting in structural and mechanical changes in the ECM. The altered ECM biomechanical properties further change the behavior of the cells within the tissue. These aspects are known as mechanobiology. This chapter will focus primarily on the changes of the ECM in various cardiac diseases, the alterations in the mechanical properties of the myocardium as a result of the ECM remodeling, and the impact of these

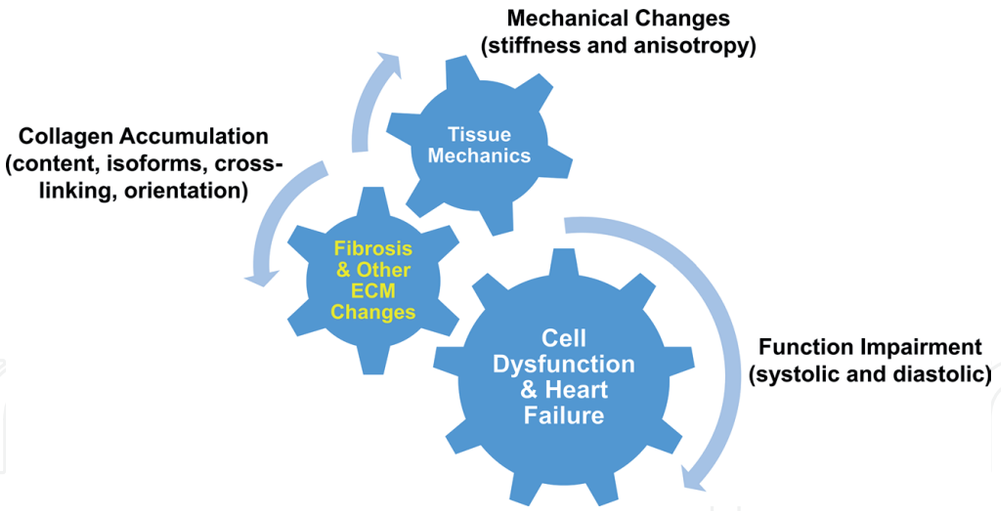

Figure 1.
A schematic plot showing the relations between cardiac ECM remodeling (fibrosis), tissue mechanics and ventricular function.

biomechanical factors on cellular and organ function in the progression of heart failure (**Figure 1**). Moreover, because collagen accumulation (fibrosis) is mostly investigated and contributes significantly to myocardial mechanical properties, we will limit our discussion on cardiac fibrosis as the main ECM remodeling event. Readers are referred to other extensive reviews that have summarized a broad category of ECM proteins during myocardial remodeling in different types of heart diseases [1, 2].

2. Extracellular matrix proteins in cardiac tissues

2.1 Overview of extracellular matrix proteins in biological tissues

Extracellular matrix (ECM) proteins found in biological tissue can serve structural or non-structural roles, depending on the location and composition of the protein. Glycoproteins primarily consist of structural ECM proteins, which include fibrillar collagen, elastin, fibronectin, and laminin [1]. These are often the main determinants of tissue's passive mechanical properties. The non-structural ECM proteins are primarily categorized as proteoglycans, which are further distinguished as four subgroups—hyalectans, cell surface proteoglycans, basement membrane proteoglycans, and small leucine-rich proteoglycans (SLRP's) [2]. These proteins play a key role in cell–cell or Cell—matrix interactions, interaction with growth factors, as well as binding to cell receptors to regulate cellular function [2].

Among the structural ECM proteins, collagen and elastin are mostly investigated in cardiovascular tissues. Collagen is the most abundant protein in the body and formed from the basic unit of tropocollagen [3]. Tropocollagens, made up of polypeptide chains, spontaneously twist together to form a triple helix structure, which form a newly synthesized procollagen fibril. Mature collagen fibers can then form via cross-linking of procollagen fibrils into bundles or sheets, conferring versatile mechanical properties in various tissues [3]. There are over 25 collagen types to date [2], each with different physical and mechanical properties [4]. For instance, type I collagen is a subtype that exhibits stiffer mechanical property and higher tensile strength compared to type III collagen. Collagen metabolism is maintained by the synthesis and degradation balance, and different tissues have different turnover rates [4, 5]. Higher turnover of collagen types I and III is observed in diseased

tissues, and is also linked to the pathological state of the tissue such as inflammation and aging [4, 5]. Overall, fibrillar collagen contributes to CV tissue's nonlinear elastic behavior at high strains due to the increased recruitment of fibers.

Elastin is another primary structural protein, contributing to the mechanical property of the CV tissue at low strains [6]. It is formed as sheets comprised of the base unit tropoelastin, which has the ability to stretch and recoil [6]. Compared to collagen fibers which have an average Young's Modulus of 250–400 MPa [7], elastin fibers are more compliant with an average Young's Modulus of 1 MPa [8]. Elastin is a highly stable protein that has very low turnover rate – once formed, it lasts almost for the entire lifespan [9]. Increased elastin degradation or damage is a sign of pathological remodeling of the tissue and is associated with aging and CV diseases [9].

2.2 Primary ECM proteins in the heart

In the myocardial tissue, the main ECM protein is collagen. Collagen is diffusively spread over in the myocardium—interstitial and perivascular collagen fibers have been revealed by histology. Collagen types I and III are the most prominent collagen types in the myocardium [10], making up 85% and 6–11% of the total collagen, respectively [11]. Other isoforms of collagen are also reported. Bashey et al. examined murine, canine, and nonhuman primate healthy hearts and found that type V collagen comprises 2 ~ 3% and type VI collagen comprises ~5% of the total collagen in the myocardium [11]. While the fractional content of type IV collagen was not evident, it appeared most prominent in the basement membrane and in the media [11]. A detailed summary of all fibrillar and non-fibrillar collagen and their roles in cardiac diseases can be found in a recent review [12].

Elastin content in the ventricle is not detailed in the same manner as collagen. The distribution of elastin is mainly limited to the epicardium, the outer layer of the ventricular wall [13, 14]. Biochemical measurements showed that elastin content is about one tenth of the collagen content in healthy heart. Furthermore, the left ventricle (LV) tended to have more elastin and collagen content (in μg/mg) than the right ventricle (RV) [15].

In heart valves, elastin is more abundant than in the myocardium. It is located primarily in the inflow layer and sparsely distributed in the outflow and central layers, comprising approximately 10% of total proteins in the tissue [16, 17]. Collagen type I is found primarily in the valve leaflets and the valve outflow layer, whereas collagen type III is distributed throughout the entire valve structure [16]. Collagen comprises approximately 60% of ECM proteins in human heart valves [17].

2.3 Cells and molecules responsible for ECM synthesis and degradation

The cellular components responsible for the cardiac collagen synthesis include interstitial fibroblasts (in healthy hearts), transdifferentiated myofibroblasts and inflammatory cells (in diseased hearts), cardiomyocytes, and adventitial fibroblasts and smooth muscle cells (SMCs) in the blood vessels [18–20]. Several biological mediators such as pro-inflammatory cytokines, growth factors and hormones are also identified to participate in collagen synthesis, which are reviewed previously [4, 21, 22]. Among them, fibroblasts and transforming growth factor beta (TGF-β) are two main contributing factors. In cardiac remodeling, fibroblasts migrate to the injured region or to the area where ECM proteins are over-degraded and secrete new ECM proteins—primarily collagen types I and III [18]. Moreover, a "new" phenotype of cells myofibroblasts, emerge in injured myocardium, which is a key step to strengthen cardiac fibrosis in both infarcted and hypertensive myocardium.

MMPs	Substrate
MMP-1	Collagens I, II, III, VII, X
MMP-8	Collagens I, II, III, V, VII, X
MMP-13	Collagens I, II, III, IV, fibronectin, laminin
MMP-2	Collagens, I, IV, V, VII, X, XI, fibronectin, laminin elastin
MMP-9	Collagens III, IV, V, VII, X, elastin
MMP-3	Collagens III, IV, V, IX, X, fibronectin, laminin
MMP-10	Collagens III, IV, V, IX, laminin, fibronectin
MMP-11	Collagen IV, fibronectin, laminin
MMP-14	Collagens I, II, III, fibronectin, laminin

Table 1.
Matrix Metalloproteinases (MMPs) and their substrates (adapted from [24]).

TGF-β is involved in signaling pathways of various cells (fibroblasts, cardiomyocytes, immune and vascular cells) to initiate fibrogenic action [19]. For a thorough review of current understanding of cardiac fibrosis in ischemic and non-ischemic heart diseases, the reader is referred to these references [12, 21].

Degradation of the ECM proteins is necessary for the turnover as well as normal protein function. Matrix metalloproteinases (MMPs) are the most significant molecules that contribute to this degradation and these enzymes are key in the CV tissue remodeling [10]. MMPs in the heart are primarily expressed by the fibroblasts and cardiomyocytes [20, 23]. **Table 1** details various types of MMPs and the ECM proteins that they target. Insoluble fibrillar collagen such as collagen type I and III or more cross-linked collagen is difficult to be enzymatically degraded [11]. To prevent excessive degradation of ECM, tissue inhibitors of metalloproteinases (TIMPs) are secreted to bind to MMPs and limit the role of activated MMPs. Therefore, the overall balance between the activated MMPs and TIMPs determines the ECM remodeling and turnover rate.

3. Measurement of ECM proteins

There is increasing agreement that the ECM is not a passive biological component but actively interferes with cellular and organ function in the dynamic remodeling process. Thus, the measurement of ECM proteins is critical to study their roles in tissue biomechanics and remodeling in various diseases. The existing measurement methods can be classified into these categories: medical imaging techniques, optical imaging techniques, biochemical and biological methods.

3.1 Medical imaging techniques

Medical imaging techniques are generally noninvasive because they can be performed on a live subject with negligible risks; they are sometimes referred to as organ-scale imaging [25]. Primary medical imaging techniques include Magnetic Resonance Imaging (MRI), ultrasound and nuclear imaging. These techniques could measure bulk quantities of the materials—concentration, volume fraction and distribution of proteins.

MRI is the most common imaging method for collagen detection because of its higher sensitivity to specific molecular probes to target specific ECM proteins [26].

For example, the feasibility of detection of collagen (predominantly collagen type I) using a gadolinium-containing molecular contrast agent (delayed or late gadolinium enhancement) has been demonstrated in preclinical animal studies [27, 28]. The technique is valuable in the detection of fibrosis in ventricles [29, 30]. Recently, T1 mapping technique has emerged as a more useful technique for diffuse interstitial fibrosis measurement [31, 32]. In addition, a particular mode of MRI, tagging and feature tracking, enables clinicians to measure tissue strain from which collagen measurements can be indirectly deduced [33]. Elastin content can be quantified directly by molecular MRI as well [34].

Ultrasound technique is another imaging method to quantify collagen or elastin. Strain elastography measures elasticity of the myocardium, from which properties of the structure and content of collagen and elastin are indirectly derived [35, 36]. This method is sensitive to the fiber angle and density, both of which give light to the health status of the tissue [35]. Using the known mechanical properties of elastin and collagen (i.e., Young's Modulus), one can distinguish the relative contributions of ECM proteins to the tissue. Finally, cardiac nuclear imaging, including single-photon emission computed tomography (SPECT) and positron-emission tomography (PET), has been used to quantify the ECM content [25]. Radioactive molecular probes are used in these techniques. The common targets include collagen type I, II and IV, as well as MMPs [37, 38].

3.2 Optical imaging techniques

Optical imaging techniques are often used to provide the images of collagen fibers including their content and fiber orientation in intact, fresh tissues using physical properties of photons [39]. Two primary optical imaging techniques are the Second-harmonic Generation (SHG) and Small Angle Light Scattering (SALS). SHG imaging is a form of nonlinear optical microscopy. It can be applied to fresh or fixed tissues at varying depths (optical section), thus revealing the 2D or 3D structure of the collagen fibers [40]. This technique is advantageous due to its high resolution and specificity for the microstructure of collagen fibers [41]. However, it only allows the imaging of samples to a certain distance (depth), and deep tissue imaging systems are currently under development to enable larger length of penetration [42]. SALS is another method to measure fiber orientation when a polarized light beam is passed through a specimen—biological or nonbiological [43, 44]. The distribution of the scattered light is used to identify fiber orientation and alignment [43]. The gross/average collagen fiber orientation can be obtained in tissues with a thickness of at least 500 microns [45]. The advantage of this technique is the capability to measure in thicker tissues than SHG; but the disadvantage is that only an average of planar (2D) fiber orientation is derived, and the information along the depth direction is unavailable.

3.3 Biochemical and biological measurements

Direct measurements of collagen can be obtained using a long-established biochemical measurement – hydroxyproline assay, which quantifies the hydroxyproline content in digested samples. Hydroxyproline is a main molecular component of collagen and its amount can indirectly reflect the collagen content or is converted to collagen content with assumed collagen to hydroxyproline ratio [46]. Different methods have been established to measure hydroxyproline including colorimetric methods, high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS) [47].

In addition, tissue staining protocols—histology methods—are convenient to examine the ECM protein content and structure without losing the local distribution information. The common staining methods for collagen are Masson's Trichrome stains [35, 48] and Picrosirius Red stains [49]. Because mature collagen is birefringent and the Picrosirius Red stain can enhance the birefringency of collagen, collagen fibers can be visualized better and in more details under polarized light. It also enables a quick examination of types I and III collagen in CV tissues due to the different fiber thickness [50, 51]. Alternatively, the elastin is often examined by the Verhoeff-Van Gieson (VVG) stain [52] and the glycosaminoglycans (GAGs) and proteoglycans are examined by the Alcian blue stain [53]. Moreover, immunohistochemistry (IHC) employs the use of antibodies to quantify the specific protein of interest [10, 11].

Non-microscopic measurement methods include enzyme-linked immunosorbent assay (ELISA) and other standard biological methods. ELISA has been applied to detect collagen types I and III, as well as elastin and cross-linking of collagen [54, 55]. Finally, like all other proteins, the ECM proteins can be quantified by the protein expression using Western blot (immunoblotting) or by the mRNA or DNA expression using qPCR (quantitative Polymerase Chain Reaction) [6, 56].

4. Alterations of biomechanical properties in cardiac disease progression

Heart failure (HF) affects approximately 6.2 million adult Americans [57]. The main causes of HF are myocardial infarction, pressure-overload (hypertension), volume-overload, arrhythmia, valve stenosis or regurgitation, etc. Ventricular dysfunction is the most common type of HF including left-sided HF with preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF), as well as right-sided HF secondary to pulmonary hypertension and congenital heart disease [58–60]. The malfunction of the myocardium can occur in the LV, RV, or both ventricles (biventricular HF).

As shown in the overall scheme (**Figure 1**), the cardiac ECM remodeling is an interactive, dynamic procedure that brings the cellular function, tissue mechanical behavior and organ function into one scenario. The ECM remodeling leads to both biological (structural) and mechanical (functional) changes of the tissue, which in turn regulates cell behaviors and alters the hemodynamics and cardiac performance. It is accepted that the remodeling often starts with an attempt to maintain the homeostasis environment of the cells and organ. This is referred to as adaptive remodeling (compensation). However, when the remodeling cannot achieve a 'stable' status of the new homeostasis but continues to deteriorate, impairments of the cells and organ will occur. This is known as adverse or mal-adaptive remodeling (decompensation). The mechanism of transition from compensation to decompensation remains a key knowledge gap. In almost all types of HF, cardiac fibrosis plays an important role in the pathogenesis; but its effects on mechanical changes of the myocardium and the physiological function are less noted. Hence, our discussion here will focus on the maintenance of the biomechanical homeostasis in common types of HF involving both LV and RV.

4.1 Biomechanical adaptations of hypertensive (pressure overload) myocardium

4.1.1 Fibrotic changes in hypertensive LV and RV

Hypertension is defined as chronically elevated blood pressure in the systemic circulation, with a systolic blood pressure greater than 120 mmHg, and/or a

diastolic blood pressure greater than 80 mmHg [61]. It is one of the most prevalent pathologies in the United States, affecting approximately one in three adults. Hypertensive heart disease (HHD) accounts for approximately a quarter of all causes of heart failure [61]. In response to the pressure overload, cardiac hypertrophy and fibrosis are the most prominent events observed. The increased interstitial and perivascular collagen is originated from several cell types including cardiac fibroblasts, activated macrophages [62], cells derived from EndMT (or epithelial-to-mesenchymal transition (EMT)) or myofibroblasts, which are transdifferentiated from EndMT and EMT [21, 63].

In preclinical animal models, HHD can be induced by aortic banding [64, 65], genetic alteration (e.g. spontaneous hypertensive rates (SHR)) [66], or other methods that overlap with the models of systemic hypertension (e.g. high-salt diet or angiotensin II infusion) [63]. From both clinical and animal studies, various fibrillar collagen types such as I, III, IV, and V were reported to increase in the hypertensive LV [12, 64, 65, 67, 68]. The regulation of collagen turnover is dynamic. For instance, collagen types I and III increased within a day after hypertension was induced [64, 65]. Collagen types I and III reached a peak content (fivefold and 1.7-fold, respectively) at day 3, then decreased at day 7 and plateaued four weeks after the aortic banding. In contrast, collagen type IV reached its peak one day after the banding, and then began to decline at day 3 and plateaued at day 7 [65]. A similar trend of an initial elevation followed by a decline of collagen types I, III and IV has been reported by Jalil et al. in a rat aortic banding model [64]. The time-dependent change has been suspected to be associated with the transition from compensative hypertrophy to decompensation. The overall ECM degradation in later stages of HHD is speculated to disrupt the mechanical support and electrical conduction for myocardial contraction and promote cardiomyocyte apoptosis, which results in impairments of cardiomyocyte contractility and organ failure [12, 69].

There is no consensus on whether type I or type III collagen plays a more significant role in the LV hypertrophy. Some studies have indicated a predominance of type I collagen accumulation [70, 71], but others reported equivalent elevations of type I and III [72]. In a patient study that examined the collagen type I and III mRNAs expression in dilated cardiomyopathy, it was shown that the collagen type III/I ratio was higher in dilated cardiomyopathy patients (idiopathic, hypertensive and alcoholic) than the healthy controls [73]. This suggests an important role of collagen type III in the hypertensive LV fibrosis. The metabolism of the two collagen isoforms may not be independent since it has been shown that type III collagen is crucial for collagen I fibrillogenesis during the normal development of cardiovascular system [74]. Therefore, a further understanding of the role of collagen isoforms in fibrosis is needed.

In the RV, similar fibrotic remodeling occurs in response to pulmonary hypertension (PH), which is defined as a mean pulmonary arterial pressure (mPAP) exceeding 20 mmHg [75]. Increases in total collagen content or collagen synthesis were consistently reported in hypertensive RVs, from clinical to preclinical studies, from large to small animals, as well as from early to late stage of RV failure [50, 76–78]. The types of collagen upregulated in the RV has been investigated but inconsistent findings are reported. In a mouse model of PH (Sugen+hypoxia), the ratio of collagen type I/III was increased in the diseased RV and it was mainly attributed to the increase in type I collagen [78]. But in a recent ovine model of PH, type III collagen rather than type I collagen was found to be increased more significantly in the RV [51]. While both studies used Picrosirius Red stained histology samples to quantify collagen isoforms in the RV, these results need to be confirmed by other quantitative methods in future studies.

Finally, not only is there a change of the collagen content, but the morphology, cross-linking, and alignment are altered in the remodeling process. First, in the hypertensive LV, collagen type I became thicker and denser, creating a tighter mesh of fibers [64]. Second, the increase in collagen content is accompanied with an increase in cross-linking as this is part of the maturation of new collagen. Cross-linking is an enzymatic driven event and two types of enzymes—the LOX (lysyl oxidase) family and TG (tissue transglutaminase)—have been found to be upregulated in hypertensive myocardium [79, 80]. It has been shown that the collagen cross-linking, not content, was associated with the LV chamber stiffness and filling pressure [80, 81]. Collagen cross-linking was also associated with a higher incidence of hospitalization in HHD patients [82]. On the other hand, the cardiac remodeling requires degradation of insoluble collagens to enable rearrangement of cells and matrix proteins, and reduction in collagen cross-linking was reported as well [70]. Therefore, the role of cross-linking has not been fully understood in HHD. Third, collagen fibers became more aligned in the hypertensive LV [65]. Similarly, enhanced fiber alignment has been reported in the RV. In rat PH RVs, the myo-fibers and collagen fibers were more strongly aligned in the longitudinal (apex-to-outflow) direction so that the tissue became more anisotropic in mechanical behavior [83].

4.1.2 Mechanical changes in hypertensive LV and RV

Myocardial stiffening is widely evident in HHD patients, particularly revealed by the increase in diastolic (passive) stiffness of the LV [65, 67]. The reduced mechanical strain is a surrogate of myocardial stiffening and became noticeable in hypertensive LVs even when the contractility was preserved [32, 84]. This indicates that the stiffening occurs in the early stage of HHD. Increased myocardial stiffness is thought to predominantly contribute to the diastolic dysfunction, which is evident by increases in isovolumetric relaxation time (IVRT), and end-diastolic volume or diameter (EDV or EDD) [85, 86]. Moreover, the persistent diastolic dysfunction with a dilatation/thinning of the myocardium is associated with impaired contractile (systolic) function, which was revealed by changes in dP/dt_{max}, dP/dt_{min}, end-systolic pressure-volume relations (ESPVR), fractional shortening (FS), ejection fraction (EF), stroke volume (SV), or cardiac output (CO) [87]. The mechanical changes are of high clinical relevance as the myocardial stiffness is significantly greater in the high-risk patients than in healthy controls, which may indicate a transition to heart failure with preserved ejection fraction (HFpEF) [88]. However, what initiates the transition from adaptive to maladaptive remodeling remains unclear.

Like the LV, the RV myocardium stiffens under chronic pressure overload [83, 89, 90]. The clinical evidence of RV stiffening in PH patients has been reported via the myocardial strain or strain rate measurements [91, 92]. Compared with the LV, the RV passive mechanics seem to play a more important role in physiological function, which is supported by the findings that the RV elasticity is more closely associated with the severity of RV failure and is better related to prognosis than the RV systolic function [93–95]. The RV stiffening is also evident from *ex vivo* measurements of RV mechanical properties from a number of preclinical animal studies. A significant increase in RV stiffness was noted in the PH group in both longitudinal and circumferential directions, with or without cardiomyocytes [96, 97]. But some reported a greater change in stiffness in the longitudinal direction [90], whereas others reported a greater change in the circumferential direction [89]. Therefore, the characterization of anisotropic mechanical changes of hypertensive RV needs to be further elucidated.

The potential clinical significance of RV stiffening has been explored in a few studies. Jang et al. found that in PH rats, the RV longitudinal elastic modulus (EM) derived at low strains was correlated with RV diastolic function (end-diastolic elastance). This is the first report on the linkage of RV tissue mechanics and in vivo hemodynamics [90]. Recently, from an ovine model of PH, the RV longitudinal stiffness was significantly increased and correlated with the long-axis end-diastolic or end-systolic diameter or area. Moreover, in the longitudinal (apex-to-outflow tract) direction, there were trends of correlations between the low-strain EM and the acceleration time, as well as between the high-strain EM and the deceleration time. These findings indicate the critical role of the RV passive mechanical properties in the organ function [98]. Nevertheless, the question remains as to how exactly the mechanical changes affect the transition from adaptive to maladaptive remodeling.

4.1.3 Different roles of fibrosis in hypertensive LV and RV?

Finally, the role of RV fibrosis in PH development may be different than the fibrosis in the hypertensive LV. For instance, RV fibrosis occurs early with the pressure overload and no report of collagen degradation has been noted in failing RVs. In contrast, collagen degradation has been documented in the late stage of LV failure (see **Figure 3** below). Second, the RV fibrosis measured by T1 mapping was correlated with pulmonary arterial stiffness and RV RAC (relative area change), but not correlated with pulmonary pressure, RV mass or ejection fraction in PH patients [99]. This indicates that the RV fibrosis may be an early marker of maladaptive RV remodeling before the deterioration in the functional metric [99]. Such prognostic role has not been reported in LV fibrosis. Third, different outcomes of anti-fibrotic treatment were found between LV and RV. In the LV, treatments that induced reduction of fibrosis had led to regression of chamber stiffness and function improvement [71, 100–103]. The beneficial outcomes of anti-fibrotic treatment are convincing and recently reviewed [104]. But interestingly, interruptions of collagen accumulation in RV had led to various consequences. The restriction of collagen accumulation using a transgenic mouse model resulted in limited RV hypertrophy and preserved RF function in PH development, indicating a protective role of anti-fibrosis therapy for the RV [78]. Other drug studies that demonstrated reduced RV fibrosis and improved RV function are briefly summarized by Bogaard et al. [105]. But recently, an anti-fibrotic intervention via suppressed Gelectin-3 expression (knock-out mice) or pharmaceutical inhibitors was insufficient to improve RV function in PH mice, despite an improvement in RV fibrosis [106]. These results raise more questions about the role of RV fibrosis in its function. Therefore, whether and how the fibrotic event precedes the functional decline in the RV may be organ specific and remains to be elucidated. Other different responses of the LV and RV to pressure overload are listed in **Table 2**.

4.2 Biomechanical adaptations in volume overload myocardium

Volume overload is initially learned as physiological responses of myocardium because of the reversible myocardial remodeling observed in athletes and women in pregnancy. But pathological responses are also found in patients with heart valve disease (regurgitation) and congenital heart disease, which alternatively lead to heart failure. As a result, different views form regarding whether the remodeling from volume overload is adaptive and irreversible [110, 111]. In contrast to pressure overload, volume overload is often treated as another type of mechanical 'insult'

	RV	LV
Fibrosis	Persistent in all stages	Mostly in early stage; degradation occurs in late stage
Cardiomyocytes	↓ α-MHC and ↑β-MHC (fetal gene expression); no atrial natriuretic peptide (ANP); inefficient energy metabolism	↑β-MHC only; ↑ ANP; Improved energy metabolism
Collagen isoforms	Type I and III	Type I, III, V, IV, VIII, and more
Collagen fiber	More aligned fibers into apex-to-outflow tract direction	Thicker, denser collagen type I, more cross-linking & aligned fibers
Dilatation	Occurs acutely in early stage	Occurs in late stage
Capillary rarefaction? (↓ blood perfusion)	Yes	No
Inflammatory response involved?	Yes (macrophages, TNF-α, IL-1β)	Yes (T lymphocytes, TNF-α, IL-1β)
Response to anti-fibrosis treatment	Discrepant findings on function improvement	Mostly effective

Table 2.
Different responses of the LV and RV to pressure overload. MHC: myosin heavy chain; ANP: atrial natriuretic peptide. Adapted from [107–109].

in which the tissue is stretched beyond its normal state during diastole [112–114]. Under this type of mechanical load, collagen loss and chamber dilatation occurred since the early stage and these changes persisted, leading to an overall decrease in cardiac ECM [112–115]. Such remodeling was attributed to an increase in MMP activity [113]. But elastin showed biphasic changes in the progression of ECM remodeling. Ruzicka et al. reported an initial (within one week of induced volume overload) increase in elastin concentration but then a decrease of elastin concentration below control levels 10 weeks after induced volume overload [114]. The reduction of ECM turnover in volume overloaded LV was related to a phenotype change of fibroblasts into hypofibrotic type [116] and increased MMP expressions from macrophages or mast cells [117–119]. An increased collagen III/I ratio was also noted in the compensated stage of the remodeling [114]. The elevated ECM degradation in volume overloaded LV seems to share common pathways as seen in late stage of HHD—to enable ventricle dilatation and thinning and weaken the cell-matrix connections, which impairs contractile function.

The RV did not undergo initial remodeling to the same extent of the LV. It is well known that the RV is better in adaptation of the volume overload whereas the LV is better in adaptation of the pressure overload [107, 120]. The chamber difference also lies in the fact that LV alterations are more widely reported on than those of the RV and that the RV has exhibited milder remodeling than the LV [121]. The less pronounced remodeling including the ECM alteration of the RV may be explained by the different origins and contractile behaviors of cardiomyocytes [120, 122] and, thus, different responses to the mechanical stimulations.

Like in pressure overloaded HF, myocardial stiffening also arises in response to volume overload and eventually leads to heart failure [123, 124]. For instance, Emery et al. reported a 10-fold increase in LV mid-wall stiffness along the fiber

direction and the cross-fiber direction six weeks after volume overload induction [125]. The underlying cause of the myocardial stiffening is investigated by collagen measurement. Interestingly, despite the decrease in the relative collagen content, there was an upregulation of collagen cross-linking [115]. A similar finding was identified using hydroxylysylpyridinoline (HP) assay in minipig LVs [126]. Therefore, despite a decrease in total collagen content, the tissue stiffening occurs due to elevated cross-linking in these ventricles [115, 125, 126].

Myocardial compliance, however, showed different trends of changes than the intrinsic (material) mechanical property of the myocardium. This is because the chamber compliance is a measurement of overall stiffness that incorporates changes in the intrinsic mechanical property, the geometry of the wall (dilatation and thinning) and the contractility of the heart (ventricular pressure). In the acute stage, the LV wall dilated and the compliance increased 2 days after induction of volume overload [112]. Similar findings are reported in the compensated stage. A significant decrease in the end-diastolic pressure-volume relationship (EDPVR) and a right shift of the pressure-volume loop were seen in the group with 8-week volume overload [127]. This indicated increased compliance and chamber dilatation. But at 15–21 weeks of volume overload, there was no significant change in the EDPVR compared to that at week 8 [127]. Thus, the maintained compliance may be a combined results of increased intrinsic stiffness and decreased myocardial thickness.

4.3 Different myocardial remodeling induced by pressure and volume overload

Both pressure overload and volume overload are categorized as the hemodynamic (mechanical) 'insult' of the myocardium. The pressure overload is considered as an afterload increase whereas the volume overload is considered as a preload increase. Therefore, the main mechanical stimulus difference lies in the 'phase' – systolic phase for pressure overload and diastolic phase for volume overload, during which the cells sense increased wall stress or stretch. The comparison of physiological changes and cellular responses are reviewed previously [110, 111, 128], and temporal myocardial responses to pressure overload and volume overload are summarized in **Figures 2** and **3**.

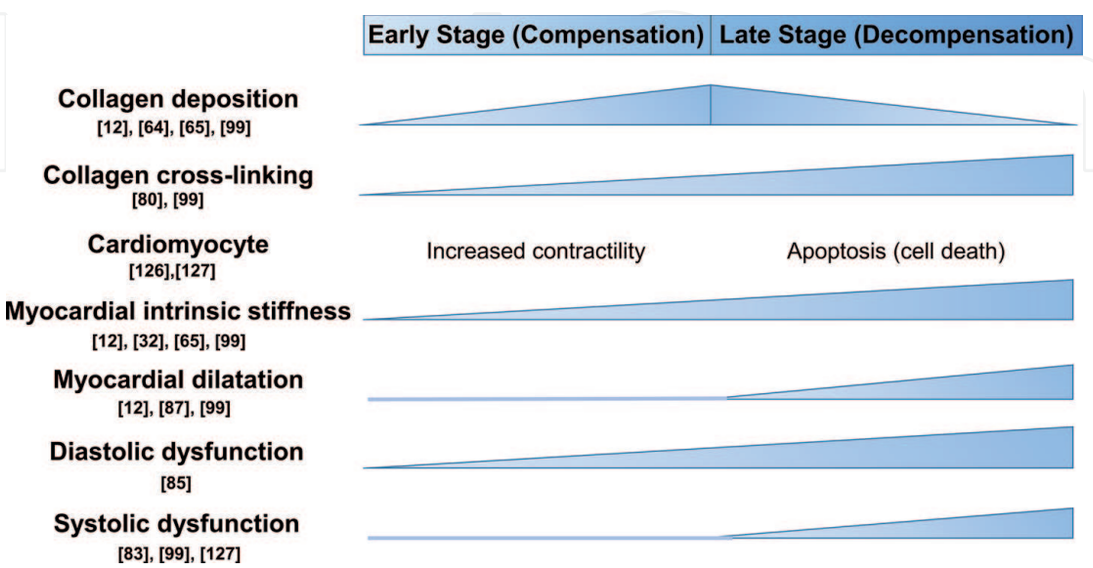

Figure 2.
Temporal changes in myocardial fibrosis, wall stiffening and physiological function in pressure overload induced heart failure progression.

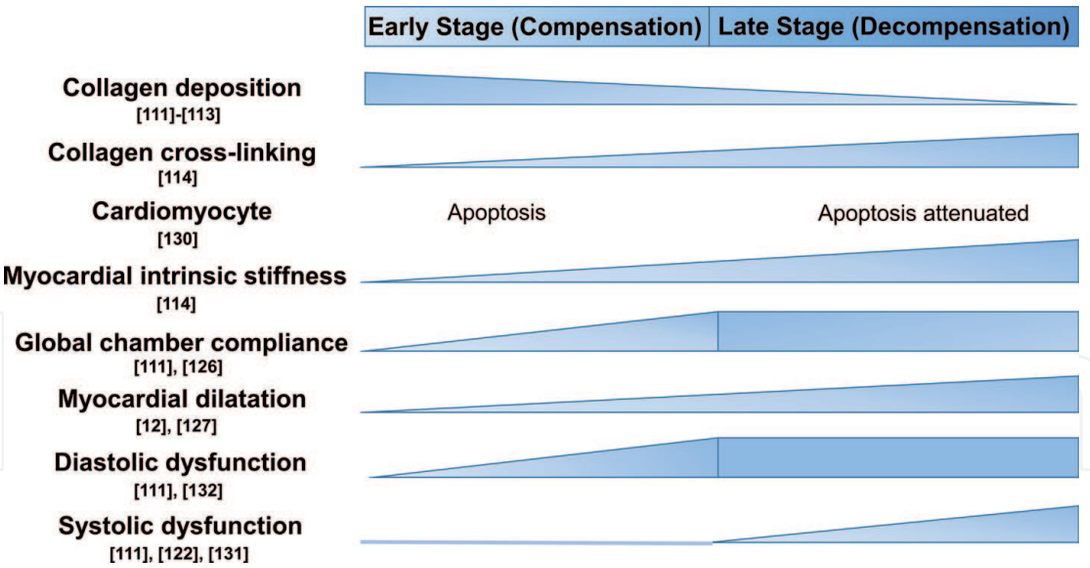

Figure 3.
Temporal changes in myocardial fibrosis, wall stiffening and physiological function in volume overload induced heart failure progression.

The initial (early) remodeling of these two types of overload is different, leading to concentric remodeling (reduced volume and increased wall thickness (h) to radius (r) ratio) in pressure overload and eccentric remodeling (increased volume and reduced h/r ratio) in volume overload. Sometimes these changes are considered as 'adaptive' since the wall stress was normalized by the remodeling (according to Law of Laplace). However, at the cellular level, the 'growth' of cardiomyocytes is in width (pressure overload) and in length (volume overload), respectively. Interestingly, it remains unclear why and how the cardiomyocytes respond to the higher stress at passive and active states differently [129]. Furthermore, as pointed by Pitoulis et al., the late remodeling of these two types of overload 'converges', indicating some common pathways shared in the decompensation stage [128]. The convergence could be caused by a co-existence of these mechanical overloads in the same patient or by the key shared pathways that lead to irreversible remodeling at the late stage of heart failure. These are important questions that remain to be investigated to further improve the clinical management of non-ischemic heart failure patients.

4.4 Biomechanical adaptations in myocardial infarction (ischemia)

Myocardial infarction (MI) is caused by a reduction of blood perfusion in coronary arteries and necrosis—cardiomyocyte cell death [130]. This is the most studied type of heart failure, and there are numerous reviews on ECM changes in MI [1, 2, 21]. Acute and chronic MI lead to significant inflammatory and fibrotic responses by recruitment and activation of neutrophils, macrophages and (myo) fibroblasts. Interstitial and perivascular fibrosis from both replacement and reactive types of collagen accumulation greatly reduces the cardiomyocyte population and muscle contractility. The failure to resolve the fibrotic region (scar) results in a continuous stiffening and dilatation of myocardium and irreversible HF [105]. Compared to the LV, the RV is less susceptible to ischemic injury and it can recover after prolonger coronary occlusion [131, 132]. Therefore, our discussion below is mainly focused on the LV.

The initial response of MI is a cascade of events including the release of MMPs and proteases, which is thought to trigger the post-MI inflammatory response and

to degrade the ECM following proliferative phase [12]. Then, collagen deposition is seen most prevalently in the infarcted region, creating a fibrotic scar [133]. The injured region can be clearly identified, with a high-density mesh of small diameter, aligned collagen fibers [134]. Both collagen types I and III were increased in infarcted LVs [134, 135]. Cleautiens et al. found that type I procollagen expression was increased and peaked by 10-fold four days post-MI, and the elevation remained 90 days after infarction. A similar trend was seen in type III procollagen, however its levels reached a peak later at day 21 post-MI [135]. Alternatively, experimental work also shows that the procollagen type III induction occurs earlier than that of procollagen I [136]. Other fibrillar collagen, such as types V and VI, increased in infarcted areas; for instance, the content of type VI peaked two weeks post-MI [137]. Elastin content was found to increase initially, but it rapidly decreased to values lower than the healthy tissue [134].

Moreover, regional changes occur in the non-infarcted zones. In the rat LV, collagen concentration increased by 50% in the infarcted region, whereas only a 27% increase was reported in the remote region [138]. Furthermore, collagen cross-linking only occurred in the infarcted zone and not in the remote region [138]. Similarly, there was a gradient of changes in the mechanical properties in different regions. The infarcted region showed mechanical alterations to the highest degree; the border zone showed moderate changes, and the remote region showed little to no change [139]. ECM remodeling also arises in the septum and RV with infarcted LV [135]. A 4–5 folds increase in expression of procollagen types I and III mRNA was observed in the septum within three weeks post-MI, followed by a decline to control levels. A less but significant elevation of collagen types I and III also occurred in the RV, with levels of type III remaining elevated 90 days post-MI [135].

The injured area post-MI is predominantly a fibrotic scar with time-dependent changes in mechanical properties. Torres et al. found that the myocardial stiffness increased initially in the MI region, then in the border zone and the remote region. At 28 days post-MI, the myocardial longitudinal stiffness in all three regions decreased, and the reduction was most notable in the MI region with a 40% decrease [139]. Decreases in stiffness in the infarcted zone in late MI have been documented in other studies: the tissue becomes more compliant and more isotropic compared to the healthy tissue [140]. The initial increase in stiffness can be attributed to deposition of collagen and increased collagen isotropy [141]. At later stages, tissue became softer (e.g., 6–8 weeks post-MI) due to collagen degradation [131, 132, 140, 141]. These biomechanical changes are speculated to contribute to the further thinning and dilation of the myocardium leading to irreversible HF. Thus, the end-stage of HF due to MI, pressure overload and volume overload seem to share some common pathways associated with similar biomechanical alterations.

4.5 Biomechanics of heart valves

4.5.1 Biomechanical alterations in heart valve disease

Heart valve disease can occur as a result of various causes such as aging, birth-defects, or infections. Depending on the mechanical abnormalities, the heart valve disease can be categorized into two types—stenosis (with reduced opening of valves) and regurgitation (with incomplete closure of valves). These mechanical changes will increase the mechanical load of the ventricle and reduce cardiac output. Often the disease progression involves a mix of pressure and volume overloads and biventricular dysfunction. Aortic valve disease (AVD) is the most studied

heart valve disease and involves high levels of ECM degradation and calcification. For instance, collagen I and III decreases resulted in decreased valve stiffness [142]. But the aggregation of calcium hydroxyapatite (calcification) that replaces degraded collagen can lead to an ultimate stiffening of the valve leaflets. We recommend the reader to these thorough reviews of the biomechanical changes of heart valves in heart valve disease [143–145].

4.5.2 Role of ECM in the mechanics of heart valves

In healthy valve leaflets, collagen makes up ~90% of the ECM and thus is the main load-bearing component. Elastin, proteoglycans, and GAGs also contribute to the mechanical properties of the heart valves. This dense connective tissue is highly organized and present special viscoelastic mechanical behavior (minimal creep but significant stress relaxation) [146]. Therefore, any changes in the collagen fiber orientation or ECM proteins will induce mechanical dysfunction and thus abnormal opening/closing of the valves. The necessity of investigation of valve ECM is recently reviewed [143]. Furthermore, for the atrioventricular valve (i.e., mitral valve or tricuspid valve), the additional connection of the leaflets (LL) to the chordae tendineae (CT) and then further to papillary muscles (PM) has extended the research into transition regions of LL-CT and CT-PM. Because CT rupture is the primary cause of valve regurgitation, the mechanical properties of these regions are critical to delineate the pathology. Advanced methodologies on collagen fiber quantification (e.g., X-ray diffraction) and computational models were recently adopted to investigate the macro- and micro-mechanical properties of these transition regions [147, 148], although the research has been mostly focused on healthy tissues. A nice review of the microstructural mechanical characterization on CT of valves is referred here [149].

Collagen fibers in the CT contribute greatly to the overall function of the valve [147]. From the mitral valve (MV), collagen fibers found in the CT form a 'core' that are oriented longitudinally, with another group of collagen fibers that wrap around the core, offset from the primary axis [150]. Conversely, it has been observed that the CT extending from the tricuspid valve (TV) consists of smaller diameter of fibers but in higher densities than the MV [151]. This difference in collagen formation is a result of the mechanical needs of each CT, as the TV experiences lower loading than the MV. In diseased valves such as myxomatous degeneration of the mitral valve (MDMV), collagen deposition and myofibroblasts activation are observed. Although accumulation of collagen fibers is often associated with increased stiffness, the CT in MDMV actually becomes more compliant. Barber et al. found that the elastic modulus (EM) of healthy CT (132 ± 15 MPa), as well as the failure strength (25.7 ± 1.8 MPa), were significantly higher than those of diseased CT in MDMV (40.4 ± 10.2 MPa and 6.0 ± 0.6 MPa, respectively) [152]. A similar finding was also observed in other valve diseases: myxomatous degeneration of the tricuspid valve by Lim et al. [153], as well as by Casado et al. in the calcified mitral valve CT [154]. In such cases, the collagen fibers in the center core of the CT became disorganized and were no longer formed in tight bundles as they do in healthy CT [153]. Therefore, the altered alignment and dis-organization of the collagen fibers reduce the overall stiffness as well as the tensile strength of the CT.

5. Mechanobiology of cardiac cells

As we discussed above, ECM remodeling is a critical part of tissue's response to altered mechanical loads or other pathological stimulations (e.g., ischemia)

[112–114, 155–160]. This leads to dynamic alterations of the myocardial mechanical properties including the anisotropic, nonlinear elastic behavior as well as the viscoelastic behavior. The clinical relevance of ventricular mechanical behavior has been recently reviewed by our group [76]. In this chapter, we will focus on another impact – the cellular response to substrate biomechanical properties. Because of the prominent role of fibrillar collagen in myocardial mechanical behavior (especially in diseased tissues), we will mainly discuss the cellular response caused by fibrillar collagen deposition. The influence of other ECM proteins on cardiac cells during HF progression is reviewed in these references [12, 161].

The cardiac cell's response to mechanical factors has been mostly investigated by exposing cells to steady or cyclic stretches to mimic myocardial contraction. Compared to the unstretched condition, there was significant increase in procollagen type I activity in cultured fibroblasts under cyclic stretching [156, 157]. Alternatively, Carver et al. evaluated the change in collagen III/I ratio in isolated cardiac fibroblasts. After 12 hours of cyclic loading, there was a 70% increase in the ratio of type III to type I collagen compared to unstretched cells [158]. Not only the collagen synthesis but also the degradation signaling pathway are upregulated by the mechanical loads. Multiple studies have found an upregulation of MMPs, particularly MMP-2 and MMP-9, in response to mechanical stretches compared to unstretched conditions [113, 155, 159]. Since the expression of collagen or procollagen was greater than that of MMP's, a net increase in collagen was observed [155]. This mechanical regulation can be attributed to higher levels of transforming growth factor beta 1 (TGF-β1), which stimulates fibroblast's growth and transdifferentiation and ECM protein synthesis [155, 160]. However, while these findings strongly advocate the mechanical regulation of cardiac fibroblasts and fibrogenesis, the unstretched condition is non-physiological and thus it is difficult to directly translate the findings into pathogenesis of fibrosis and heart failure.

More in-depth investigation of the substrate's mechanical regulation is performed by varying the magnitudes of mechanical strains or comparing the effects of tensile stretch and compression. For example, after 24 hours of culture of cardiac fibroblasts, Lee et al. found that the expression of collagen type I mRNA significantly increased at uniaxial strain of 10% but showed no change at 20% strain. In contrast, type III collagen mRNA expression significantly increased at 10% strain but decreased at 20% strain. The response of collagen type III was more prominent than collagen type I [160]. In compression though, no significant change was noted in type I or III collagen mRNA. The different response between stretch and compression is intriguing as it implies that it is the mechanical signal in diastole (stretching of wall), not in systole (compression of wall), that stimulates the fibrotic response in fibroblasts. But a discrepant result is also reported. Kong et al. observed at compressive cyclic loading (5–20% strain) an upregulation in collagen type I and TGF-β1 expression [155], whereas Lee et al. reported significant increases only in TGF-β1 expression and no notable changes in collagen type I at 6% compressive strain compared to unstretched cells [160].

Besides the mechanical forces, it is also critical to investigate if and how the mechanical stiffness of the substrate affects cellular function. To date, a few *in vitro* studies have reported the regulation of matrix stiffness using synthetic hydrogels tuned to match the stiffness of healthy and diseased myocardial tissues. When cultured on different stiffness of poly (ethylene glycol) (PEG) hydrogels, cardiac fibroblasts were activated into myofibroblasts by increased stiffness (6 vs. 60 kPa) and addition of TGF-β1 stimulation. Furthermore, only the condition medium from fibroblasts cultured on stiff matrix treated with TGF-β1 caused neonatal rat ventricular myocytes enlargement, indicating a synergistic effect of matrix stiffness and TGF-β1 on the activation of myofibroblasts and myocyte hypertrophy [162]. In

addition, the fibroblasts responded to the dynamic stiffening of the PEG hydrogel (from 10 to 50 kPa) by increasing cell spread area and reducing nuclei roundness within 2–5 days of culture, mimicking the *in vivo* observation of phenotype changes of fibroblasts [163]. Increased fibroblast cells spreading and collagen type I expression, and decreased collagen III expression were reported in stiffer matrix

Biomechanical Cue	Cell Type	Main Finding	Ref
Polyacrylamide gel with varying stiffnesses: 8 kPa, 15 kPa, 50 kPa, 100 kPa (stiffness range from healthy to diseased heart	Adult Rat Cardiomyocytes (CM)	Contracting force of the CM tends to increase with matrix stiffness. Contractile function, though, is normalized when the matrix stiffness was returned to healthy levels	[165]
PDMS surfaces with varying stiffnesses: 1 kPa, 6 kPa, 20 kPa, 130 kPa (embryonic stiffness to fibrotic heart stiffness)	Adult Rat Cardiomyocytes (CM)	On soft surfaces, CM exhibits greater contraction than those on stiff surfaces. Sarcomeres show faster shortening speeds on soft surfaces. TGF-β1 is more prevalent on stiffer surfaces	[166]
PEG hydrogels with varying stiffnesses: 10 kPa (healthy neonatal heart), 35 kPa (diseased neonatal heart)	Neonatal Rat Ventricular Cardiomyocytes (CM)	Regardless of matrix stiffness, myocytes spontaneously contract and form healthy, organized sarcomeres. However, fractional shortening increased only on soft gels	[167]
Collagen type I matrix morphology (fibrous vs. non-fibrous) and stiffness (48–170 kPa)	Alveolar-like Macrophages	Non-fibrous collagen leads to cells with higher filopodia (actin-rich protrusions) and higher CD206 expression, whereas stiffness has no significant impact on cell behavior	[168]
Fibronectin coated polyacrylamide gels with varying stiffness: soft (1–5 kPa) to represent healthy artery and stiff (280 kPa-70 GPa) to represent atherosclerotic plaque	Human Macrophages	Stiffer substrates leads to larger cell spread area, faster migration speed, less dense but more organized F-actin, and increased proliferation rate	[169]
Polyacrylamide Gel with varying stiffness: 1.2 kPa to represent normal lungs and 150 kPa to represent fibrotic lungs	Mouse macrophages	Cell elasticity increases with increased matrix stiffness; and cell elasticity is a major determinant of innate macrophage function	[170]
PEG-RGD hydrogel with tangent (compressive) modulus from 130 kPa to 840 kPa	Mouse macrophages	Stiffer hydrogel leads to larger cell spread area and more extended branches of actin, activation of pro-inflammatory cytokines, and more severe foreign body response	[171]

Table 3.
Summary of in vitro studies of matrix stiffness dependent changes in cell behavior and function.

(3 vs. 8 kPa), which is postulated to imitate the late stage MI heart with matured scar formation [164]. In the same study, it was also found that the cross-linking of collagen was 'triggered' by non-equibiaxial static stretch mimicking *in vivo* strains in the border region rather than the matrix stiffening. While this experiment design de-couples the mechanical stretch and stiffness, it must be noted that in physiological conditions, these two factors are not independent – a stiffer material will result in reduced stretch/strain under the same pressure. We have listed a few *in vitro* studies that investigated the responses of cardiomyocytes or macrophages to substrate stiffness in **Table 3**. These pilot studies highly support a role of ECM mechanical properties in cellular regulation relevant to cardiac ECM remodeling. Overall, the area of cardiac mechanobiology is still very young, as we reviewed recently [76]. The discrepant elastic moduli reported from literature and different matrix stiffness ranges selected by various groups have increased the difficulty to delineate the cellular responses in the progression of cardiac diseases. Therefore, future studies should aim to further elaborate the role of matrix mechanics (stretch/strain, stiffness, etc.) in heart failure development.

6. Conclusions

The cardiac ECM is critical in maintaining cardiac tissue structure and function. Many studies have been conducted to measure ECM proteins in healthy and diseased myocardium to better understand their roles in cardiac remodeling – including the biomechanical changes. Our review shows that the ECM remodeling (particularly collagen accumulation) in HF is both spatially and temporally dependent. We have compared the myocardial collagen deposition, wall stiffening and systolic and diastolic dysfunction between early and late stages of various types of HF. While the initial remodeling events being quite different among these diseases, common biomechanical changes are shared in the end-stage of HF – ECM degradation with persisted cross-linking, which are associated with thinning and dilatation of the myocardial wall. However, the relation of cardiac fibrosis to the transition from compensation to decompensation remains to be elucidated. Furthermore, we have high-lighted different responses of the LV and RV to 'identical stimulus' (pressure overload, volume overload and ischemia). The interventricular difference should be another important future direction of research, which may help to bring new insights into the pathogenesis and treatment for ventricular failure.

Conflict of interest

The authors declare no conflict of interest.

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