Advances in Experimental Medicine and Biology 1098

Eric G. Schmuck · Peiman Hematti
Amish N. Raval *Editors*

Cardiac Extracellular Matrix

Fundamental Science to Clinical Applications

Springer

Advances in Experimental Medicine and Biology

Volume 1098

More information about this series at http://www.springer.com/series/5584

Eric G. Schmuck • Peiman Hematti
Amish N. Raval
Editors

Cardiac Extracellular Matrix

Fundamental Science to Clinical Applications

Springer

Editors
Eric G. Schmuck
School of Medicine & Public Health
University of Wisconsin-Madison
Madison, WI, USA

Peiman Hematti
School of Medicine & Public Health
University of Wisconsin-Madison
Madison, WI, USA

Amish N. Raval
School of Medicine & Public Health
University of Wisconsin-Madison
Madison, WI, USA

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-97420-0 ISBN 978-3-319-97421-7 (eBook)
https://doi.org/10.1007/978-3-319-97421-7

Library of Congress Control Number: 2018954646

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

For their unwavering support and encouragement, I dedicate this book to my wife, Katie and my children, Owen and Lillian.

Eric

To my wife, Shirin, and my daughters Neeloufar and Faranak,
For their Endless Love and Never-ending Support.

Peiman

With loving adoration and with warm gratitude for their support of me, I dedicate this book to the strong women of my life, Nilam, Serena, Kirin, and Layla.

Amish

Introduction

Cardiac extracellular matrix (ECM) forms the structural and biochemical foundation of the heart. Historically, cardiac ECM was thought to be an inert web of fibrous proteins, providing structural support and synergizing myocyte contraction from the cellular to the organ level. It is now understood that cardiac ECM plays a much broader foundational role by directing cell survival, migration, proliferation, anoikis, apoptosis, and inflammatory responses. Although all the mechanisms by which cardiac ECM regulates cell behavior are not fully understood, variables such as stiffness, architecture, availability of integrin-binding domains, and the presence of bioactive molecules have been shown to play important roles.

Our evolving understanding of the complex nature of cardiac ECM has led to important discoveries in the areas of cardiac development, normal cardiac ECM deposition and maintenance, cardiac ECM-associated pathologies, and the potential therapeutic value of cardiac ECM in regenerative medicine. As our understanding of cardiac ECM has grown, so has the potential to manipulate and/or harness cardiac ECM for bioengineering, tissue engineering, and therapeutic applications. Cardiac ECM applications include in vitro culture systems for pharmaceutical testing, and development of cardiac ECM and cardiac ECM inspired materials as stand-alone therapeutics or in combination with various therapeutic cell types as a means to regenerate damaged or lost heart tissue. Whole decellularized hearts have been used as a framework for growing new hearts. This approach, combined with induced pluripotent stem cell technology, may one day result in personalized whole heart transplants.

This book explores a broad range of topics related to cardiac ECM. Topics range from the basic structural and biochemical properties of cardiac ECM to translational topics including bioengineering and therapeutic application. Finally, this book incorporates important clinical considerations including manufacturing and clinical trial design to transferring cardiac ECM technologies from academia to the private sector for further clinical development. The editors would like to sincerely thank all of the authors who contributed to the completion of this book. Without their outstanding contributions, this book would not have been possible.

Contents

About the Editors

Eric G. Schmuck, PhD is a senior scientist in the Division of Cardiovascular Medicine at the University of Wisconsin-Madison School of Medicine and Public Health where he leads multidisciplinary research teams focused on bringing regenerative medicine therapies to the clinic. He received his PhD in Human Physiology from the University of Wisconsin-Madison and is an expert in cardiac extracellular matrix and translational research models, specifically focusing on cardiac regeneration. His major research interests are in cardiac extracellular matrix, bioengineering, and regenerative medicine therapies for the heart. He has authored numerous peer-reviewed manuscripts and book chapters in the regenerative medicine field and holds multiple patents on cardiac extracellular matrix technologies. Finally, he is a co-founder and chief scientific officer of Cellular Logistics Inc., a biotech company developing innovative biomaterial solutions to enhance stem cell retention for the treatment of advanced cardiovascular disease.

Peiman Hematti, MD is professor of Medicine in the Section of Hematology/Oncology, University of Wisconsin-Madison School of Medicine and Public Health, with joint appointments in departments of pediatrics, surgery, and biomedical engineering. He is director of the Clinical Hematopoietic Cell Processing Laboratory and medical director of Apheresis and Hematopoietic Cell Collection facility at the University of Wisconsin-Madison. As a bone marrow stem cell transplant physician, his major clinical research interest is in immunology of transplantation and use of novel

cellular therapies for malignant and nonmalignant disorders, including the use of bone marrow stem cells in cardiovascular medicine. His current laboratory research focuses on immunobiology of stem cell transplantation with a special focus on investigating the immunomodulatory and anti-inflammatory properties of macrophages, including their interactions with biomaterials used in regenerative medicine. He has been a coauthor in more than 100 peer-reviewed manuscripts and 10 book chapters and has been coeditor of two other books in the field of stem cells and regenerative medicine. He has been inventor of several patents issued and filed in the field of cellular therapy and regenerative medicine. He is a co-founder and chair of the Scientific Advisory Board for Cellular Logistics Inc.

Amish N. Raval, MD is an associate professor of Medicine and Biomedical Engineering at the University of Wisconsin. He received his MD at the University of Western Ontario, where he completed Internal Medicine and Cardiology Residency. He subsequently completed his Interventional Cardiology Fellowship at Georgetown University/Washington Hospital Center, and later, he joined the National Heart, Lung, and Blood Institute, Bethesda. Thereafter, he became a faculty member at the University of Wisconsin School of Medicine and Public Health where he is director of Clinical Cardiovascular Research. He is an accomplished interventional cardiologist with expertise in structural heart and endovascular and coronary interventions. His translational research is focused on therapeutic delivery of biologic agents, including stem cells and scaffolds for cardiac and vascular disease. He has numerous peer-reviewed publications, national scientific statements, and patents in this field. He is the principal investigator of >12 cardiovascular regenerative medicine clinical trials ranging from Phase 1 to Phase 3. He has served as a scientific advisor for several biotech companies and is chairman of the Board for Cellular Logistics Inc., which is a biotech company that is developing innovative biomaterial solutions to enhance stem cell retention for the treatment of advanced cardiovascular disease.

Chapter 1
Biomechanical Properties and Mechanobiology of Cardiac ECM

Michael Nguyen-Truong and Zhijie Wang

Abstract The heart is comprised of cardiac cells and extracellular matrix (ECM) which function together to pump blood throughout the body, provide organs with nutrients and oxygen, and remove metabolic wastes. Cardiac ECM provides a scaffold to cardiac cells and contributes to the mechanical properties and function of the cardiac tissue. Recently, more evidence suggests that cardiac ECM plays an active role in cardiac remodeling in response to mechanical loads. To that end, we provide an overview of the structure and function of the heart and the currently available in vivo and ex vivo mechanical measurements of cardiac tissues. We also review the biomechanical properties of cardiac tissues including the myocardium and heart valves, with a discussion on the differences between the right ventricle and left ventricle. Lastly, we go into the mechanical factors involved in cardiac remodeling and review the mechanobiology of cardiac tissues, i.e., the biomechanical responses at the cellular and tissue level, with an emphasis on the impact on the cardiac ECM. The regulation of cardiac ECM on cell function, which is a new and open area of research, is also briefly discussed. Future investigation into the ECM deposition and the interaction of cardiac cells and ECM components for mechanotransduction can assist to understand cardiac remodeling and inspire new therapies for cardiac diseases.

Keywords Left ventricle · Right ventricle · Collagen · Fibrosis · Biomechanics · Mechanobiology · Mechanical response · Viscoelasticity

M. Nguyen-Truong
School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA

Z. Wang (✉)
School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA

Department of Mechanical Engineering, Colorado State University, Fort Collins, CO, USA
e-mail: Zhijie.Wang@colostate.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_1

1.1 Introduction

1.1.1 Overall Structure and Function of the Heart

Central to human life, the heart serves to maintain the systemic and pulmonary blood circulations which provide nutrients and oxygen while removing wastes from all organs in the body. The heart, often analogous to a "pump," is made up of four chambers (i.e., left and right atriums, left and right ventricles) and four valves (i.e., mitral valve, aortic valve, tricuspid valve, and pulmonary valve) (Fig. 1.1). In this unique architecture of the organ, the blood is "pumped" out of the heart into systemic or pulmonary circulation via the contraction of the left ventricle (LV) or right ventricle (RV), respectively. The open and close of the valves during systole (contractile) and diastole (relaxing or dilated) phases are also important steps to ensure the sequential, unidirectional blood flow between the chambers.

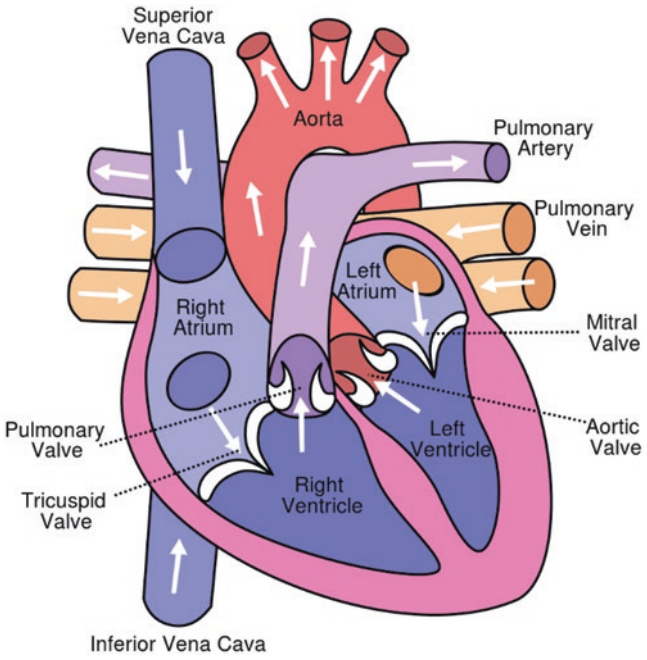

Fig. 1.1 Structure of a heart with major connecting blood vessels, valves, and the four chambers (left atrium, left ventricle, right atrium, and right ventricle). (The figure is from Wikipedia (https://en.wikipedia.org/wiki/Heart))

1.1.2 Different Cardiac Cell Types

The composition of cardiac tissues varies depending on the location of the tissue. In the myocardium (i.e., the muscular layer of the ventricular wall), the typical cell types include cardiomyocytes (cardiac muscle cells), cardiac fibroblasts (CFs), endothelial cells (ECs), and smooth muscle cells (SMCs). The cardiomyocytes occupy around 70% of the volumes of the ventricular wall under normal circumstances [72]. However, the CF is the most abundant cell type in the myocardium, and the main role of fibroblasts lies in the synthesis and maintenance of the extracellular matrix (ECM) in the myocardium [45]. The number of CFs (or the ratio of fibroblasts to myocytes) varies depending on tissue regions, species, and age [4].

In the heart valves, the main cells include the interstitial cells and endothelial cells. The interstitial cells are specialized cells that mostly exhibit the characteristics of smooth muscle cells and myofibroblast cells. They are responsible for the synthesis and maintenance of ECM in the heart valves [69]. Valvular ECs play essential roles in establishing the valve structures during embryonic development and are important for maintaining lifelong valve integrity and function [68].

1.1.3 The ECM Composition in Cardiac Tissues

The cells in the heart are supported by the architecture maintained by the ECM components. The conventional wisdom considers the ECM as a passive component that mainly affects the mechanical properties of cardiac tissue. However, more and more evidence suggests that cardiac ECM can play an active role in the tissue remodeling process in response to altered mechanical loadings, which is reviewed in the later sections of this chapter.

In the myocardium, the ECM consists of proteins such as collagen, elastin, fibronectin, proteoglycan, and laminin. Collagen is the most abundant ECM protein in the heart, with at least five different types of collagen (I, III, IV, V, and VI) that have been identified in the myocardium [72]. Whereas types IV and V collagen are mostly found in the basement membrane of the myocytes, types I and III collagen are the main constituents in the ECM: type I collagen represents 75~80% of total collagen content and type III collagen represents approximately 15~20% of the total collagen [41]. A small percentage of collagen is composed of type V collagen (less than 5%) [5, 72]. The collagen content in the heart depends on species: the larger the mammal, the greater the collagen content of the heart [4]. Cardiac collagen metabolism, i.e., the balance of synthesis and degradation, is profoundly affected by various cardiac cells and secreted cytokines, growth factors, hormones, enzymes, and other reagents [72]. Lastly, because of the branching of cardiomyocytes and the spiral orientation of these cells called the “myocardial band,” the investigation on the structure and arrangement of ECM surrounding the myocytes as well as its interaction with the cells has been challenging and remains a matter of debate.

The heart valves are leaflets that can be divided into three layers (the fibrosa, spongiosa, and ventricularis layers) when viewed from the cross-sectional structure. Each layer has a distinct ECM composition that aids in normal mechanical behavior of the valve. The mechanical function of valves is achieved by a highly organized and hydrated network of ECM proteins including collagen (rich in fibrosa layer), elastin (rich in ventricularis layer), glycosaminoglycans (GAGs), and proteoglycans (PGs) (rich in spongiosa layer) [32, 81]. Water comprises about 60–70% of the valves by weight, which is similar to other soft tissues such as arteries, skin, cartilages, etc. The hydrated nature may be especially important for heart valves because it enables the diffusion of nutrients and oxygen through the valvular tissue, which is largely avascular in adults [61].

1.2 Mechanical Measurement of Cardiac Tissues

The mechanical properties of cardiac tissues have a great impact on the overall function of the organ, and changes in mechanical properties are often associated with various cardiovascular diseases (e.g., hypertension, ischemic heart failure, and diabetes). Therefore, the measurement of mechanical behavior of cardiac tissues is important to the understanding of the disease progression and the development of new therapies.

1.2.1 Ex Vivo Measurement

In materials science, the general principle to obtain a material's mechanical behavior is to characterize its force-deformation relationship. That is, the researcher simultaneously records the applied force (e.g., pressure or stretch force) and the changes in dimension/morphology (e.g., diameter or length) to derive a stress-strain curve (see an example in Fig. 1.2).

To date, both uniaxial and biaxial mechanical tests have been performed on a tissue strip harvested from the heart to quantify the mechanical properties [20, 32, 48, 59]. Not surprisingly, the tissue presents different mechanical behaviors under uniaxial and biaxial conditions (e.g., the tissue is stiffer under biaxial conditions [20]). As a result, the biaxial mechanical test is especially critical for cardiac tissues because of the presence of anisotropy between different fiber directions in the heart wall [20, 72] and valves [67]. The biaxial mechanical test can be combined with 2D or 3D imaging techniques such as tracking markers or 3D ultrasound speckle tracking (3D-UST) which assess the strain (deformation) of the tissue. The mechanical behavior coupled with the strain information can then be used to obtain mechanical properties and compute fiber orientation from the strain energy function (SEF) [36, 49].

Furthermore, depending on the protocol of the test, the tissue can be tested in static or dynamic conditions, and in the latter case, viscoelastic properties are

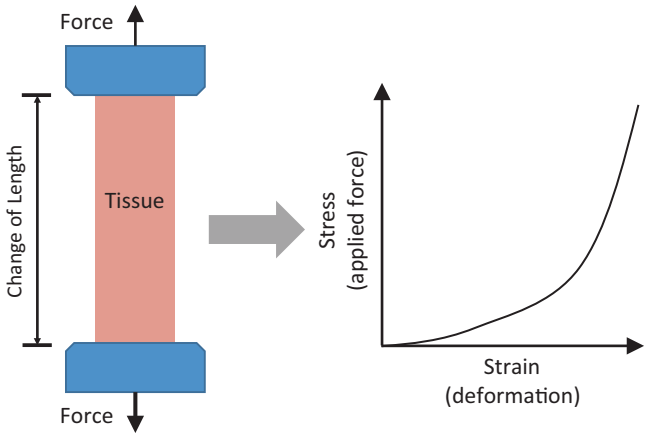

Fig. 1.2 Schematic of a uniaxial mechanical test. The biological tissue is cut into a rectangular shape and mounted on a tensile testing system

obtained with the cyclic loading and unloading of the tissue specimen. The viscoelasticity is the best characterization of the mechanical properties of cardiac tissues because this is the true, in vivo behavior of a living tissue under pulsatile blood flows [79]. The understanding of the viscoelasticity and the molecular basis for the viscoelastic properties is an important future direction of research to better restore cardiac tissues' performance with treatment. In either steady or pulsatile mechanical testing, the pre-conditioning of the specimen, which is a number of loading and unloading cycles applied to the tissue after mounting to the apparatus and before the real test, is a key step that induces a constant mechanical response to a load or deformation and thus allows for a more accurate measurement of the mechanical properties [79].

In addition, another uncommon method called ball burst mechanical testing can determine the biaxial burst strength (maximal force) of the tissue [76]. For heart valves, more testing methods are developed to measure the flexural properties, which include the three-point bending, cantilever bending, and macro-indentation tests. These methods are recently reviewed [32].

Finally, because the myocardium is a specialized biomaterial that processes active contractions from the myocytes, the mechanical measurement needs to incorporate both passive and active force-deformation relationships under static and dynamic mechanical loadings to mimic in vivo conditions. This can be achieved by single- and multicellular cardiac tissue force-length mechanical testing. In these in vitro experiments, single myocytes isolated from the ventricle or groups of myocytes surrounded by cardiac ECM (such as trabeculae or papillary muscles) are used to measure the force-length relationship under different calcium concentrations. Then, a whole metric of biomechanical parameters at the myocyte level can be derived. Myocytes are composed of repeating contractile units called sarcomeres, which contain titin, actin, and myosin proteins. Therefore, sometimes these experiments are

also referred to as the measurement of sarcomere mechanics. More details on the myocyte measurement methods and their relation to the cardiac organ function have been recently reviewed [26].

1.2.2 In Vivo Measurement

The in vivo measurement of myocardium compliance can be obtained from the pressure-volume (PV) loop relationship. The PV loop measurement is the gold standard for assessment of ventricular performance at the whole-organ level. With an open- or closed-chest surgery, a PV catheter is inserted into the ventricle, and the synchronized pressure-volume data is recorded. The end-diastolic pressure-volume relationship (EDPVR) and chamber compliance (i.e., the ratio of volume change over pressure change (dV/dP) during a cardiac cycle) derived from the PV loop are used as the indirect and direct measurements of ventricular stiffness, respectively.

The development in medical imaging techniques enables the in vivo strain measurement for cardiac tissues. For the heart valves, a pioneering work was done by Thubrikar et al. in the 1980s using biplane X-ray imaging to quantify the circumferential and radial strain rates of the valves (reviewed in Sacks and Yoganathan [62]). The first dynamic in vivo 3D strain tensor of the mitral valve was measured by crystal placement and sonomicrometry [60]. This in vivo strain measurement can then be combined with the simultaneous LV pressure measurement to provide the dynamic stiffness of the tissue. Lately, efforts have made significant progress toward in vivo strain analysis of heart valves [40, 53] including the use of the latest transesophageal 3D echocardiographic imaging technology and segmentation algorithm [1]; however, all current methods require invasive procedures, and the establishment of a noninvasive measurement should be the future direction in heart valve research.

For the myocardium, in vivo strain has been obtained by cardiovascular magnetic resonance imaging (CMR/MRI), a noninvasive method developed since the late 1980s and modified to enable different quantities of measurement including structure and function parameters [13, 39, 66]. The use of phase-contrast MRI to measure blood flow precludes the need of placing markers in the myocardial wall and makes this method completely noninvasive. Currently, CMR has been considered as the gold standard in measuring anatomical, functional, vascular, and metabolic information in the cardiac tissues. A detailed review of this technique is published lately [37].

Besides CMR, myocardial deformation (i.e., strains) can also be obtained noninvasively by another common standard developed recently: Doppler or speckle-tracking echocardiography (STE) [64, 75]. A variety of strains including strain rates can be calculated from this technique. From the in vivo strain measurements, twist or torsion force of the ventricular wall can be derived. Most importantly, these techniques enable the ability to noninvasively and accurately measure myocardial motion both regionally and globally, which greatly assist in the diagnosis, prognosis, and management of heart diseases. The main limitations of the in vivo strain

measurement lie in the large variability between different vendors and the lack of reference values. Overall, the in vivo strain measurement, although not a direct measurement of material stiffness, provides valuable information on the cardiac function.

The direct measurement of myocardium stiffness can be achieved by an emerging technique called MR elastography (MRE) [24]. In this imaging modality, the tissue's elasticity can be derived from the detected 3D motion of the tissue, which reveals a spatial distribution of the tissue's stiffness in the myocardium.

1.3 Biomechanical Properties of Cardiac Tissues

1.3.1 Myocardium

The ECM protein predominated by collagen is a major determinant of the passive myocardial stiffness and contributes to the mechanics of the sarcomere. Another significant contributor is the intracellular component called titin, a huge protein that is viewed as an adjustable spring to actively adjust the myocardial passive stiffness. The exact contributions of collagen vs. titin on sarcomere mechanical properties are not fully understood, but they are found to depend on sarcomere length (SL) and location within the heart [19, 26, 45]. At shorter SL the mechanical properties are dominated by titin, whereas at longer SL they are dominated by collagen. In addition, an examination of length-tension relationship in bovine left atrium and left ventricle shows that the slope of the force-SL curve is larger in the ventricle compared to the atrium [82].

Mechanical properties of myocardium can be altered in a number of physiological conditions. It is well known that in athletes, the myocardium wall thickens and undergoes adaptive hypertrophy (i.e., enlargement of the myocytes) that is physiological and reversible [21]. Another example of physiological change-induced myocardium remodeling is pregnancy. It is found in rats that pregnancy leads to decreased stiffness as well as viscoelasticity (reduced hysteresis) in both LV and RV myocardium, but the changes are reversible in postpartum [73].

In contrast, under pathological conditions such as volume overload- or pressure overload-induced myocardial hypertrophy, the changes in biomechanical properties can be maladaptive. Tissue-level (ventricular) stiffening with increased interstitial and/or perivascular collagen deposition has been observed in ventricles under chronic pressure overload, regardless of an adaptive or maladaptive phenotype of cardiac hypertrophy [6, 23, 36, 80]. The accumulation in collagen is associated with impaired ventricular function: decreased longitudinal shortening, identified by myocardial strains, occurs in parallel with the subendocardial fibrosis in hypertensive rat LVs [38]. On the other hand, ventricular stiffening under volume overload is inconsistently reported: using the same isolated heart perfusion method, Herrmann et al. reported an increased longitudinal stiffness in rat LVs [33], whereas Brower

et al. found decreased ventricular stiffness in rat LVs [12] from the early adaptive to late decompensated stage of the remodeling.

Furthermore, the distribution of ventricular stiffness and mechanical function (i.e., contractility) is heterogeneous and affected by the orientation of the myofibers and surrounding collagen fibers. In hypertrophied RVs, the longitudinal stiffness increase is larger than the circumferential stiffness increase, and there is a loss of myofiber and collagen fiber spiral variation as observed in healthy RVs [36, 49]. The loss of helical fiber orientation, with the global changes of shape from ellipse to sphere in diseased ventricles, leads to a reduction in contractile force and the impairment in ventricular performance. Such phenomena have been consistently observed in ischemic, idiopathic, and dilated ventricular cardiomyopathy [14, 15].

1.3.2 The Right Ventricle Is Different from the Left Ventricle

Historically, the cardiovascular performance is considered to be mainly determined by the LV, and the investigation on its counterpart, the RV, has been neglected. Over the last decade, there was an increasing recognition that the RV is distinct from the LV in its embryologic origin, anatomy, myofiber structure and function, as well as the molecular response to adverse loading [22, 31, 52, 77]. In terms of the mechanical function, first, the two chambers work in different physiological ranges of blood pressures (80–120 mmHg in LV and 8–25 mmHg in RV), which results in different ventricular afterloads to the muscle wall. The hemodynamic and mechanical differences between LV and RV are summarized Table 1.1. The physiological differences between LVs and RVs are also found in the coronary circulations and characteristics of oxygen demand/supply balance. While the RV is experiencing lower blood

Table 1.1 Hemodynamic and mechanical differences between the LV and RV [50, 65, 76, 77, 85]

Properties	LV	RV
Mass (g/m^2)	87 ± 12	26 ± 5
Wall thickness (mm)	7–11	2–5
Ventricular systolic pressure (mmHg)	90–140	15–40
Ventricular elastance (mmHg/mL)	5.48 ± 1.23	1.30 ± 0.84
Vascular resistance ($dyne/s/cm^5$)	1238 ± 407	97 ± 55
Passive stiffness in normal strain (N/mm)	5.2 (in porcine LV)	1.2 (in porcine RV)
Myocardial compliance ($mmHg^{-1}$)	0.006 ± 0.002	0.313 ± 0.084
Coronary blood flow (ml/min/g)	High (0.5–2.0)	Low (0.5–1.0)
Pressure flow autoregulation	Presence	Absence
Accommodation to imposed load	Better in response to pressure overload	Better in response to volume overload

pressure and arterial afterloads in normal conditions, the oxygen requirements and coronary blood flow and conductance are also lower compared to the left compartment [85].

An in vitro mechanical test on intact porcine hearts showed that the LV and RV have distinct mechanical properties when the stretch is relatively small (within the physiological stretch range), with RV being more compliant than the LV. Interestingly, they have similar maximal burst force which suggests the tissue "strength" is identical [76]. On the other hand, an examination of the mechanical properties of female rat ventricles showed that the RV is stiffer than the LV [73]. These rare but inconsistent reports suggest that the tissue's mechanical difference between healthy (as well as diseased) LV and RV is unclear and awaits further investigation.

At the myocyte level, differences in LV and RV myocyte morphology and function are occasionally reported. For example, Campbell et al. found that myocytes from the RV were shorter than myocytes from the LV in rats and hamsters, while the opposite was observed in guinea pigs [16]. The contractile function in RV myocytes was higher than in LV myocytes in normal and supraventricular tachycardia (SVT) cardiomyopathy subjects [46]. In addition, RV myocytes have faster twitch velocities than LV myocytes [57].

The entire research field of right heart physiology and its failure is very young and in 2006, RV research was named a cardiovascular research priority by the USA NIH [30, 74]. RV failure (RVF) is a common cause of death in patients suffering from pulmonary hypertension (PH), congenital heart disease (CHD), chronic pulmonary disease in the absence of LV failure, RV myocardial infarction, LV failure, and valvular heart disease [30, 55, 74]. Extensive reviews on RVF including the mechanical adaptations can be found in recent publications [8, 36, 77]. However, the biomechanical mechanisms underlying the decompensated RV remodeling are not fully understood and likely unique and differ from those inherent to the LV.

1.3.3 Heart Valves

The investigation on the mechanical properties of the native heart valves is another important area of research due to the increasing number of heart valve diseases. The urgent clinical need to fix or replace diseased heart valves requires an accurate measurement of the valve's mechanical properties to better guide the manufacture of artificial valves and the treatment strategies.

Currently, most of the mechanical properties are obtained from the tensile mechanical tests and flexural or bending mechanical tests [32, 61, 62, 67]. The understanding of heart valve mechanical behavior has been contributed greatly to by a group of studies performed by Michael Sacks and his colleagues. The physiological movement of valvular tissues involves the experience of three different types of mechanical forces: in-plane tensile forces, flexural force, and shear stress. From in-plane mechanical property measurement alone, the leaflets demonstrated a nonlinear, viscoelastic, highly-anisotropic mechanical behavior. The functional

behavior is closely related to the structural alignment and recruitment of collagen fibrils and the internal slipping mechanism modulated by non-collagenous components (e.g., proteoglycan). Furthermore, the flexural response, which is an important and necessary component of the mechanical properties of the valve leaflets, is thought to be a result of the unique structure of the valve composed of three distinct, highly organized ECM-dominated layers. Detailed review on the biomechanical function of heart valves can be found in [32, 61, 62]. Overall, the investigation on human and animal native heart valves is still limited, especially in terms of its dynamic fatigue, creep behavior, and other associated viscoelastic properties.

1.4 Biomechanical Responses and Mechanobiology of Myocardium

1.4.1 Types of Mechanical Factors

The mechanical factors resulting in myocardium remodeling originate from the pulsatile blood pressure and flow. Laplace's law clearly reveals how transmural blood pressure can be translated into the circumferential wall stress by the force balance (wall stress = pressure × radius / 2 × wall thickness). Therefore, the impact of blood pressure on the ventricular wall can often be studied by the investigation of wall stress or strain of the ventricular wall. The wall stress is sometimes considered as an expression of *afterload* of the ventricle. The mechanical force generated by the blood flow is essentially the frictional force between the moving fluid and the wall, i.e., wall shear stress (WSS). WSS plays a critical role in the embryonic heart development and heart valve diseases, but its role in adult myocytes is not extensively explored. The WSS distribution in the myocardium is complex and arises from the pulsatile, oscillatory, or even turbulent blood flow in the chamber. To date, there is limited data on the in vivo WSS measurements in adult hearts. Nevertheless, a preliminary study has showed a change in myocyte ion channel recruitment in response to laminar flow [10]. In this section, we will neglect the effects of WSS and focus on other mechanical factors. The mechanical stresses described above are illustrated in Fig. 1.3.

Besides the different types of mechanical stresses stated above, we can also assess the mechanical loadings on the heart via the measurement of preload and afterload of the ventricle; this method considers the entire ventricle as a whole to receive "load" from upstream and downstream compartments. The arterial afterload can be quantified by two entities: pressure-volume relationship or vascular impedance. The pressure-volume (PV) loop is obtained by in vivo catheterization, and two parameters are often derived as a measure of arterial afterload: effective arterial elastance (E_a) and total vascular resistance R (R = systolic pressure / cardiac output) [80]. The vascular impedance is a comprehensive measurement of ventricular afterload and includes a whole metric of parameters, including the contributions from

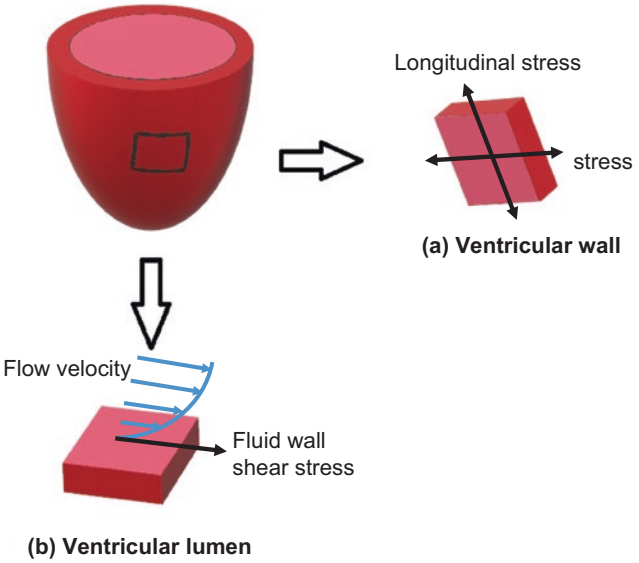

Fig. 1.3 Different mechanical stresses in ventricular tissue. (**a**) The wall stresses (longitudinal and circumferential) in the ventricular wall. (**b**) The wall shear stress at the luminal surface of the ventricle

proximal to distal vessels as well as wave reflections. However, the methodology is more complicated and often involves Fourier transform analysis of the dynamic pressure and flow relationships [78].

1.4.2 Mechanical Response of Cardiac Tissue

Alterations in mechanical factors lead to acute or chronic changes in the myocardium, and the responses occur at multiple levels (cells, tissues, or the entire organ). At the tissue level, for instance, the temporally increased cardiac chamber (due to blood volume increase) or cardiac wall stress (due to the pressure elevation) can result in a thicker ventricular wall over time, so that the eventual wall stress (determined by Law of Laplace) is maintained within the physiological range. In the meanwhile, the ventricular contractility increases to match the increased afterload of the ventricle [80]. Such remodeling is called adaptive or compensated hypertrophy [21]. With the prolonged pressure overload, the cardiac contractility becomes unable to bear the increased afterload, and the hypertrophied ventricle gets thinner and dilated. The failure to normalize the increased wall stress has been thought as a trigger for the progression of ventricular dilation and adverse outcomes. In this stage, the remodeling is called maladaptive or decompensated, and heart failure

occurs [21]. Over the past decades, efforts have been made to elucidate the mechanisms of transition from compensation to decompensation for both ventricles.

Depending on the type of mechanical loading, the ECM remodeling and biomechanical properties vary differently. A study by Chaturvedi et al. compared volume-overloaded versus pressure-overloaded LVs in human. It was shown that the pressure-overloaded LV was 2.6- to 7.0-fold stiffer than the volume-overloaded LV, which was correlated with the increased number of elastic units of the ECM. Unexpectedly, collagen content, determined through hydroxyproline content, was similar between pressure-overloaded and volume-overloaded LVs. It is thus speculated that a larger increase in collagen cross-linking led to the larger overall stiffening of the ECM in the pressure-overloaded LVs [18]. The RV is also found to respond to pressure-overload and volume-overload differently. While the Frank-Starling mechanism (i.e., an increased blood volume in ventricles before contraction leads to increased stroke volume) was maintained in both cases, Borgdorff et al. found that the RV had increased contractility under pressure overload but no change in contractility under volume overload. There was no structural examination, so it is unknown whether the ECM accumulation was altered differently in these RVs [9].

1.4.3 Mechanical Response of Cardiac Cells

Cardiac cells actively respond to mechanical cues, which may directly or indirectly affect cardiac ECM accumulation. For example, the cardiomyocyte under stretch can release paracrine factors that induce the proliferation of CFs [34], which indirectly impacts cardiac ECM remodeling. On the other hand, the biomechanical response of CFs and its direct impact on cardiac ECM have been well demonstrated in a number of in vitro studies [29, 45]. For instance, static and cyclic mechanical deformation of the laminin-coated Silastic membranes seeded with CFs differently increased in the ratio of collagen III to collagen I synthesis compared to the non-stretch condition [17], which mirrors the event occurring in the early phase of dilated cardiomyopathy [51]. Moreover, CFs plated on silicone elastic membranes and subject to a cyclic mechanical stretch (4% or 8% at 1 Hz for 1 h/day) showed increased hydroxyproline and GAG content in their ECM when compared to the ECM of unstretched cells [28]. In experiments done where CFs were seeded on type I collagen and exposed to a 10% uniaxial stretch, CF ECM gene expression such as collagen was increased. Interestingly, a 20% uniaxial stretch on CFs showed an inhibition of ECM mRNA [45]. Similarly, a 3% equibiaxial strain led to increased collagen III and fibronectin mRNA, but a 6% equibiaxial strain led to a decrease in collagen III and unchanged fibronectin mRNA [45]. These observations suggest that CFs are highly sensitive to mechanical stimuli and that CF-mediated ECM deposition is stretch dependent.

Next, cardiac cells vary their morphology and orientation in response to mechanical stretch, and the response is ECM-dependent. A variety of cardiac cells (cardiac myocytes, endothelial cells, CFs), when exposed to cyclical distention, showed

elongated morphology and reorientation perpendicular to the direction of stretch [70, 83]. The change is speculated to be mediated by the interaction with collagen α-helix. Atance et al. demonstrated that neonatal heart fibroblasts (NHFs) plated on collagen fibers (either aligned or randomly aligned) showed an elongated, stellate morphology and low confluence. In contrast, NHFs plated on fibronectin and laminin showed round morphology and high confluence. These differences disappeared after a 12-h stretch where NHFs on all ECM substrates displayed increased and similar round spreading [2]. Thus, cell morphology and confluence are highly sensitive to the mechanical stimuli mediated by ECM substrates, which probably contributes to the adaptive or maladaptive processes involved in myocardial remodeling.

1.4.4 Importance of Cardiac ECM

Collagen is an essential component of cardiac ECM. The myocardium collagen content is normally ~2–4% from morphometric assessment [11], and the rate of collagen turnover in the normal heart is ~5% per day [72]. Different types of collagen can be synthesized by various cells in the heart: fibroblasts, SMCs, and ECs. The collagen metabolism is highly sensitive to mechanical loadings and leads to rapid changes in cardiac ECM composition and mechanical properties [7, 11, 27].

Under chronic pressure elevation such as in the hypertensive heart disease, CFs transition into myofibroblasts, alter ECM composition, and modify the balance of MMPs and their inhibitors (TIMPs) to promote fibrosis [6, 71]. In hypertensive and ischemic failing hearts, the ventricle initiates with a loss of collagen and imbalanced MMP/TIMP ratio due to the acute inflammatory response; despite this acute response, increased collagen accumulation (i.e., fibrosis) is then consistently found in the early remodeling phase [41]. However, with the progressive LV dilation and wall thinning as often seen in late-stage heart failure, conflicting results are given in collagen metabolism: some report that (type I) collagen is degraded and the extent of collagen cross-linking is reduced [6, 11, 42], whereas others report elevated collagen content or cross-linking [43, 56]. In the case of volume overload-induced heart failure, changes of collagen seem to be opposite (i.e., a loss in collagen) of that observed in pressure overload in the initial phase. Moreover, there is some disagreement on collagen deposition in the ventricles: increased collagen [12], decreased collagen [58, 84], and no change in collagen [47] in the ventricles are all reported. The variations in collagen deposition depending on the type of heart disease or the specific phase in the disease development suggest that collagen metabolism is a key factor contributing to the heterogeneity of the heart failure. Future studies should elaborate on the mechanobiology of cardiac ECM in these pathological mechanical environments.

Cardiac ECM is not only a key outcome of mechanical responses of the heart but also an active system that senses and adapts to mechanical loading changes. The ECM probably elicits the earliest responses to mechanical stimuli by actively interacting with surrounding cells through integrins, focal adhesions, collagen receptors, and toll-like receptors [25]. For example, β3 integrins can bind to an RGD peptide

sequence in fibronectin, collagen, or vitronectin and induce force-dependent cell growth and differentiation of CFs to myofibroblasts [44, 54, 63]. β3 integrin is also responsible for the accumulation of collagen and fibronectin in mice with pressure overload-induced cardiac fibrosis [3]. In the fibrotic activities of CFs, the mechanical stretch is transmitted by ECM to the integrins on CFs, which causes the release of active TGFβ and initiates pro-fibrotic cascades. In fact, the ECM can respond to mechanical stretch by activation of TGFβ in the absence of cells [35]. In addition, ECM-derived molecules named danger-associated molecular patterns (DAMPs) can activate CFs through toll-like receptors (TLRs) in response to pressure overload, which ultimately causes pro-fibrotic gene expression in CFs [35]. Lastly, matrix stiffening can increase CF spreading and smooth muscle actin fiber formation and cause an increase in collagen I/III ratio [34]. The overall roles of cardiac ECM are summarized in Table 1.2.

The change in ECM modifies the signals that cardiac cells receive from their scaffolding environment, leading to changes in gene and protein expression associated with myocyte hypertrophy and contractile dysfunction. This topic forms another large area of research that studies the influence of ECM in the biology and function of cardiac cells, which is covered in later chapters of the book. Overall, the active roles of ECM on cell function, including adult cardiac cells and other cells like stem cells, are an open area of research, and its significance may gain more recognition with the development of new cell-based therapies.

1.5 Summary

Cardiac ECM, thought to be a passive component of cardiac tissue, is now viewed as an active and essential component in cardiac function. Mechanical changes, partly mediated by the ECM, affect the heart at both the tissue and cellular level. Current in vivo and ex vivo mechanical measurement techniques combined with advanced

Table 1.2 Overview of the roles of cardiac ECM in mechanobiology during cardiac remodeling

Effects on cardiac ECM	
Passive roles (regulated by surrounding cells)	ECM mechanical properties
	ECM composition (different ECM proteins and their subtypes, cross-linking formation)
	ECM synthesis and degradation
Functions of cardiac ECM	
Active roles	Contribute to mechanical properties of cardiac tissues
	Contribute to passive tension and mechanical function
	Provide scaffold and mechanical environment to surrounding cells
	Alter cell function with chemical or mechanical cues
	Assist mechanotransduction via cell-ECM binding sites (e.g., integrins, focal adhesions, receptors)

biological tools have improved our understanding of the mechanobiology in the heart. However, a lot of questions remain unanswered for this complex human organ system including the potential different biomechanical mechanisms between the left and right heart. Moreover, further study of cardiac ECM deposition and the role of various ECM receptors in the mechanosensing/mechanotransduction will increase understanding of the ECM-mediated cardiac remodeling. Ultimately, this knowledge can aid in designing therapies that will prevent and treat cardiac diseases.

References

1. Aggarwal A, et al. In-vivo heterogeneous functional and residual strains in human aortic valve leaflets. J Biomech. 2016;49:2481–90. https://doi.org/10.1016/j.jbiomech.2016.04.038.
2. Atance J, Yost MJ, Carver W. Influence of the extracellular matrix on the regulation of cardiac fibroblast behavior by mechanical stretch. J Cell Physiol. 2004;200:377–86. https://doi.org/10.1002/jcp.20034.
3. Balasubramanian S, et al. β3 integrin in cardiac fibroblast is critical for extracellular matrix accumulation during pressure overload hypertrophy in mouse. PloS one. 2012;7:e45076. https://doi.org/10.1371/journal.pone.0045076.
4. Banerjee I, Fuseler JW, Price RL, Borg TK, Baudino TA. Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. Am J Physiol Heart Circ Physiol. 2007;293:H1883–91. https://doi.org/10.1152/ajpheart.00514.2007.
5. Bashey RI, Donnelly M, Insinga F, Jimenez SA. Growth properties and biochemical characterization of collagens synthesized by adult rat heart fibroblasts in culture. J Mol Cell Cardiol. 1992;24:691–700.
6. Berk BC, Fujiwara K, Lehoux S. ECM remodeling in hypertensive heart disease. J Clin Invest. 2007;117:568–75. https://doi.org/10.1172/JCI31044.
7. Bishop JE, Laurent GJ. Collagen turnover and its regulation in the normal and hypertrophying heart. Eur Heart J. 1995;16(Suppl C):38–44.
8. Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest. 2009;135:794–804. https://doi.org/10.1378/chest.08-0492.
9. Borgdorff MAJ, Bartelds B, Dickinson MG, Steendijk P, de Vroomen M, RMF B. Distinct loading conditions reveal various patterns of right ventricular adaptation. Am J Physiol-Heart C. 2013;305:H354–64. https://doi.org/10.1152/ajpheart.00180.2013.
10. Boycott HE, et al. Shear stress triggers insertion of voltage-gated potassium channels from intracellular compartments in atrial myocytes. Proc Natl Acad Sci U S A. 2013;110:E3955–64. https://doi.org/10.1073/pnas.1309896110.
11. Brower GL, Gardner JD, Forman MF, Murray DB, Voloshenyuk T, Levick SP, Janicki JS. The relationship between myocardial extracellular matrix remodeling and ventricular function. Eur J Cardio-Thorac. 2006;30:604–10. https://doi.org/10.1016/j.ejcts.2006.07.006.
12. Brower GL, Janicki JS. Contribution of ventricular remodeling to pathogenesis of heart failure in rats. Am J Physiol Heart Circ Physiol. 2001;280:H674–83.
13. Buchalter MB, Rademakers FE, Weiss JL, Rogers WJ, Weisfeldt ML, Shapiro EP. Rotational deformation of the canine left ventricle measured by magnetic resonance tagging: effects of catecholamines, ischaemia, and pacing. Cardiovasc Res. 1994;28:629–35.
14. Buckberg GD, Coghlan HC, Torrent-Guasp F. The structure and function of the helical heart and its buttress wrapping. . V. Anatomic and physiologic considerations in the healthy and failing heart. Semin Thorac Cardiovasc Surg. 2001a;13:358–85.

15. Buckberg GD, Coghlan HC, Torrent-Guasp F. The structure and function of the helical heart and its buttress wrapping. VI. Geometric concepts of heart failure and use for structural correction. Semin Thorac Cardiovasc Surg. 2001b;13:386–401.
16. Campbell SE, Gerdes AM, Smith TD. Comparison of regional differences in cardiac myocyte dimensions in rats, hamsters, and guinea pigs. Anat Rec. 1987;219:53–9. https://doi.org/10.1002/ar.1092190110.
17. Carver W, Nagpal ML, Nachtigal M, Borg TK, Terracio L. Collagen expression in mechanically stimulated cardiac fibroblasts. Circ Research. 1991;69:116–22.
18. Chaturvedi RR, et al. Passive stiffness of myocardium from congenital heart disease and implications for diastole. Circulation. 2010;121:979–88. https://doi.org/10.1161/CIRCULATIONAHA.109.850677.
19. Chung CS, Granzier HL. Contribution of titin and extracellular matrix to passive pressure and measurement of sarcomere length in the mouse left ventricle. J Mol Cell Cardiol. 2011;50:731–9. https://doi.org/10.1016/j.yjmcc.2011.01.005.
20. Demer LL, Yin FC. Passive biaxial mechanical properties of isolated canine myocardium. J Physiol. 1983;339:615–30.
21. Dorn GW 2nd. The fuzzy logic of physiological cardiac hypertrophy. Hypertension. 2007;49:962–70. https://doi.org/10.1161/HYPERTENSIONAHA.106.079426.
22. Friedberg MK, Redington AN. Right versus left ventricular failure: differences, similarities, and interactions. Circulation. 2014;129:1033–44. https://doi.org/10.1161/CIRCULATIONAHA.113.001375.
23. Gaasch WH, Zile MR. Left ventricular diastolic dysfunction and diastolic heart failure. Annu Rev Med. 2004;55:373–94. https://doi.org/10.1146/annurev.med.55.091902.104417.
24. Glaser KJ, Manduca A, Ehman RL. Review of MR elastography applications and recent developments Journal of magnetic resonance imaging. JMRI. 2012;36:757–74. https://doi.org/10.1002/jmri.23597.
25. Goldsmith EC, et al. Organization of fibroblasts in the heart. Dev Dyn. 2004;230:787–94. https://doi.org/10.1002/dvdy.20095.
26. Golob M, Moss RL, Chesler NC. Cardiac tissue structure, properties, and performance: a materials science perspective. Ann Biomed Eng. 2014;42:2003–13. https://doi.org/10.1007/s10439-014-1071-z.
27. Golob MJ, Wang Z, Prostrollo AJ, Hacker TA, Chesler NC. Limiting collagen turnover via collagenase-resistance attenuates right ventricular dysfunction and fibrosis in pulmonary arterial hypertension. Physiol Rep. 2016;4:e12815. https://doi.org/10.14814/phy2.12815.
28. Guo Y, et al. Extracellular Matrix of Mechanically Stretched Cardiac Fibroblasts Improves Viability and Metabolic Activity of Ventricular Cells. In J Med Sci. 2013;10:1837–45. https://doi.org/10.7150/ijms.6786.
29. Gupta V, Grande-Allen KJ. Effects of static and cyclic loading in regulating extracellular matrix synthesis by cardiovascular cells. Cardiovas Res. 2006;72:375–83. https://doi.org/10.1016/j.cardiores.2006.08.017.
30. Haddad F, Doyle R, Murphy DJ, Hunt SA. Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. Circulation. 2008a;117:1717–31. https://doi.org/10.1161/CIRCULATIONAHA.107.653584.
31. Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in cardiovascular disease, part I: anatomy, physiology, aging, and functional assessment of the right ventricle. Circulation. 2008b;117:1436–48. https://doi.org/10.1161/CIRCULATIONAHA.107.653576.
32. Hasan A, et al. Biomechanical properties of native and tissue engineered heart valve constructs. J Biomech. 2014;47:1949–63. https://doi.org/10.1016/j.jbiomech.2013.09.023.
33. Herrmann KL, McCulloch AD, Omens JH. Glycated collagen cross-linking alters cardiac mechanics in volume-overload hypertrophy. Am J Physiol Heart Circ Physiol. 2003;284:H1277–84. https://doi.org/10.1152/ajpheart.00168.2002.

34. Herum KM, Choppe J, Kumar A, Engler AJ, McCulloch AD. Mechanical regulation of cardiac fibroblast profibrotic phenotypes. Mol Biol Cell. 2017a;28:1871–82. https://doi.org/10.1091/mbc.E17-01-0014.
35. Herum KM, Lunde IG, McCulloch AD, Christensen G. The Soft- and Hard-Heartedness of Cardiac Fibroblasts: Mechanotransduction Signaling Pathways in Fibrosis of the Heart. J Clin Med. 2017b;6:E53. https://doi.org/10.3390/jcm6050053.
36. Hill MR, Simon MA, Valdez-Jasso D, Zhang W, Champion HC, Sacks MS. Structural and mechanical adaptations of right ventricle free wall myocardium to pressure overload. Ann Biomed Eng. 2014;42:2451–65. https://doi.org/10.1007/s10439-014-1096-3.
37. el Ibrahim SH. Myocardial tagging by cardiovascular magnetic resonance: evolution of techniques--pulse sequences, analysis algorithms, and applications. J Cardiovasc Magn Reson. 2011;13:36. https://doi.org/10.1186/1532-429X-13-36.
38. Ishizu T, et al. Left ventricular strain and transmural distribution of structural remodeling in hypertensive heart disease. Hypertension. 2014;63:500–6. https://doi.org/10.1161/HYPERTENSIONAHA.113.02149.
39. Khan JN, Singh A, Nazir SA, Kanagala P, Gershlick AH, McCann GP. Comparison of cardiovascular magnetic resonance feature tracking and tagging for the assessment of left ventricular systolic strain in acute myocardial infarction. Eur J Radiol. 2015;84:840–8. https://doi.org/10.1016/j.ejrad.2015.02.002.
40. Krishnamurthy G, et al. Material properties of the ovine mitral valve anterior leaflet in vivo from inverse finite element analysis. Am J Physiol Heart Circ Physiol. 2008;295:H1141–9. https://doi.org/10.1152/ajpheart.00284.2008.
41. Leonard BL, Smaill BH, LeGrice IJ. Structural remodeling and mechanical function in heart failure. Microsc Microanal. 2012;18:50–67. https://doi.org/10.1017/S1431927611012438.
42. Lopez B, Gonzalez A, Querejeta R, Larman M, Diez J. Alterations in the pattern of collagen deposition may contribute to the deterioration of systolic function in hypertensive patients with heart failure. J Am Coll Cardiol. 2006;48:89–96. https://doi.org/10.1016/j.jacc.2006.01.077.
43. Lopez B, Querejeta R, Gonzalez A, Beaumont J, Larman M, Diez J. Impact of treatment on myocardial lysyl oxidase expression and collagen cross-linking in patients with heart failure. Hypertension. 2009;53:236–42. https://doi.org/10.1161/HYPERTENSIONAHA.108.125278.
44. Luo Y, Sun Y, Zhu Z, Li F. Is the change of integrin alpha(v)beta(3) expression in the infarcted myocardium related to the clinical outcome? Clin Nucl Med. 2014;39:655–7. https://doi.org/10.1097/RLU.0000000000000426.
45. MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. Cardiovasc Res. 2000;46:257–63.
46. McMahon WS, Mukherjee R, Gillette PC, Crawford FA, Spinale FG. Right and left ventricular geometry and myocyte contractile processes with dilated cardiomyopathy: myocyte growth and beta-adrenergic responsiveness. Cardiovasc Res. 1996;31:314–23.
47. Namba T, et al. Regulation of fibrillar collagen gene expression and protein accumulation in volume-overloaded cardiac hypertrophy. Circulation. 1997;95:2448–54.
48. Novak VP, Yin FC, Humphrey JD. Regional mechanical properties of passive myocardium. J Biomech. 1994;27:403–12.
49. Park DW, Sebastiani A, Yap CH, Simon MA, Kim K. Quantification of coupled stiffness and Fiber orientation remodeling in hypertensive rat right-ventricular myocardium using 3D ultrasound speckle tracking with biaxial testing. PloS one. 2016;11:e0165320. https://doi.org/10.1371/journal.pone.0165320.
50. Pasipoularides A. Right and left ventricular diastolic pressure-volume relations: a comprehensive review. J Cardiovasc Transl Res. 2013;6:239–52. https://doi.org/10.1007/s12265-012-9424-1.
51. Pauschinger M, et al. Dilated cardiomyopathy is associated with significant changes in collagen type I/III ratio. Circulation. 1999;99:2750–6.

52. Perreault CL, Bing OH, Brooks WW, Ransil BJ, Morgan JP. Differential effects of cardiac hypertrophy and failure on right versus left ventricular calcium activation. Circ Res. 1990;67:707–12.
53. Rausch MK, Kuhl E. On the effect of prestrain and residual stress in thin biological membranes. J Mech Phys Solids. 2013;61:1955–69. https://doi.org/10.1016/j.jmps.2013.04.005.
54. Roca-Cusachs P, Gauthier NC, del Rio A, Sheetz MP. Clustering of α(5)β(1) integrins determines adhesion strength whereas α(v)β(3) and talin enable mechanotransduction. Proc Natl Acad Sci U S A. 2009;106:16245–50. https://doi.org/10.1073/pnas.0902818106.
55. Roche SL, Redington AN. The failing right ventricle in congenital heart disease. Can J Cardiol. 2013;29:768–78. https://doi.org/10.1016/j.cjca.2013.04.018.
56. Rossi MA. Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans. J Hypertens. 1998;16:1031–41.
57. Rouleau JL, Paradis P, Shenasa H, Juneau C. Faster time to peak tension and velocity of shortening in right versus left ventricular trabeculae and papillary muscles of dogs. Circ Res. 1986;59:556–61.
58. Ryan TD, Rothstein EC, Aban I, Tallaj JA, Husain A, Lucchesi PA, Dell'Italia LJ. Left ventricular eccentric remodeling and matrix loss are mediated by bradykinin and precede cardiomyocyte elongation in rats with volume overload. J Am Coll Cardiol. 2007;49:811–21. https://doi.org/10.1016/j.jacc.2006.06.083.
59. Sacks MS, Chuong CJ. Biaxial mechanical properties of passive right ventricular free wall myocardium. J Biomech Eng. 1993;115:202–5.
60. Sacks MS, et al. In-vivo dynamic deformation of the mitral valve anterior leaflet. Ann Thorac Surg. 2006;82:1369–77. https://doi.org/10.1016/j.athoracsur.2006.03.117.
61. Sacks MS, Merryman WD, Schmidt DE. On the biomechanics of heart valve function. J Biomech. 2009;42:1804–24. https://doi.org/10.1016/j.jbiomech.2009.05.015.
62. Sacks MS, Yoganathan AP. Heart valve function: a biomechanical perspective philosophical transactions of the Royal Society of London Series B. Biol Sci. 2007;362:1369–91. https://doi.org/10.1098/rstb.2007.2122.
63. Schroer AK, Merryman WD. Mechanobiology of myofibroblast adhesion in fibrotic cardiac disease. J Cell Sci. 2015;128:1865–75. https://doi.org/10.1242/jcs.162891.
64. Seo Y, Ishizu T, Aonuma K. Current status of 3-dimensional speckle tracking echocardiography: a review from our experiences. J Cardiovasc Ultrasound. 2014;22:49–57. https://doi.org/10.4250/jcu.2014.22.2.49.
65. Skowronski EW, et al. Right and left ventricular function after cardiac transplantation Changes during and after rejection. Circulation. 1991;84:2409–17.
66. Soleimanifard S, Abd-Elmoniem KZ, Sasano T, Agarwal HK, Abraham MR, Abraham TP, Prince JL. Three-dimensional regional strain analysis in porcine myocardial infarction: a 3T magnetic resonance tagging study. J Cardiovasc Magn Reson. 2012;14:85. https://doi.org/10.1186/1532-429X-14-85.
67. Stella JA, Sacks MS. On the biaxial mechanical properties of the layers of the aortic valve leaflet. J Biomech Eng. 2007;129:757–66. https://doi.org/10.1115/1.2768111.
68. Tao G, Kotick JD, Lincoln J. Heart valve development, maintenance, and disease: the role of endothelial cells. Curr Top Dev Biol. 2012;100:203–32. https://doi.org/10.1016/B978-0-12-387786-4.00006-3.
69. Taylor PM, Batten P, Brand NJ, Thomas PS, Yacoub MH. The cardiac valve interstitial cell. Int J Biochem Cell Biol. 2003;35:113–8.
70. Terracio L, Miller B, Borg TK. Effects of cyclic mechanical stimulation of the cellular components of the heart. In vitro Cell Dev Biol. 1988;24:53–8. https://doi.org/10.1007/bf02623815.
71. van Putten S, Shafieyan Y, Hinz B. Mechanical control of cardiac myofibroblasts. J Mol Cell Cardiol. 2016;93:133–42. https://doi.org/10.1016/j.yjmcc.2015.11.025.
72. Villarreal FJ. Interstitial fibrosis in heart failure. Boston: Springer Science + Business Media, Inc.; 2005.

73. Virgen-Ortiz A, Marin JL, Elizalde A, Castro E, Stefani E, Toro L, Muniz J. Passive mechanical properties of cardiac tissues in heart hypertrophy during pregnancy. J Physiol Sci. 2009;59:391–6. https://doi.org/10.1007/s12576-009-0047-5.
74. Voelkel NF, et al. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. Circulation. 2006;114:1883–91. https://doi.org/10.1161/CIRCULATIONAHA.106.632208.
75. Voigt JU, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/industry task force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging. 2015;16:1–11. https://doi.org/10.1093/ehjci/jeu184.
76. Wainwright JM, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, Badylak SF. Preparation of cardiac extracellular matrix from an intact porcine heart tissue engineering Part C. Methods. 2010;16:525–32. https://doi.org/10.1089/ten.TEC.2009.0392.
77. Walker LA, Buttrick PM. The right ventricle: biologic insights and response to disease: updated. Current cardiology reviews. 2013;9:73–81.
78. Wang Z, Chesler NC (2011) Pulmonary vascular wall stiffness: An important contributor to the increased right ventricular afterload with pulmonary hypertension Pulmonary Circulation 1:212–223 https://doi.org/10.4103/2045-8932.83453
79. Wang Z, Golob M, Chesler NC. Viscoelastic properties of cardiovascular tissues. In: El-Amin MF, editor. Viscoelastic and viscoplastic materials: InTech; 2016. https://doi.org/10.5772/61921.
80. Wang Z, Schreier DA, Hacker TA, Chesler NC. Progressive right ventricular functional and structural changes in a mouse model of pulmonary arterial hypertension. Physiol Rep. 2013;1:e00184. https://doi.org/10.1002/phy2.184.
81. Wiltz D, Arevalos CA, Balaoing LR, Blancas AA, Sapp MC, Zhang X, Grande-Allen KJ. Extracellular matrix organization, structure, and function. In: Aikawa E, editor. Calcific aortic valve disease: InTech; 2013. https://doi.org/10.5772/52842.
82. Wu Y, Cazorla O, Labeit D, Labeit S, Granzier H. Changes in titin and collagen underlie diastolic stiffness diversity of cardiac muscle. J Mol Cell Cardiol. 2000;32:2151–62. https://doi.org/10.1006/jmcc.2000.1281.
83. Yost MJ, Simpson D, Wrona K, Ridley S, Ploehn HJ, Borg TK, Terracio L. Design and construction of a uniaxial cell stretcher. Am J Physiol Heart Circ Physiol. 2000;279:H3124–30.
84. Zheng J, et al. Microarray identifies extensive downregulation of noncollagen extracellular matrix and profibrotic growth factor genes in chronic isolated mitral regurgitation in the dog. Circulation. 2009;119:2086–95. https://doi.org/10.1161/CIRCULATIONAHA.108.826230.
85. Zong P, Tune JD, Downey HF. Mechanisms of oxygen demand/supply balance in the right ventricle. Exp Biol Med (Maywood). 2005;230:507–19.

Chapter 2
Imaging the Cardiac Extracellular Matrix

Michael A. Pinkert, Rebecca A. Hortensius, Brenda M. Ogle, and Kevin W. Eliceiri

Abstract Cardiovascular disease is the global leading cause of death. One route to address this problem is using biomedical imaging to measure the molecules and structures that surround cardiac cells. This cellular microenvironment, known as the cardiac extracellular matrix, changes in composition and organization during most cardiac diseases and in response to many cardiac treatments. Measuring these changes with biomedical imaging can aid in understanding, diagnosing, and treating heart disease. This chapter supports those efforts by reviewing representative methods for imaging the cardiac extracellular matrix. It first describes the major biological targets of ECM imaging, including the primary imaging target of fibrillar collagen. Then it discusses the imaging methods, describing their current capabilities and limitations. It categorizes the imaging methods into two main categories: organ-scale noninvasive methods and cellular-scale invasive methods. Noninvasive methods can be used on patients, but only a few are clinically available, and others require further development to be used in the clinic. Invasive methods are the most established and can measure a variety of properties, but they cannot be used on live patients. Finally, the chapter concludes with a perspective on future directions and applications of biomedical imaging technologies.

Keywords Cardiac · Extracellular matrix · Collagen · Imaging · Composition · Fibers

M. A. Pinkert · K. W. Eliceiri (✉)
Laboratory for Optical and Computational Instrumentation and Department of Medical Physics, University of Wisconsin at Madison, Madison, WI, USA

Morgridge Institute for Research, Madison, WI, USA
e-mail: eliceiri@wisc.edu

R. A. Hortensius · B. M. Ogle
Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN, USA

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_2

2.1 Introduction

Cardiovascular diseases are the leading cause of death across the globe, and efforts to address this public health problem are increasingly focused on the cardiac extracellular matrix (ECM) [75]. The cardiac ECM is a dynamic environment that varies in organization and composition throughout the course of cardiac disease and during normal cardiac development. Knowledge of these changes can help researchers and clinicians understand, detect, and treat cardiac disease [20, 62]. Biomedical imaging is one of the main tools for measuring the cardiac extracellular matrix (ECM), encompassing a range of research and clinical imaging methods that are capable of visualizing different ECM properties and components. However, prospective researchers of the ECM should be aware that there are significant capability gaps in ECM imaging.

ECM imaging is a still-developing field currently defined by the divide between invasive and noninvasive imaging methods [25, 74]. Invasive imaging methods are dominated by microscopy-based applications and are the gold standard for measuring the ECM, but they are limited in their clinical application. They require either cardiac biopsies, which can be damaging to patient health, or are restricted to imaging small areas during surgical interventions or through endoscopes. They are used extensively in engineered tissue and animal models of cardiac disease. In return, they obtain detailed information on the ECM, visualizing fiber networks, proteins, and other ECM components at the cellular and subcellular scales (< 2 μm). By contrast, noninvasive imaging methods can safely be applied to patients but can only provide limited information about the ECM. Noninvasive methods currently image at the organ scale (> 0.5 mm), measuring a small range of bulk ECM properties such as the concentrations of some ECM components, extracellular volume, or tissue mechanical properties. This divide means that invasive imaging methods are used for either ex vivo imaging or preclinical imaging of research models or human tissues, whereas noninvasive methods are suitable for in vivo clinical imaging.

This chapter covers the targets, methods, and applications of imaging the ECM. It begins by discussing the biological targets for ECM imaging, explaining that collagen is one of the primary components targeted by ECM images and why this is so. The chapter then reviews the methods themselves, classifying them into two categories based on common usages and limitations. These categories are noninvasive organ-scale imaging, which is predominant for imaging patients, and invasive cellular-scale imaging, which is largely restricted to ex vivo tissue imaging or in vivo research model imaging but gives the most information about the ECM. Finally, it discusses the future directions of ECM imaging in terms of current research focuses and unmet needs.

2.2 Collagen, Fibrosis, and Other Biological Targets for ECM Imaging

The families of proteins known as fibrillar collagen are the main targets for ECM imaging, with a focus on imaging collagen types I and III. Collagens I and III are the most common ECM components, and they respectively account for 85–90% and 5–11% of collagens in the cardiac ECM [20]. These collagens compose a fibrillar network in cardiac tissue and provide both structural stability and take part in cellular signaling. They are an important imaging target because most cardiac diseases cause increased deposition or rearrangement of collagen in the ECM. These changes are part of the tissue repair and the compensatory mechanism known as fibrosis (Fig. 2.1) [10, 25]. Fibrosis is a process where the ECM thickens, increasing the concentration and arrangement of ECM components. This process increases collagen concentration the most of any component, and changes the organization of collagen networks in ways that can be seen both qualitatively and quantitatively [56]. Fibrosis occurs either in a single focal site of injury or diffusely throughout the diseased tissue, with certain patterns corresponding to particular diseases. Thus, the imaging of collagen can reveal fibrosis, and in doing so screen for or monitor most

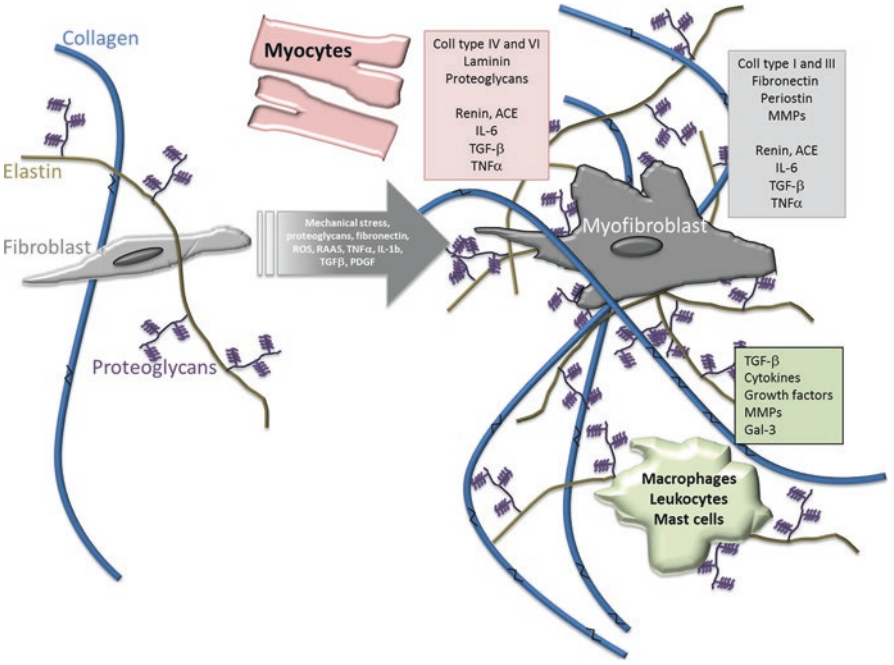

Fig. 2.1 Stress or damage to the heart triggers myocardial fibrosis, where cells synthesize increased amounts of extracellular matrix components. The largest increase in synthesis is for collagen. (This work is attributed to Gyöngyösi et al. and used under a Creative Commons Attribution-NonCommercial 4.0 International license [24])

cardiac diseases [25]. Consequently, the majority of noninvasive imaging methods aim to find areas of fibrosis or to measure collagen concentration, with relatively few examining other ECM components. Invasive imaging is less focused on collagen than noninvasive imaging, but there is also a large body of work with invasive imaging devoted to analyzing collagen networks and structure.

Most imaging methods are sensitive to fibrillar collagen types I and III, but only a few measure other collagens in the heart [42, 73, 74]. Collagen types I and III form into chains of molecules known as fibrils, which are arranged into a fibrous scaffold. Methods that image types I and III are sensitive to their molecular properties or to the discrete and recognizable fiber network they form [56]. Other collagens are harder to target because they are less prevalent, are non-fibrillar, or are difficult to isolate from other ECM components due to their location. Collagen type IV is part of the basement membrane in the ECM, is intermixed with other proteins, and due to its non-fibrillar makeup lacks the structural imaging contrast of fibrillar collagens. Collagen type V occupies the core of some type I fibers, making them difficult to detect. Finally, type VI collagen forms fibrous structures surrounding individual myocardial cells, arteries, and capillaries (Fig. 2.2) [38, 42, 73]. For cardiac collagen imaging, the collagen properties of interest include the absolute abundance of collagen, the relative abundance of collagen types, the structure/location of the fiber network, and the connection to other constituents of the ECM [23, 49, 52, 56, 73].

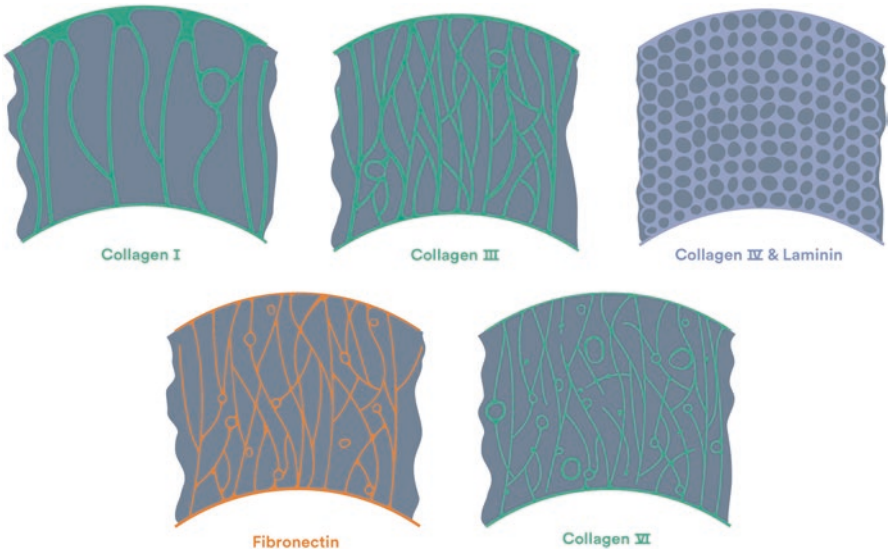

Fig. 2.2 The fibrous structures in the cardiac extracellular matrix vary in appearance and location. Collagens type I and III are major structural components, which span from major tissue septa such as the epicardium and endocardium. Fibronectin and collagen type VI span from minor septa to the connective tissue of the capillaries and myocardial cells. Finally, laminin and collagen type IV compose the basement membrane [5]. (Figure Permission granted through RightsLink)

Collagen is the most common target for ECM imaging, but less frequent targets include elastin, fibronectin, various receptors, myofibroblasts, as well as other proteins and protein compounds. These targets are less frequently targeted because their sparse concentration and their location in the ECM makes them difficult to distinguish from other targets in non-specific forms of imaging. These targets are typically imaged using high-specificity molecular imaging methods on both the organ and cellular scales. Elastin is a fibrillar protein which complements collagen in structuring the ECM. Collagen contributes to tissue strength, but elastin contributes to tissue flexibility and elasticity, which is important for the constriction and relaxation of the beating heart [71]. Fibronectin is also a fibrillar protein, but its primary role is to convey information between cells and other components of the ECM. It connects between cellular surfaces and to other components, coordinating the development and growth of new cells, and affecting how the cells deposit more components into the ECM [77]. This cellular signaling also involves protein molecules known as receptors, which mediate the cellular response to chemical signals. These receptors can be targeted using protein-specific imaging probes or stains [25]. Myofibroblasts are cells in the ECM that deposit ECM components and can also be a sign of fibrosis. Beyond these targets, specific probes can also target some of the milieu of proteins, and other protein compounds dispersed through the ECM [25].

2.3 ECM Imaging Methods

This chapter divides ECM imaging methods into two categories based on the scale at which they are typically used: organ-scale imaging and cellular-scale imaging (Table 2.1). Organ-scale imaging methods measure bulk properties across large regions of the heart, e.g., the left ventricle. These methods can be used for noninvasive patient imaging, with applications including detection of myocardial fibrosis and monitoring scar tissue formation after myocardial infarction [52, 58]. Cellular-scale imaging methods visualize the ECM components directly at high-resolutions. These methods are invasive, requiring either a biopsy or an imaging probe near the cardiac tissue. Consequently, these methods are currently used to image patient tissue samples or for preclinical research. For example, they have been used to characterize how diseases alter collagen networks in the myocardium and vasculature [23, 28, 46, 71]. Each of these two categories contains several imaging methods, which range in sensitivity (signal strength), specificity (signal source), and cost. These methods are further described in the sections below.

Table 2.1 Commonly used methods for imaging the ECM in cardiac models

Imaging spatial scale	Imaging category	Biological source of contrast	Invasiveness	Other limitations
 Organ scale (>100 μm resolution)	Magnetic resonance imaging	ECM volume, focal fibrosis, diffuse fibrosis, collagen, tropoelastin	Noninvasive, use of contrast agents	Expensive, variable specificity/ sensitivity by technique
	Ultrasound	Collagen, diffuse fibrosis	Noninvasive	Low specificity for collagen
	Nuclear imaging	Collagen, matrix metalloproteinases (MMP), integrins, angiotensin receptor, blood coagulation factor FXIII	Noninvasive, requires extrinsic probes	Low resolution, limited probe availability for ECM
 Cellular scale (< 100 μm resolution)	Tissue staining	Collagen, elastin, fibronectin, various proteins	Invasive, requires biopsy	Tissue processing artifacts, 2D
	Nonlinear optical microscopy	Fibrillar collagen, elastin, various proteins through probes, chemical environment	Semi-invasive, ~100–300 μm penetration depth or requires biopsy	Low availability, expensive
	Electron microscopy	Highest-resolution subcellular and molecular structures	Invasive, requires biopsy, destructive to imaged tissue	Low specificity, low availability, expensive, 2D

2.4 Organ-Scale Imaging

Organ-scale imaging methods are noninvasive and capable of being used on live patients, making them ideal for clinical imaging. There are strong research efforts going into developing these methods, finding new ways to measure ECM components with molecular imaging, or in better quantifying fibrosis with all approaches. However, these methods cannot directly visualize the ECM because they measure bulk (> 0.5 mm area) properties across large regions of cardiac tissue, such as a ventricle or heart wall. These properties include the volume of the extracellular matrix, the amount or bulk distribution of collagen and other ECM components, and the tissue mechanical properties (an indirect measurement of collagen/elastin structure).

2.4.1 Magnetic Resonance Imaging

The main clinical method for organ-scale collagen imaging is magnetic resonance imaging (MRI) [52]. MRI, also known as cardiac magnetic resonance (CMR), is a noninvasive imaging method that perturbs atomic protons/neutrons (typically hydrogen protons) with radiofrequency energy and measures the behavior of those perturbed atoms. The behavior depends on the molecular composition of the tissue

and the sequence of radiofrequency pulses used. The sensitivity of the MRI measurements to different molecules (e.g., collagen) varies based on the sequence used and any potential contrast agent. Thus, MRI can be performed through different techniques with varying sensitivity and specificity to collagen or collagen-related properties (e.g., extracellular matrix volume).

There are several techniques used in clinical cardiac MRI. Late gadolinium enhancement (LGE) MRI is considered the gold standard for identifying scar tissue regions for myocardial fibrosis [60]. However, LGE depends on contrast between the heart regions and so cannot detect diffuse increases in collagen content (Fig. 2.3). Such diffuse increases are associated with early stages of fibrosis. Early stage fibrosis can be detected using the developing class of MRI techniques known as T1 mapping [68]. These T1 mapping MRI sequences provide semiquantitative measures voxel-by-voxel. These maps are useful in clinical diagnosis but have biological and practical limitations. Measured values can be different for normal and diseased tissue, and changes in these values are also associated with other ECM altering diseases (e.g., edema). T1 mapping sequences are not standardized, making measurements difficult to reproduce or interpret across institutions [52]. A final set of techniques, tagging and feature tracking MRI, are reproducible and quantitative, but only indirectly measure collagen or other ECM biology [55]. Tagging and feature tracking measure the mechanical deformation (strain) of the myocardium,

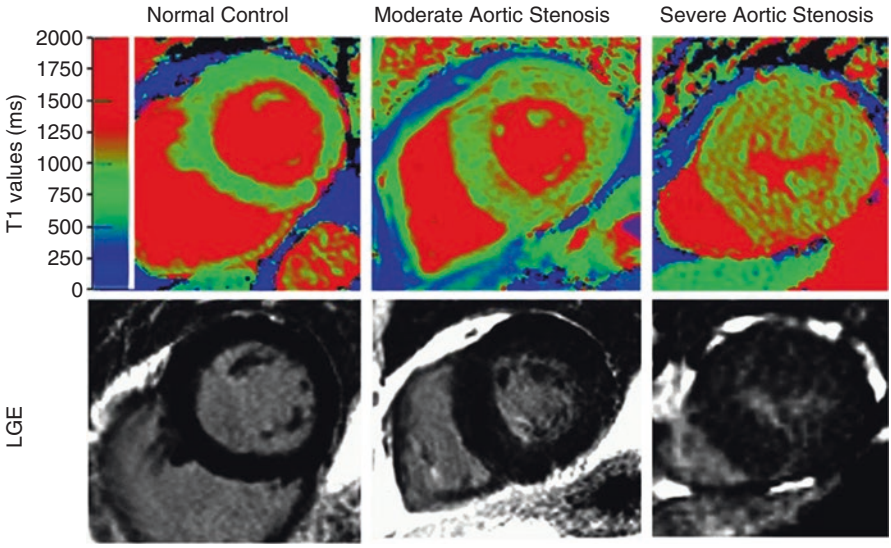

Fig. 2.3 T1 mapping and late gadolinium enhancement MRI depict regions of high collagen content and fibrosis. This can be seen in aortic valve images from patients. This figure shows three cases in order of increasing fibrosis: a normal control, moderate aortic stenosis, and severe aortic stenosis. (This work is attributed to Bull et al. and used under a Creative Commons Attribution-NonCommercial 3.0 Unported license [7])

which is affected by the collagen structure [52]. These techniques are valuable clinical tools but are not wholly specific to collagen or the ECM.

Molecular MRI imaging is a preclinical MRI technique that has very high specificity to collagen and other ECM components. Molecular MRI uses probes that bind to target molecules and which include an MRI contrast agent. These probes are still in the preclinical stage and need further safety and efficacy studies before being brought to the clinic, but they have already shown promise for ECM imaging [52]. Collagen targeted molecular MRI more accurately visualizes scar tissue than other methods of MRI [25]. Molecular MRI targeted at other molecules can study the underlying processes behind fibrosis (apoptosis, necrosis, inflammation, and scar maturation) [52]. Additionally, molecular MRI using an elastin−/tropoelastin-specific agent allowed for the in vivo assessment of ECM remodeling in a mouse model of myocardial infarction [53]. There are no probes for many existing ECM components; however, any molecule can potentially be targeted given the development of an appropriate probe. Such a probe would be both specific to the molecule in question and would include an MRI contrast agent. Molecular MRI has high potential for future clinical work as a noninvasive way of measuring various ECM components in bulk. However, its use will depend on the continued development of imaging probes and their validation through clinical trials.

Overall, MRI is considered a standard tool for assessing fibrosis in the clinic at the organ scale. However, the major limitation of MRI is high expense. This difficulty compounds the issue of imaging standards, where the field of MRI is still developing standards for data acquisition and analysis [52]. The lack of these standards means that MRI data can vary from location to location, which can impact the results. Other limitations include that there are few clinical trials using ECM MRI techniques and that molecular MRI is preclinical and has only a small collection of probes. As such, other imaging methods complement it in the clinic and for research.

2.4.2 Ultrasound

Ultrasound (US), also known as echocardiography, is a widely used clinical imaging modality that is noninvasive, inexpensive, and can assess mechanical properties related to the tissue collagen structure [13]. For example, strain and elastography US assess myocardial deformation and elasticity, both of which are heavily affected by collagen and elastin structure [59]. Integrated backscatter US can detect changes in cardiac collagen density, which is a major feature of fibrosis [21]. In addition, US is sensitive to the angle and amount of collagen fiber alignment in a region [32, 54, 57]. However, these techniques have low collagen specificity because they are also affected by other biological structures, e.g., muscle fibers. In addition, these techniques currently have low reproducibility between different US labs and require high image quality, leading to variable results between labs [74]. As such, while US is a valuable clinical modality that is widely used for qualitative cardiac imaging, it

is not currently used for quantitative cardiac ECM imaging. This may change in the future, as researchers improve current methods or develop more reproducible methods of differentiating regions in US images. One example of this are alternative metrics that rely on tissue texture, paired with computer models to automate image assessment [66].

2.4.3 Nuclear Imaging

Nuclear imaging methods have the highest specificity and sensitivity but are limited by low resolution and the need to develop clinically compatible imaging probes [25]. Cardiac nuclear imaging uses two methods, known as single-photon emission computed tomography (SPECT) and positron-emission tomography (PET). Nuclear imaging uses radioactive probes that bind to target molecules. Currently, there are no clinical probes that target collagen, but there are several at the preclinical stage that have been tested on animal models for fibrosis. In cardiac models, SPECT and PET probes based on a peptide known as collagelin accumulate in areas of myocardial infarction (Fig. 2.4) [33, 47]. In lung and liver models, other peptide-based probes accumulate in fibrotic tissue ([14, 78], p. 201). There have been few other probes demonstrated in the literature, but a recent review has highlighted potential targets for collagen probes [72]. These targets include intact collagens I, II, and IV but also include degraded collagens I–V, which are found in fibrotic tissue and other sites of collagen remodeling. Beyond collagen, there are also many probes that target components of the ECM, including matrix metalloproteinases, integrins, a blood coagulation factor, and angiotensin receptors [76]. As such, nuclear imaging of the cardiac ECM is a promising imaging method for preclinical imaging. Ultimately, the clinical use of nuclear imaging depends on further developing these probes to be reproducible and to demonstrate their safety through clinical trials.

2.5 Cellular–Scale Imaging

Imaging methods at the cellular scale visualize the organization of the ECM but are invasive and cannot currently be used on live patients. For fibrous components, this organization includes properties such as fiber density, diameter, alignment, cross-linking between fibers, and more. These methods either require patient biopsies or use surgical and endoscopic tools to image the tissue in the clinic. Some of these methods can be used in vivo in cardiac research models such as the fish [70]. There are three major categories of methods for imaging collagen on the cellular size scale: tissue staining protocols, nonlinear optical microscopy, and electron microscopy.

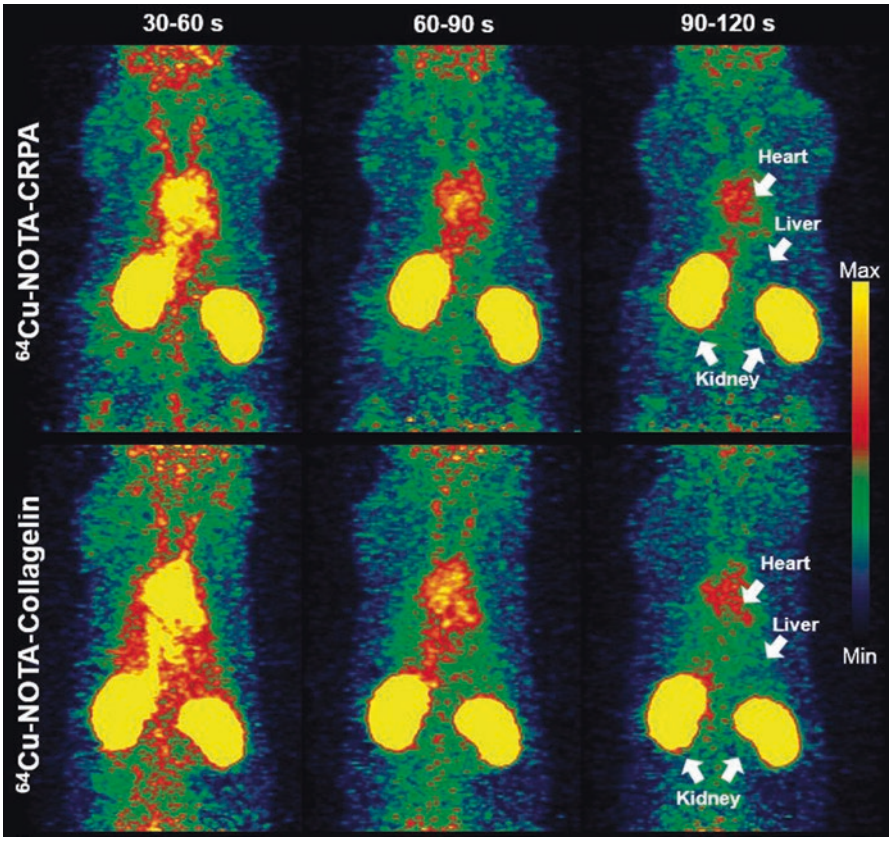

Fig. 2.4 Collagelin and collagelin analogue probes accumulate in regions of myocardial infarction and fibrosis in rat models. This figure depicts positron-emission tomography images taken at several time points after probe injection. The top row shows a probe based on the collagelin analogue CRPA, and the bottom a probe based on collagelin [33]. (Figure Permission granted through RightsLink)

2.5.1 Tissue Staining Protocols

Tissue staining protocols (histochemistry and immunohistochemistry) are the most clinically accessible means of visualizing collagen in the ECM. Staining protocols take thin (1–10 μm) tissue sections and label components of the ECM and cellular microenvironments. Histochemistry uses chemical dyes that bind to various tissue components, coloring them in the process. Immunohistochemistry (IHC) relies on antibodies that bind to protein antigens in the tissue. These antibodies localize to the areas containing the protein of interest and are labeled with a reporter molecule (e.g., a fluorescently labeled secondary antibody) for imaging. For both methods, the resulting slides can be analyzed using a standard wide field optical microscope, equipped with fluorescence in the case of IHC.

The primary advantages of staining methods are the ability to target many different components and ease-of-use, but there are many limitations on application. There is a large library of stains for comparing different biological components, giving staining methods applicability for many research problems. IHC in particular can be protein specific, allowing stains to target many of the proteins of the ECM. Staining methods have a widespread availability because optical microscopy is relatively inexpensive and already available in most clinical and research environments. Finally, these methods are the oldest and best established, meaning they are considered the gold standard and are prevalent in the literature. However, tissue staining protocols are also subject to several limitations. They require biopsies of cardiac tissue and so cannot image the environment of live cells. These biopsies can be difficult to acquire without hurting the patient and are often unavailable [10]. The biopsies may not be representative of the diseased tissue, as they cover only a small portion of the tissue, and thus are subject to sampling errors. In addition, staining protocols involve processing steps which often deform or alter the tissue from the original tissue structure [1, 12]. Finally, the tissue sections are typically imaged in 2D and so provide a limited view of collagen organization across the whole tissue, preventing robust 3D quantitative analysis. Researchers are addressing the 3D issue by developing rapid imaging and image fusion for series of adjacent slides, but the difficulties of using such image fusion schemes mean that other 3D imaging methods may remain more attractive options [3]. For these reasons, tissue staining protocols have a limited quantitative value in measuring collagen or other components of the ECM; however, they remain the standard for cardiac care and research because of their qualitative value [10].

Stains are a common method for looking at collagen structure. In the clinic, they can be used to assess fibrosis based on clinician judgment. In addition, researchers are developing quantitative computer-based methods to analyze microscopy slides [64]. Some notable collagen stains include the Picrosirius red, hematoxylin and eosin, trichrome, and antibody stains [11, 36, 45]. The Picrosirius red stain is the highly specific to collagen and can further serve as a contrast agent when combined with polarized light microscopy (PLM). This combination can quantify the collagen fiber orientation and organization in 2D [36]. Other stains, such as hematoxylin and eosin (H&E) and trichrome stains, are less specific to collagen and more difficult to quantify but allow collagen to be viewed in the context of the rest of the ECM. The hematoxylin and eosin (H&E) protocol stains components of the ECM in shades of blue and pink, with collagen a pink shade, yielding shades for each component that reflect how well they bind either dyes [11]. Trichrome stains are a collection of over one hundred stains which dye collagen, muscle, and a third biological component in three different colors [45]. Finally, antibody stains use antibodies to bind collagen molecules, and certain procedures can use them to distinguish between collagen types I and V [39, 48]. These four stains are prevalent for imaging collagen, but it should be noted that there are other collagen stains and stains that can be used to differentiate other elements of the ECM.

IHC stains are also the primary method for imaging elastin and fibronectin in the heart. Early work has shown fibronectin labeling in endocardial and myocardial

tubes of a stage 22 embryonic chick heart [40]. Additional work in embryonic chick hearts over several stages of embryonic development demonstrates the dynamics of elastin expression [27]. More recent studies have revealed how the expression and distribution of fibronectin and elastin vary across the developmental stages of the murine heart [26]. For example, Hanson et al. found that there is fibronectin in the left ventricle's epicardium and endocardium in embryonic and early postnatal mouse hearts. However, fibronectin fibrils did not form in the myocardium until embryonic day 14.5 (E14.5), at which point they continued to increase in density through early postnatal development. Similarly, elastin did not appear in the heart until E16.5, where it began to organize into fibrils in all three regions of the ventricle as well as the blood vessel walls. Fluorescent images from IHC were quantified using mean intensity per pixel values in order to compare relative protein amounts. This, along with the IHC mapping of other ECM proteins found in the heart (Collagen I, Collagen IV) (Fig. 2.5), begin to provide a blueprint for the spatial and temporal organization of proteins critical for cardiac development and function.

2.5.2 Nonlinear Optical Microscopy

Nonlinear optical microscopy (NLO) methods generate label-free high-resolution images at greater imaging depths and higher signal-to-noise ratio than conventional optical imaging such as laser scanning confocal microscopy [35]. These methods are based on nonlinear interactions between light and biological tissues. NLO methods can be used to perform nondestructive imaging that is capable of quantifying collagen and other ECM components in 3D, does not require tissue-damaging labels, and minimizes phototoxic effects in live tissue. They can typically image through 100–300 μm thick cardiovascular tissue, deriving contrast from intrinsic biology and optionally from extrinsic contrast agents [9, 10]. They are currently used to study biopsies and live animal models, but they can potentially be used on patients through endoscopic or surgical probes [4, 10]. The downside of these methods is low availability, expensive equipment requirements, and the need for experienced technicians. However, there are many efforts to develop cheaper and more clinically friendly NLO equipment such as endoscopes that could make these methods more widespread and available for ECM imaging [41]. In addition, NLO methods share most of the same equipment and so can often be implemented on the same workstation and used alongside each other [10, 56].

Combinations of NLO methods are highly effective in characterizing the ECM, as each method derives contrast from a different source. Thus far, the two primary NLO methods for cardiac imaging are second harmonic generation (SHG) and two-photon excited fluorescent (2PEF), which we review below. Another emerging NLO method, Coherent anti-Stokes Raman (CARS) microscopy [35], has been used for imaging atherosclerosis and heart valve stenosis, but its applications overall for ECM imaging are still being explored [8, 37]. Fluorescent methods can also be combined with NLO for additional contrast such as the NLO compatible method of

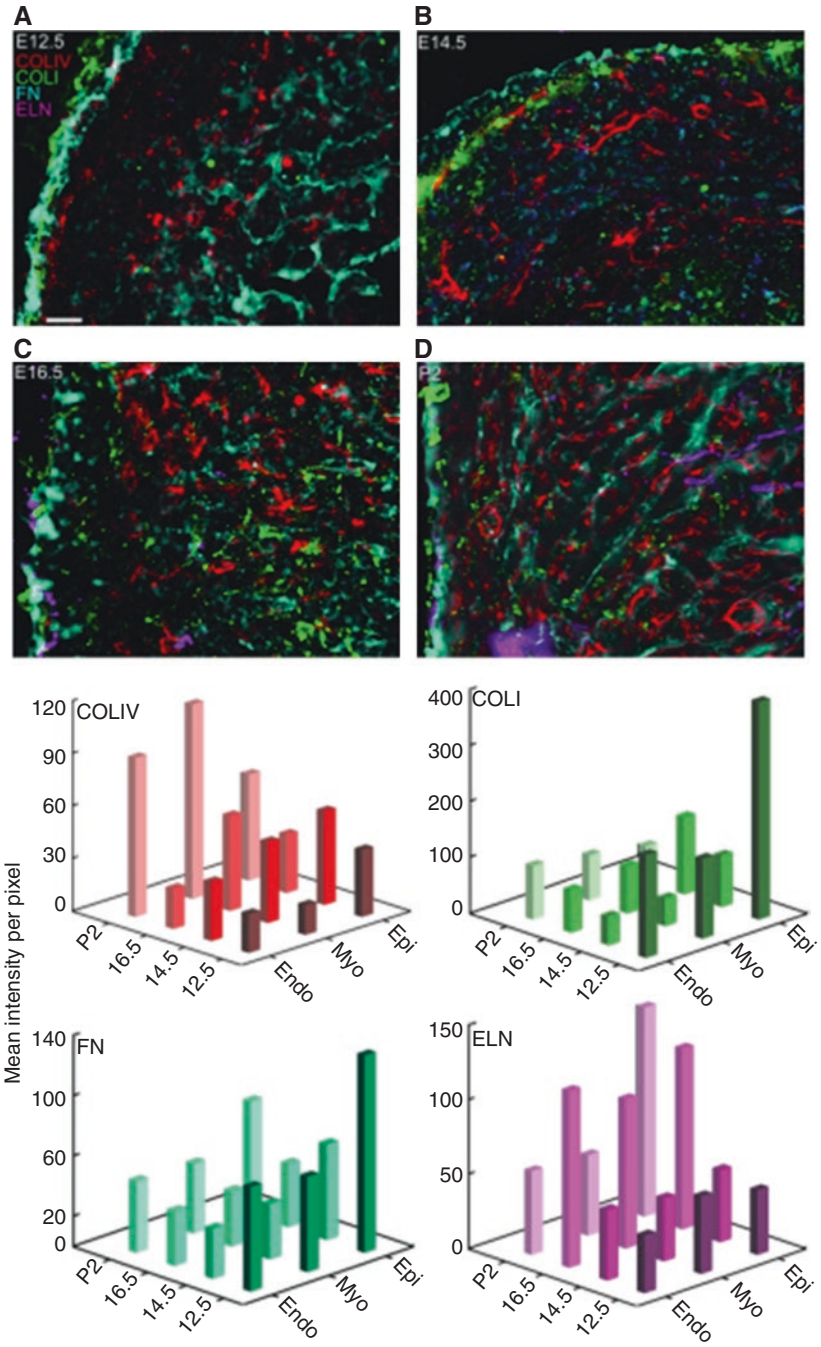

Fig. 2.5 IHC labeled sections of the murine ventricle over the course of development (**a**–**d**). Red, Col IV; green, Col I; cyan, fibronectin; magenta, Elastin. (**e**) Quantitative comparison of ECM proteins across development in each cardiac tissue layer. Endo, endocardium; Myo, myocardium; Epi, epicardium (Hanson, [31]). (Copyright granted as paper coauthor (Eliceiri))

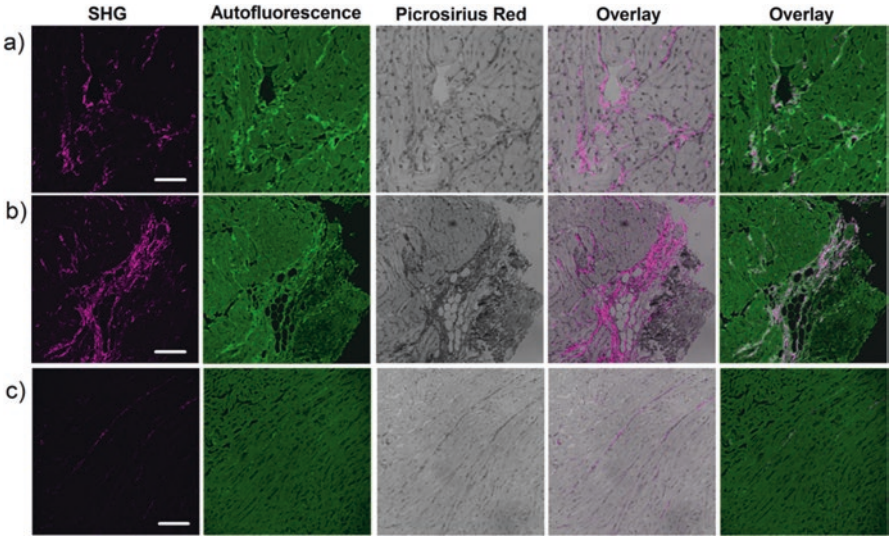

Fig. 2.6 Second harmonic generation (SHG) can image cardiac fibrillar collagen at high specificity and high resolution. These panels show SHG, autofluorescence, and Picrosirius red stained imaging of a myocardial infarction rat model (**a** and **b**) and an age-matched control (**c**). The SHG is overlaid onto the Picrosirius red and autofluorescence stains in the fourth and fifth columns, respectively. (This work is attributed to Caorsi et al. and used through the Creative Commons Attribution license [10])

fluorescence lifetime imaging microscopy (FLIM) to examine the chemical microenvironment of the ECM [35].

SHG can obtain highly specific images of fibrillar collagen in the ECM without the use of extrinsic probes, allowing it to image collagen in live tissue (Fig. 2.6). SHG derives contrast from non-centrosymmetric molecules such as fibrillar collagen (I/III/V), elastin, and myosin. Both collagen and myosin are present in cardiovascular tissue; however, the SHG signal is highly specific to collagen (>95%) at low laser powers (10–20 mw) (Fig. 2.6) [10]. These qualities allow SHG to quantify collagen fiber organization in many cardiovascular diseases, including fibrosis, cardiomyopathy, scar formation after a heart attack, and aortic stenosis [10, 16, 49, 58, 63]. The main limitation of SHG for clinical studies is limited depth due to scattering and refractive index effects of the tissue that attenuate the collected signal. As well, the polarization state of the laser and the orientation of the fibers to the laser can both affect the signal strength. The former can be mitigated using circularly polarized light, but the latter is an intrinsic limitation and affects the signal from collagen fibers between imaging planes [9].

2PEF images fluorescent molecules and so can derive contrast from both intrinsic and biocompatible extrinsic sources, which means that it can be used on live tissue and be enhanced using probes. The intrinsic sources include collagen, elastin, and myocyte proteins [10]. Extrinsic 2PEF is currently less common than intrinsic imaging for cardiac ECM studies, as there are few suitable fluorescent probes.

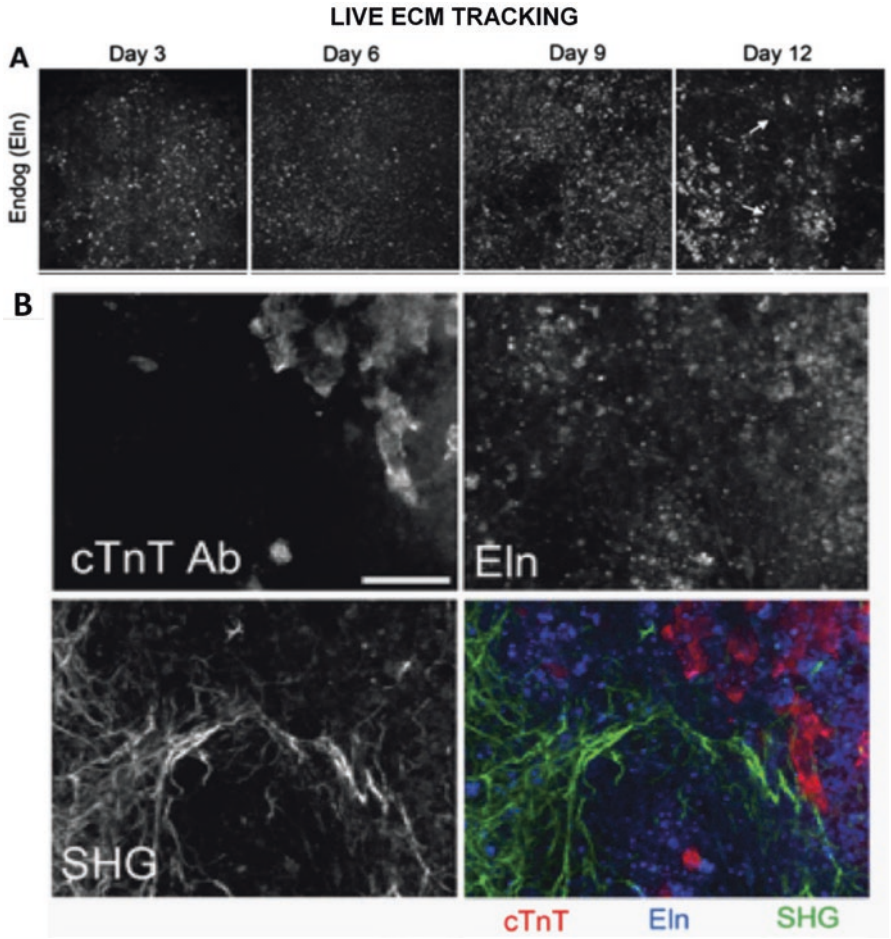

Fig. 2.7 Panel (**a**) Endogenous elastin fluorescence over time. Panel (**b**) Spatial relationship between elastin, collagen, and cardiomyocytes in embryoid bodies [69]. (Copyright granted as paper coauthor (Eliceiri))

However, researchers are facing this problem and developing new methods and identifying suitable probes [15, 67]. 2PEF is easily implemented on the same system as SHG, and so they are often used in conjunction. This pairing visualizes several components of the ECM in the same imaging volume and allows collagen to be separated from other components. For example, tracking elastin deposition during in vitro mouse embryoid body development reveals that the ECM expression transitions from a punctate to fibril-like structure over time (Fig. panel 2.7a) and is spatially associated with cells exhibiting markers of cardiomyocyte maturity (Fig. panel 2.7b) [69].

Fluorescence lifetime imaging microscopy (FLIM) is a 2PEF compatible quantitative imaging method that measures the time a fluorophore stays in the excited state

and is sensitive to changes in the microenvironment such as pH and protein binding [6]. FLIM is currently less common than SHG or 2PEF but can offer unique capabilities for ECM imaging. The lifetime is related to molecular properties including the pH of its surroundings, the concentration of oxygen near the molecule, and the molecule's bonds to other molecules [6]. FLIM has been used to image atherosclerotic plaques, helping characterize the plaque composition [30, 51]. FLIM may have uses in cardiac myopathy, where the ratio of collagen I and III fibers matters. A previous study showed that FLIM can be used in combination with SHG to separate intrinsic fluorescence signal from collagens I and III, though it was not demonstrated in cardiac tissue [56].

In summation, these NLO imaging methods offer 3D imaging on the cellular size scale and in live tissue. They are currently used in research applications but can potentially be implemented in clinic given emerging advances in less expensive, more modular NLO implementation and endoscope-based NLO imaging.

2.5.3 Electron Microscopy

Electron microscopy (EM) is the highest resolution imaging category mentioned in this chapter and is capable of resolving biology smaller than the resolution limits of light microscopy. There are other similarly high-resolution imaging methods with potential for cardiac ECM imaging such as atomic force microscopy (AFM) and super resolution optical imaging [17, 19], but we do not review them due their infrequent use in cardiac ECM imaging. EM is a category of imaging methods that use electrons to create the image, achieving much higher resolution than optical-based methods. Cardiac imaging studies primarily use transmission electron tomography (TEM) and scanning electron microscopy (SEM). However, there are also powerful EM variants such as environmental S/TEM (E-S/TEM) [34], immuno-EM [43], and Cryo-EM [18] that can image the structure of individual cardiac ECM proteins or offer advantages in contrast and visualization. The high-resolutions EM can visualize the molecular structure of ECM components, e.g., collagen fibrils. Such high-resolution molecular information is valuable for many research applications.

However, EM is subject to several limitations that prevent it from being used clinically. EM is expensive and requires highly specialized training, limiting the number of locations that can perform it. It requires tissue samples, such as from biopsies, which are often unavailable in clinical care. In addition, most forms of EM currently involve extensive sample preparation and process or destroy the tissue samples during imaging, making it impossible to image with other methods [44]. As such, EM can offer valuable research insights about disease biology but is less likely to be used routinely for patient care due to specimen preparation requirements and high cost.

EM has been used in a range of cardiac applications from disease models to basic physiology to cardiac tissue engineering. In models for early stage heart failure, TEM was able to detect fibrotic changes in collagen before other collagen imaging methods [64]. TEM has also been used in the field of cardiac tissue engineering to

validate the structure of collagenous scaffolds meant for cardiac tissue patches [65]. Additionally, TEM has been useful in cardiac physiology research. The bulbus arteriosus, an elastic chamber in the heart, appears to be a dense (presumably rigid) collagen matrix under immunohistochemistry, but EM showed an interwoven collagen-elastin matrix, giving a much more accurate view of the physiology [29]. In models of myocardial infarction, SEM analysis of collagen fibril structure showed that osteoglycin promotes proper collagen maturation and improves tissue healing outcomes [2]. In models for calcific aortic stenosis, a disease where minerals are heavily deposited in the ECM, SEM showed how the mineral deposits develop in the interior of collagen fibers [50]. Finally, Sands et al. used E-SEM to visualize the cardiac ventricular trabeculae carneae, strands of axially arranged tissue in the

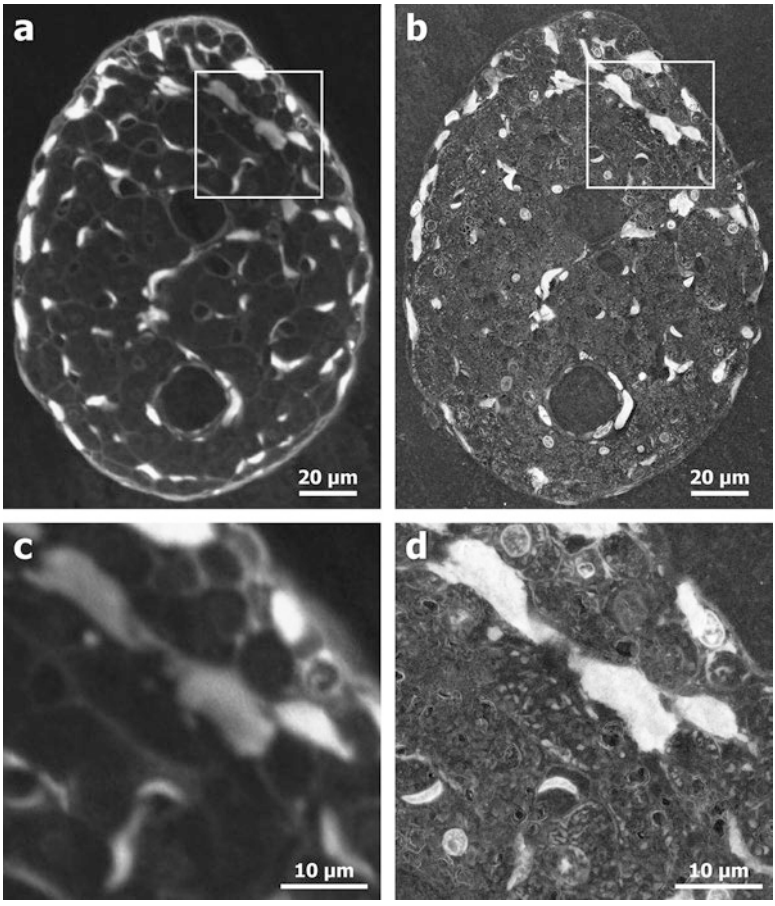

Fig. 2.8 Electron microscopy (**b**, **d**) can resolve biological structures in the cardiac trabecula that are beyond the resolution limit of confocal microscopy (**a**, **c**) [61]. (Figure permission granted through RightsLink)

heart. They showed that collagen content and microstructure varied widely, ranging from 1% to 100% of the tissue cross section in different areas (Fig. 2.8) [61].

2.6 Future Directions

Knowledge of the composition and topography of the human heart can improve clinical evaluation as well as help develop treatment strategies; however, acquiring this knowledge in a noninvasive method is a continuing challenge of the field. There is a need for noninvasive imaging methods that are component-specific, high-resolution, and cost-effective for use in the clinic. Researchers are making progress on each of these areas, but there still remains much to be done. The best developed methods for imaging individual components of the ECM are invasive, e.g., histochemistry requiring biopsies. Recent advances in molecular MRI and nuclear imaging show promise for low-resolution organ-scale imaging of ECM components, providing probes for imaging collagen, elastin, receptors, and matrix metalloproteinases [25]. However, the existing probes still require development and validation through clinical trials, and there are no probes for many other ECM components of interest. Other advances may address the difficulty of high-resolution imaging. NLO methods can image ECM components at the cellular-scale without labels and in 3D up to a depth of several hundred microns. However, they are currently expensive, require trained technicians, and most implementations cannot be used on patients. Future advances may change this with the development of endoscopes, simplification of equipment, and corresponding reduced cost. Overall, ECM imaging is heading in promising directions that may help improve clinical outcomes and the role of the ECM in heart physiology.

The previous sections have covered ECM imaging in regard to disease screening, therapy targeting, and diagnostics, but there is also a strong need for such noninvasive ECM imaging in the field of tissue engineering and regeneration. The native composition and organization of ECM proteins can serve as a template for ECM-mimicking tissue engineering strategies. Hydrogels that incorporate ECM molecules such as collagen and laminin support mouse-induced pluripotent stem cell (miPSC) cardiomyocyte differentiation [31], while tissue regeneration structures modeled after the fibronectin distribution in a mouse heart support cardiomyocyte function in a mouse model of myocardial infarction [22]. The field of tissue regeneration can also benefit from knowing the role and organization of specific ECM molecules in cardiac tissue development. Many tissue regeneration strategies are based on mimicking the native ECM composition and architecture. Future advances in cardiac tissue regeneration will rely on the discovery of the critical combinations and organizations of ECM molecules for cardiac function. Thus, both tissue engineering and tissue regeneration would benefit from future developments for advanced ECM imaging methods.

2.7 Conclusion

The field of cardiac ECM imaging is in a rapidly advancing state; it is in the middle of an evolution from primarily invasive imaging with small ex vivo samples to more noninvasive imaging that can measure large regions of the human heart in the clinic. In the past, the only option was for invasive imaging of small samples, but this is beginning to change as researchers approach the problem from two angles. The first is by developing low-resolution, but high-specificity, imaging methods that be used clinically to measure the bulk composition of the ECM. The second is in improving high-resolution methods by reducing costs and developing better form factors, so they can be used with minimal invasiveness in the clinic. The combination of these approaches will improve researcher and clinician ability to nondestructively evaluate the composition of the heart. In the future, these advanced imaging methods will propel both the clinical and research fields toward strategies for enhanced patient care, aiding in the fields of cardiac development, disease, tissue engineering, and tissue regeneration.

Acknowledgment We thank Brenda Ogle and Ellen Arena for useful input. We also acknowledge funding from the Laboratory for Optical and Computational Instrumentation, the Morgridge Institute for Research, NIH R01 HL137204 (RAH), NIH R01 131017 (RAH), NIH T32 CA009206 (MAP), and the NIH T32 GM008349 (MAP). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

References

1. Abe M, Takahashi M, Horiuchi K, Nagano A. The changes in crosslink contents in tissues after formalin fixation. Anal Biochem. 2003;318:118–23. https://doi.org/10.1016/S0003-2697(03)00194-5
2. Aelst LNLV, Voss S, Carai P, Leeuwen RV, Vanhoutte D, Wijk SS, Eurlings L, Swinnen M, Verheyen FK, Verbeken E, Nef H, Troidl C, Cook SA, Rocca H-PB-L, Möllmann H, Papageorgiou A-P, Heymans S. Osteoglycin Prevents Cardiac Dilatation and Dysfunction After Myocardial Infarction Through Infarct Collagen StrengtheningNovelty and Significance. Circ Res. 2015;116:425–36. https://doi.org/10.1161/CIRCRESAHA.116.304599
3. Al-Janabi S, Huisman A, Van Diest PJ. Digital pathology: current status and future perspectives. Histopathology. 2012;61:1–9. https://doi.org/10.1111/j.1365-2559.2011.03814.x
4. Bao H, Boussioutas A, Jeremy R, Russell S, Gu M. Second harmonic generation imaging via nonlinear endomicroscopy. Opt Express. 2010;18:1255. https://doi.org/10.1364/OE.18.001255
5. Bashey RI, Martinez-Hernandez A, Jimenez SA. Isolation, characterization, and localization of cardiac collagen type VI Associations with other extracellular matrix components. Circ Res. 1992;70:1006–17.
6. Becker W. Fluorescence lifetime imaging – techniques and applications. J Microsc. 2012;247:119–36. https://doi.org/10.1111/j.1365-2818.2012.03618.x
7. Bull S, White SK, Piechnik SK, Flett AS, Ferreira VM, Loudon M, Francis JM, Karamitsos TD, Prendergast BD, Robson MD, Neubauer S, Moon JC, Myerson SG. Human non-contrast

T1 values and correlation with histology in diffuse fibrosis. Heart. 2013;99:932–7. https://doi.org/10.1136/heartjnl-2012-303052
8. Büttner P, Galli R, Jannasch A, Schnabel C, Waldow T, Koch E. Heart valve stenosis in laser spotlights: Insights into a complex disease. Clin Hemorheol Microcirc. 2014;58:65–75. https://doi.org/10.3233/CH-141882
9. Campagnola P. Second harmonic generation imaging microscopy: applications to diseases diagnostics. Anal Chem. 2011;83:3224–31. https://doi.org/10.1021/ac1032325
10. Caorsi V, Toepfer C, Sikkel MB, Lyon AR, MacLeod K, Ferenczi MA. Non-Linear Optical Microscopy Sheds Light on Cardiovascular Disease. PLoS One. 2013;8:e56136. https://doi.org/10.1371/journal.pone.0056136
11. Chan JKC. The Wonderful Colors of the Hematoxylin–Eosin Stain in Diagnostic Surgical Pathology. Int J Surg Pathol. 2014;22:12–32. https://doi.org/10.1177/1066896913517939
12. Chatterjee S. Artefacts in histopathology. J Oral Maxillofac Pathol JOMFP. 2014;18:S111–6. https://doi.org/10.4103/0973-029X.141346
13. Dave JK, Mc Donald ME, Mehrotra P, Kohut AR, Eisenbrey JR, Forsberg F. Recent technological advancements in cardiac ultrasound imaging. Ultrasonics. 2018;84:329–40. https://doi.org/10.1016/j.ultras.2017.11.013
14. Désogère P, Tapias LF, Rietz TA, Rotile N, Blasi F, Day H, Elliot J, Fuchs BC, Lanuti M, Caravan P. Optimization of a collagen-targeted positron emission tomography probe for molecular imaging of pulmonary fibrosis. J Nucl Med jnumed. 2017;117:193532. https://doi.org/10.2967/jnumed.117.193532
15. Drobizhev M, Makarov NS, Tillo SE, Hughes TE, Rebane A. Two-photon absorption properties of fluorescent proteins. Nat Methods. 2011;8:393–9. https://doi.org/10.1038/nmeth.1596
16. Echegaray K, Andreu I, Lazkano A, Villanueva I, Sáenz A, Elizalde MR, Echeverría T, López B, Garro A, González A, Zubillaga E, Solla I, Sanz I, González J, Elósegui-Artola A, Roca-Cusachs P, Díez J, Ravassa S, Querejeta R. Role of Myocardial Collagen in Severe Aortic Stenosis With Preserved Ejection Fraction and Symptoms of Heart Failure. Rev Esp Cardiol Engl Ed. 2017;70:832–40. https://doi.org/10.1016/j.rec.2016.12.038
17. Eghiaian F, Rico F, Colom A, Casuso I, Scheuring S. High-speed atomic force microscopy: Imaging and force spectroscopy. FEBS Lett. 2014;588:3631–8. https://doi.org/10.1016/j.febslet.2014.06.028
18. Fernandez-Leiro R, Scheres SH. Unravelling biological macromolecules with cryo-electron microscopy., Unravelling the structures of biological macromolecules by cryo-EM. Nature. 2016;537:339–46. https://doi.org/10.1038/nature19948. https://doi.org/10.1038/nature19948.
19. Fornasiero EF, Opazo F. Super-resolution imaging for cell biologists. Bioessays. 2015;37:436–51. https://doi.org/10.1002/bies.201400170
20. Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. J Clin Invest. 2017;127:1600–12. https://doi.org/10.1172/JCI87491
21. Galetta F, Franzoni F, Bernini G, Poupak F, Carpi A, Cini G, Tocchini L, Antonelli A, Santoro G. Cardiovascular complications in patients with pheochromocytoma: A mini-review. Biomed Pharmacother. 2010;64:505–9. https://doi.org/10.1016/j.biopha.2009.09.014
22. Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, Da Sie Y, Tran Q, Ajeti V, Freeman BT, Fast VG, Campagnola PJ, Ogle BM, Zhang J. Myocardial Tissue Engineering With Cells Derived From Human-Induced Pluripotent Stem Cells and a Native-Like, High-Resolution, 3-Dimensionally Printed Scaffold. Circ Res. 2017;120:1318–25. https://doi.org/10.1161/CIRCRESAHA.116.310277
23. Goergen CJ, Chen HH, Sakadžić S, Srinivasan VJ, Sosnovik DE. Microstructural characterization of myocardial infarction with optical coherence tractography and two-photon microscopy. Physiol Rep. 2016;4:e12894. https://doi.org/10.14814/phy2.12894
24. Gyöngyösi M, Winkler J, Ramos I, Do Q-T, Firat H, McDonald K, González A, Thum T, Díez J, Jaisser F, Pizard A, Zannad F. Myocardial fibrosis: biomedical research from bench to bedside. Eur J Heart Fail. 2017;19:177–91. https://doi.org/10.1002/ejhf.696

25. de Haas HJ, Arbustini E, Fuster V, Kramer CM, Narula J. Molecular Imaging of the Cardiac Extracellular Matrix. Circ Res. 2014;114:903–15. https://doi.org/10.1161/CIRCRESAHA.113.302680
26. Hanson KP, Jung JP, Tran QA, Hsu S-PP, Iida R, Ajeti V, Campagnola PJ, Eliceiri KW, Squirrell JM, Lyons GE, Ogle BM. Spatial and Temporal Analysis of Extracellular Matrix Proteins in the Developing Murine Heart: A Blueprint for Regeneration. Tissue Eng Part A. 2013;19:1132–43. https://doi.org/10.1089/ten.tea.2012.0316
27. Hurle JM, Kitten GT, Sakai LY, Volpin D, Solursh M. Elastic extracellular matrix of the embryonic chick heart: an immunohistological study using laser confocal microscopy. Dev Dyn. 1994;200:321–32. https://doi.org/10.1002/aja.1002000407
28. Hutson HN, Marohl T, Anderson M, Eliceiri K, Campagnola P, Masters KS. Calcific Aortic Valve Disease Is Associated with Layer-Specific Alterations in Collagen Architecture. PLoS One. 2016;11:e0163858. https://doi.org/10.1371/journal.pone.0163858
29. Icardo JM. Collagen and elastin histochemistry of the teleost bulbus arteriosus: False positives. Acta Histochem. 2013;115:185–9. https://doi.org/10.1016/j.acthis.2012.03.002
30. Jo JA, Park J, Pande P, Shrestha S, Serafino MJ, Jimenez Jde J R, Clubb F, Walton B, Buja LM, Phipps JE, Feldman MD, Adame J, Applegate BE. Simultaneous morphological and biochemical endogenous optical imaging of atherosclerosis. Eur Heart J Cardiovasc Imaging. 2015;16:910–8. https://doi.org/10.1093/ehjci/jev018
31. Jung JP, Sprangers AJ, Byce JR, Su J, Squirrell JM, Messersmith PB, Eliceiri KW, Ogle BM. ECM-incorporated hydrogels cross-linked via native chemical ligation to engineer stem cell microenvironments. Biomacromolecules. 2013;14:3102–11. https://doi.org/10.1021/bm400728e
32. Khan Z, Boughner DR, Lacefield JC. Anisotropy of High-Frequency Integrated Backscatter from Aortic Valve Cusps. Ultrasound Med Biol. 2008;34:1504–12. https://doi.org/10.1016/j.ultrasmedbio.2008.02.001
33. Kim H, Lee S-J, Kim JS, Davies-Venn C, Cho H-J, Won SJ, Dejene E, Yao Z, Kim I, Paik CH, Bluemke DA. Pharmacokinetics and microbiodistribution of 64Cu-labeled collagen binding peptides in chronic myocardial infarction. Nucl Med Commun. 2016;37:1306–17. https://doi.org/10.1097/MNM.0000000000000590
34. Kirk S e, Skepper J n, Donald A m. Application of environmental scanning electron microscopy to determine biological surface structure. J Microsc. 2009;233:205–24. https://doi.org/10.1111/j.1365-2818.2009.03111.x
35. Krafft C. Modern trends in biophotonics for clinical diagnosis and therapy to solve unmet clinical needs. J Biophotonics. 2016;9:1362–75. https://doi.org/10.1002/jbio.201600290
36. Lattouf R, Younes R, Lutomski D, Naaman N, Godeau G, Senni K, Changotade S. Picrosirius Red Staining: A Useful Tool to Appraise Collagen Networks in Normal and Pathological Tissues. J Histochem Cytochem. 2014;62:751–8. https://doi.org/10.1369/0022155414545787
37. Le TT, Langohr IM, Locker MJ, Sturek M, Cheng J-X. Label-free molecular imaging of atherosclerotic lesions using multimodal nonlinear optical microscopy. J Biomed Opt. 2007;12:054007. https://doi.org/10.1117/1.2795437
38. Leeming, D.J., Karsdal, M.A., 2016. Chap. .5 – Type V Collagen. In: Biochemistry of Collagens, Laminins and Elastin. Academic Press. p. 43–48. https://doi.org/10.1016/B978-0-12-809847-9.00005-2
39. Li Y, Ho D, Meng H, Chan TR, An B, Yu H, Brodsky B, Jun AS, Yu SM. Direct detection of collagenous proteins by fluorescently labeled collagen mimetic peptides. Bioconjug Chem. 2013;24:9–16. https://doi.org/10.1021/bc3005842
40. Little CD, Piquet DM, Davis LA, Walters L, Drake CJ. Distribution of laminin, collagen type IV, collagen type I, and fibronectin in chicken cardiac jelly/basement membrane. Anat Rec. 1989;224:417–25. https://doi.org/10.1002/ar.1092240310
41. Liu, J., Lim, K.C., Li, H., Seck, H.L., Yu, X., Kok, S.W., Zhang, Y., 2015. Low cost and compact nonlinear (SHG/TPE) laser scanning endoscope for bio-medical application. 93041 K–93041 K–6. https://doi.org/10.1117/12.2078809

42. López B, González A, Díez J. Circulating Biomarkers of Collagen Metabolism in Cardiac Diseases. Circulation. 2010;121:1645–54. https://doi.org/10.1161/CIRCULATIONAHA.109.912774
43. Mayhew TM, Lucocq JM. Developments in cell biology for quantitative immunoelectron microscopy based on thin sections: a review. Histochem Cell Biol. 2008;130:299–313. https://doi.org/10.1007/s00418-008-0451-6
44. McDonald KL. Out with the old and in with the new: rapid specimen preparation procedures for electron microscopy of sectioned biological material. Protoplasma. 2014;251:429–48. https://doi.org/10.1007/s00709-013-0575-y
45. McGavin MD. Factors Affecting Visibility of a Target Tissue in Histologic Sections. Vet Pathol. 2014;51:9–27. https://doi.org/10.1177/0300985813506916
46. Mostaço-Guidolin L, Rosin NL, Hackett T-L. Imaging Collagen in Scar Tissue: Developments in Second Harmonic Generation Microscopy for Biomedical Applications. Int J Mol Sci. 2017;18:1772. https://doi.org/10.3390/ijms18081772
47. Muzard J, Sarda-Mantel L, Loyau S, Meulemans A, Louedec L, Bantsimba-Malanda C, Hervatin F, Marchal-Somme J, Michel JB, Le Guludec D, Billiald P, Jandrot-Perrus M. Non-Invasive Molecular Imaging of Fibrosis Using a Collagen-Targeted Peptidomimetic of the Platelet Collagen Receptor Glycoprotein VI. PLoS One. 2009;4:e5585. https://doi.org/10.1371/journal.pone.0005585
48. Pataridis S, Eckhardt A, Mikulíková K, Sedláková P, Mikšík I. Identification of collagen types in tissues using HPLC-MS/MS. J Sep Sci. 2008;31:3483–8. https://doi.org/10.1002/jssc.200800351
49. Pauschinger M, Knopf D, Petschauer S, Doerner A, Poller W, Schwimmbeck PL, Kühl U, Schultheiss H-P. Dilated Cardiomyopathy Is Associated With Significant Changes in Collagen Type I/III ratio. Circulation. 1999;99:2750–6. https://doi.org/10.1161/01.CIR.99.21.2750
50. Perrotta I, Davoli M. Collagen Mineralization in Human Aortic Valve Stenosis: A Field Emission Scanning Electron Microscopy and Energy Dispersive Spectroscopy Analysis. Ultrastruct Pathol. 2014;38:281–4. https://doi.org/10.3109/01913123.2014.901468
51. Phipps JE, Sun Y, Fishbein MC, Marcu L. A Fluorescence Lifetime Imaging Classification Method to Investigate the Collagen to Lipid Ratio in Fibrous Caps of Atherosclerotic Plaque. Lasers Surg Med. 2012;44:564–71. https://doi.org/10.1002/lsm.22059
52. Podlesnikar T, Delgado V, Bax JJ. Cardiovascular magnetic resonance imaging to assess myocardial fibrosis in valvular heart disease. Int J Cardiovasc Imaging. 2017:1–16. https://doi.org/10.1007/s10554-017-1195-y
53. Protti A, Lavin B, Dong X, Lorrio S, Robinson S, Onthank D, Shah AM, Botnar RM. Assessment of Myocardial Remodeling Using an Elastin/Tropoelastin Specific Agent with High Field Magnetic Resonance Imaging (MRI). J Am Heart Assoc. 2015;4:e001851. https://doi.org/10.1161/JAHA.115.001851
54. Qin X, Fei B. Measuring Myofiber Orientations from High-frequency Ultrasound Images Using Multiscale Decompositions. Phys Med Biol. 2014;59:3907–24. https://doi.org/10.1088/0031-9155/59/14/3907
55. Qin X, Riegler J, Tiburcy M, Zhao X, Chour T, Ndoye B, Nguyen M, Adams J, Ameen M, Denney TS, Yang PC, Nguyen P, Zimmermann WH, Wu JC. Magnetic Resonance Imaging of Cardiac Strain Pattern Following Transplantation of Human Tissue Engineered Heart Muscles. Circ Cardiovasc Imaging. 2016;9:e004731. https://doi.org/10.1161/CIRCIMAGING.116.004731
56. Ranjit S, Dvornikov A, Stakic M, Hong S-H, Levi M, Evans RM, Gratton E. Imaging Fibrosis and Separating Collagens using Second Harmonic Generation and Phasor Approach to Fluorescence Lifetime Imaging. Sci Rep. 2015;5:13378. https://doi.org/10.1038/srep13378
57. Reusch LM, Feltovich H, Carlson LC, Hall G, Campagnola PJ, Eliceiri KW, Hall TJ. Nonlinear optical microscopy and ultrasound imaging of human cervical structure. J Biomed Opt. 2013;18:031110. https://doi.org/10.1117/1.JBO.18.3.031110
58. Richardson W, Clarke S, Quinn T, Holmes J. Physiological Implications of Myocardial Scar Structure. Compr Physiol. 2015;5:1877–909. https://doi.org/10.1002/cphy.c140067

59. Romito E, Shazly T, Spinale FG. In vivo assessment of regional mechanics post-myocardial infarction: A focus on the road ahead. J Appl Physiol. 2017;123:728–45. https://doi.org/10.1152/japplphysiol.00589.2015
60. Salerno M, Kramer CM. Advances in Parametric Mapping With CMR Imaging. JACC Cardiovasc Imaging. 2013;6:806–22. https://doi.org/10.1016/j.jcmg.2013.05.005
61. Sands G, Goo S, Gerneke D, LeGrice I, Loiselle D. The collagenous microstructure of cardiac ventricular trabeculae carneae. J Struct Biol. 2011;173:110–6. https://doi.org/10.1016/j.jsb.2010.06.020
62. Schelbert EB, Fonarow GC, Bonow RO, Butler J, Gheorghiade M. Therapeutic Targets in Heart Failure: Refocusing on the Myocardial Interstitium. J Am Coll Cardiol. 2014;63:2188–98. https://doi.org/10.1016/j.jacc.2014.01.068
63. Schenke-Layland K, Stock UA, Nsair A, Xie J, Angelis E, Fonseca CG, Larbig R, Mahajan A, Shivkumar K, Fishbein MC, MacLellan WR. Cardiomyopathy is associated with structural remodelling of heart valve extracellular matrix. Eur Heart J. 2009;30:2254–65. https://doi.org/10.1093/eurheartj/ehp267
64. Schipke J, Brandenberger C, Rajces A, Manninger M, Alogna A, Post H, Mühlfeld C. Assessment of cardiac fibrosis: a morphometric method comparison for collagen quantification. J Appl Physiol. 2017;122:1019–30. https://doi.org/10.1152/japplphysiol.00987.2016
65. Srinivasan A, Sehgal PK. Characterization of Biocompatible Collagen Fibers—A Promising Candidate for Cardiac Patch. Tissue Eng Part C Methods. 2009;16:895–903. https://doi.org/10.1089/ten.tec.2009.0475
66. Sudarshan V, Acharya UR, Ng EYK, Meng CS, Tan RS, Ghista DN. Automated Identification of Infarcted Myocardium Tissue Characterization Using Ultrasound Images: A Review. IEEE Rev Biomed Eng. 2015;8:86–97. https://doi.org/10.1109/RBME.2014.2319854
67. Tao W, Rubart M, Ryan J, Xiao X, Qiao C, Hato T, Davidson MW, Dunn KW, Day RN. A practical method for monitoring FRET-based biosensors in living animals using two-photon microscopy. Am J Physiol Cell Physiol. 2015;309:C724–35. https://doi.org/10.1152/ajpcell.00182.2015
68. Taylor AJ, Salerno M, Dharmakumar R, Jerosch-Herold M. T1 Mapping: Basic Techniques and Clinical Applications. JACC Cardiovasc Imaging. 2016;9:67–81. https://doi.org/10.1016/j.jcmg.2015.11.005
69. Thimm TN, Squirrell JM, Liu Y, Eliceiri KW, Ogle BM. Endogenous Optical Signals Reveal Changes of Elastin and Collagen Organization During Differentiation of Mouse Embryonic Stem Cells. Tissue Eng Part C Methods. 2015;21:995–1004. https://doi.org/10.1089/ten.TEC.2014.0699
70. Trivedi V, Truong TV, Trinh LA, Holland DB, Liebling M, Fraser SE. Dynamic structure and protein expression of the live embryonic heart captured by 2-photon light sheet microscopy and retrospective registration. Biomed Opt Express. 2015;6:2056–66. https://doi.org/10.1364/BOE.6.002056
71. Tsamis A, Krawiec JT, Vorp DA. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. J R Soc Interface. 2013;10:20121004. https://doi.org/10.1098/rsif.2012.1004
72. Wahyudi H, Reynolds AA, Li Y, Owen SC, Yu SM. Targeting collagen for diagnostic imaging and therapeutic delivery. J Control Release. 2016;240:323–31. https://doi.org/10.1016/j.jconrel.2016.01.007
73. White JF, Werkmeister JA, Hilbert SL, Ramshaw JAM. Heart valve collagens: cross-species comparison using immunohistological methods. J Heart Valve Dis. 2010;19:766–71.
74. White SK, Sado DM, Flett AS, Moon JC. Characterising the myocardial interstitial space: the clinical relevance of non-invasive imaging. Heart. 2012;98:773–9. https://doi.org/10.1136/heartjnl-2011-301515
75. WHO | Cardiovascular diseases (CVDs) [WWW Document], 2017. WHO. URL. http://www.who.int/mediacentre/factsheets/fs317/en/. (Accessed 6 Mar 2018).

76. Won S, Davies-venn C, Liu S, Bluemke DA. Noninvasive imaging of myocardial extracellular matrix for assessment of fibrosis. Curr Opin Cardiol. 2013;28:282–9. https://doi.org/10.1097/HCO.0b013e32835f5a2b
77. Wu X, Sun Z, Foskett A, Trzeciakowski JP, Meininger GA, Muthuchamy M. Cardiomyocyte contractile status is associated with differences in fibronectin and integrin interactions. Am J Physiol Heart Circ Physiol. 2010;298:H2071–81. https://doi.org/10.1152/ajpheart.01156.2009
78. Zheng L, Ding X, Liu K, Feng S, Tang B, Li Q, Huang D, Yang S. Molecular imaging of fibrosis using a novel collagen-binding peptide labelled with ^{99m}Tc on SPECT/CT. Amino Acids. 2017;49:89–101. https://doi.org/10.1007/s00726-016-2328-7

Chapter 3
Animal Models and Cardiac Extracellular Matrix Research

Timothy A. Hacker

Abstract Cardiovascular disease has been the leading cause of death worldwide for the last 15 years, accounting for 15 million deaths per year. While interventions are saving more lives, more than 20% of survivors will end up in heart failure. Cell-based and other types of therapy for advanced heart and vascular disease may offer new hope for those afflicted. Although a variety of cell types are under investigation, common issues include cell survival, retention, engraftment, and proliferation. Cardiac extracellular matrix (C-ECM) has compelling features that offer advantages to not only aid cell survival, retention, engraftment, and proliferation but likely has independent therapeutic (paracrine) and mechanical effects. Animal studies and clinical trials are underway to characterize the role of C-ECM and demonstrate efficacy for acute and chronic heart disease. This chapter reviews animal models used to enhance our knowledge of C-ECMs in heart disease and its use in the treatment of heart disease.

Keywords Animal models · Extracellular matrix · Mice · Rats · Swine · Dog · Cardiac disease · Heart failure · Myocardial infarction · Surgical models

3.1 Animal Models of Heart Disease

3.1.1 Introduction

Cardiovascular disease is a complex multifactorial disease with different etiologies that may act synergistically to promote disease development and progression. Animal models are necessary to recapitulate the disease in order to investigate mechanisms

T. A. Hacker (✉)
Cardiovascular Research Center, Department of Medicine, University of Wisconsin, Madison, WI, USA

Cardiovascular Physiology Core Facility, Madison, WI, USA
e-mail: th2@medicine.wisc.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_3

of disease and to provide a test system for mechanical, pharmacological, and cell-based treatments. Since no single model perfectly recreates the human condition, one must consider several factors in choosing the correct model. These would include cost, equipment, infrastructure, specialized personnel (skill), and stage of investigation. Consideration of inherent lack of comorbidities such as smoking, inactivity, age, diabetes, and hypertension in animal models can make translation to the bedside challenging. Differences in size, anatomy, muscle fiber type, heart rates, and electrical activity can create additional issues in moving from bench to bedside. However, if an appropriate model exists and when proper consideration of the question is assessed, the results of the investigation will be widely useful. The final steps to moving therapies into clinical trials will require animal testing as demanded by the FDA in relevant animal models that have yielded reproducible results in the past. This will be essential for approval of investigational new drugs or cellular therapies and for advancing our understanding of human cardiovascular disease.

3.2 Transgenic Models of ECM

A search of the Jackson Labs database reveals over 40 transgenic mice with mutations related to cardiac extracellular matrix. Many of these mice do not have an overt cardiac phenotype, but the phenotype is revealed under stress such as myocardial infarction or pressure overload. Typically, these interventions cause increases in left ventricular dilation or acceleration to heart failure with deletions of TIMP family of proteins. On the other hand, overexpression of these proteins often leads to some level of protection of cardiac structure and function post MI. Details of the popular transgenic models are reviewed in several excellent manuscripts as listed in Table 3.1.

While alteration of genes related to the ECM has given us insights to the ECM functional roles in the developing heart and with stressors such as myocardial infarctions and pressure overload, additional roles of the ECM in the heart and vascular system can be discovered using other animal models of disease.

The ECM undergoes extensive changes in the composition and mechanical properties in diseased hearts, and various surgical animal models will help to understand these changes. These animal models can then be used to help develop and test therapies that will alter the course of the disease or remodeling associated with it. Choosing the correct animal model is paramount to successful experiments. The various considerations to choosing an animal model include cost, equipment available, infrastructure, personnel skill, and the stage of the investigation.

Cost can often be the deciding factor when choosing an animal.

model. Rodents are the least expensive to purchase and house, although some transgenic mice can cost nearly as much as a young pig. The cost of equipment to

Table 3.1 Common mouse models of ECM

Gene altered	Expression level	Cardiac phenotype	Effect of MI	Effect of pressure overload	Others	Reference
TIMP1	Deletion	Modest LV dilation with age	Increased dilation	No worsening of function or structure	Enhanced aortic aneurysm	[1–4]
TIMP2	Deletion	None	Increase MI size, dilation, decreased function	Dilation, decreased function		[5] [6] [7]
TIMP2	Overexpression	N/A	Decreased MI expansion, deceased dilation improved function			[8]
TIMP3	Deletion	Dilation in aged mice	Increased rupture	Dilation, decreased function		[9–13]
TIMP3	Overexpression	N/A	Decrease MI expansion, decreased dilation improved function			[14]
TIMP4	Deletion	No effect	Increased rupture	No effect		[15]
TIMP4	Overexpression	No effect	Decreased dilation, improved function	Improved function		[16, 17]

assist with rodent surgeries and physiologic monitoring are typically similar to equipment needed for larger animals, so costs may be wildly different depending on the equipment needed to complete the experiments.

Specialized equipment to create and analyze animal models can quickly add to the high cost both for small and large animal research. Basic equipment for these types of studies will include surgical instruments, microscopes, warming pads, lights, vital sign monitoring systems, ventilators, vaporizers, physiological recording and analysis machines (ECG, pressure, pressure-volume, cardiac output monitoring), imaging (ultrasound, MRI, angiography, etc.), and disposables (suture, drugs, catheters, wires, sheaths, etc.). Core facilities are vitally important in today's research environment to justify the cost of specialized equipment used only once or twice per year in an individual lab.

Infrastructure to house and care for animals is also an important consideration in choosing an animal model. Infrastructure includes the physical spaces for housing and maintaining animals, surgical suites, imaging, and necropsies but also animal care staff, veterinarians, and institutional care and use committees to approve animal protocols and other oversight. The use of university core facilities and contract research organizations can provide cost-effective ways to expand into additional animal models.

All enterprises are only as good as their personnel. Surgical animal models are particularly challenging, due to factors that cannot be controlled such as variable anatomy and physiology from animal to animal. A highly skilled and experienced animal surgeon can play a major role in alleviating the effects some of these variabilities leading to outcomes that are more consistent and have lower mortalities. Well-qualified animal care staff and veterinarians can also influence the quality of the animal models by providing consistent care, minimizing animal stress, and keeping ahead of animal health issues. Surgical skill is the largest single factor in producing reproducible animal models. Microsurgical models (mice, rats) are particularly challenging to learn. Hand skills with excellent vision are required as there is almost no room for error in creating surgical models of injury in these small-sized animals. A single-nicked vessel can cause the animal to bleed out instantly. However, once through the surgery, rodents are particularly resilient and less prone to infections and therefore more likely to survive. Large animals have a different set of challenges. Hand-eye coordination is still crucially important, as is a thorough understanding of anatomy. While errors are more likely to be correctable in a larger animal, large animals are often more difficult to recover and more prone to infections. Teaching a student or intern to make the surgical models may work if you are planning a short-term set of experiments, but if the surgical model needs repeating year after year, a surgical tech should be employed. University-based core facilities and contract labs specializing surgical animal models are an excellent option to standardize animal models.

The stage and type of investigation will also dictate the animal model employed. Early stage/proof of principle investigations are typically performed in mice or rats. Currently nearly all physiologic measurements and imaging that can be done clinically can be in accomplished in rodents. Such clinically relevant measurements increase the relevance and translatability of the data. As discussed above, transgenic animals are vitally important in early stage investigations to determine mechanistic parameters, but later stage investigations, particularly, testing therapies require animal models as close to the clinical disease state as possible. These can be rodents or larger animals, and generally, the FDA will require safety and toxicity studies in two different species. Often venture and capital funding will be needed for efficacy studies in an animal model very close to the target disease for the intended therapy. Initial candidate, dosing levels,

and timing of the test compound administration can be done in rodent models to narrow down the best candidate. However, later optimization and efficacy would be repeated in a larger animal with physiology or disease state closer to humans. Unfortunately, poor experimental design that results in lack of proper randomization, blinding, sample size estimation, and consideration of sex differences is prevalent in preclinical research [18]. Careful attention to these elements of experimental design is crucial to remove bias and improve validity and the predictive value of animal models.

3.3 Small Animal Models of Cardiovascular Disease (Fig. 3.1)

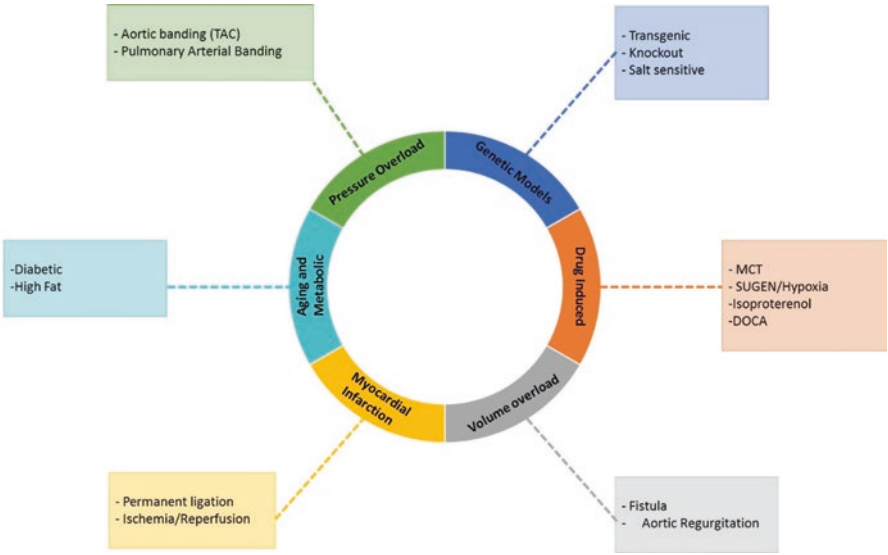

Fig. 3.1 Outlines broad categories of small animal models of cardiovascular disease, which range from surgically created models to genetically modified to drug induced to models of aging, renal, and metabolic disease. Table 3.2 includes additional information and references for small animal models of cardiovascular disease with alterations of the ECM

Table 3.2 Surgical rodent models for ECM research

Animal	Method	Phenotype	Advantages	Disadvantages	Reference
Mouse/ rat/rabbit	MI, permanent ligation	LV hypertrophy remodeling contractile dysfunction fibrosis	Cost, higher throughput, proof of concept, can be combined with other models	Technically demanding, lower clinical correlation	[19–21]
Mouse/ rat/rabbit	MI, I/R	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Cost, higher throughput, proof of concept, better clinical correlation, can be combined with other models	Technically demanding	[19–21]
Mouse/ rat/rabbit	Pressure overload Transaortic banding	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Cost, clinical correlation, levels of overload can be titrated, used with transgenic models	Technically demanding, generally abrupt	[22, 23]
Mouse/ rat/rabbit	Pressure overload Transpulmonary banding	RV hypertrophy, remodeling, RV contractile dysfunction, fibrosis	Cost, overload can be titrated, used with transgenic models	Technically demanding, undetermined clinical correlation, abrupt	[24–27]
Mouse/ rat/rabbit	Volume overload AV fistula	Moderate LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Cost Hormone/electrolyte changes Slower onset	Technically demanding	[28–30]
rabbit	Aortic regurgitation	LV dilation, heart failure	LVH occurs slowly	Technically demanding	[31, 32]
Mouse/rat	Drug-induced MCT	RV dilation, failure, PAH	Cost RVH occurs slowly, clinically relevant	Animals do not always go to failure	[33]
Mouse/rat	Drug-induced Dox	MMP changes, LV dilation, failure	Easy, cost, reproducible	Clinical correlation?	[34–36]

Mouse/rat	Drug-induced isoprotenol	LV dilation, contractile dysfunction	Easy, cost, reproducible	Clinical correlation?	[37, 38]
Mouse/rat	Drug-induced SUGEN/hypoxia	RV dilation, failure, PAH	RVH occurs slowly	Animals do not always go to failure	[39, 40]
Mouse/rat	Aging	Fibrotic remodeling	Clinically relevant can be combined with other models	Expensive time-consuming	[41, 42]
Mouse/rat	Metabolic	Streptozotocin-induced, high-fat models	Clinically relevant, best combined with other models	More challenging, time-consuming	[43, 44]
Mouse/rat	Renal	Uninephrectomy, subnephrectomy, others	Clinically relevant, best combined with other models	More challenging Higher mortality	[45–47]
Mouse	Genetically modified	Hundreds			
Rat	DOCA salt sensitive	Volume overload/hypertension	Transition from hypertrophy to failure		[48, 49]

3.4 Large Animal Models of Cardiovascular Disease (Fig. 3.2)

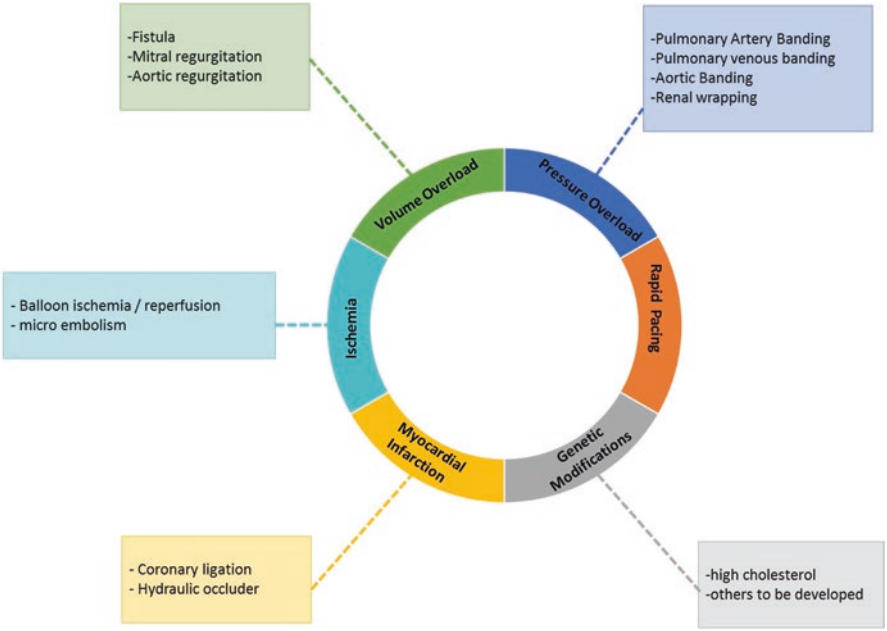

Fig. 3.2 Outlines broad categories of large animal models of cardiovascular disease, which range from surgically created models to drug induced to models of aging, renal, and metabolic disease. Table 3.3 includes additional details and references for large animal (swine, dogs, sheep) models used in ECM research

Table 3.3 Surical large animal models for ECM research

Method	Phenotype	Advantages	Disadvantages	Reference
Rapid pacing	Dilated hypertrophy	Easy, closed chest, reversible	Mechanisms unclear, cost	[50–53] [54]
Coronary ligation	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Better control of MI size, hydraulic occluders create a slow onset	Cost, technically more demanding, open chest, arrhythmias, dogs have more collateral formation	[55, 56]
Ischemia/reperfusion	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Clinically relevant, closed chest, easy	Cost, cath lab necessary, higher mortality rate, arrhythmias	[55, 57, 58]
Microembolism	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Slower onset, closed chest, clinical correlation	Time-consuming, cost, small window of effectiveness	[51–53]
Volume overload/ fistula	Moderate LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Slower onset, closed chest, clinical correlation, extracellular matrix changes	Cost, technically more demanding	[59, 60]
Volume overload/ mitral regurgitation	Moderate LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Closed chest, clinical correlation	Faster onset	[61]
Pressure overload/ TAC	LV hypertrophy, remodeling, contractile dysfunction, fibrosis	Clinical correlation, extracellular matrix changes	Faster onset technically more demanding, open chest	[59]
Pressure overload/ pulmonary artery banding, pulmonary venous banding	RV hypertrophy, remodeling, contractile dysfunction, fibrosis, PAH	Clinical correlation	Technically very demanding, open chest	[62, 63]
Renal wrapping	Volume overload, LV dilation, heart failure	No adhesions, easier surgery	Clinical correlation	[64, 65]
Genetic modifications	Various	Mechanistic, potentially better preclinical models comorbidities	Expensive/ time-consuming	[66, 67]

3.5 Summary

Large and small animal models are critical to our understanding of how heart disease alters the ECM and how changes in the ECM may alter the course of cardiovascular disease. Animal models are also necessary to develop products, including ECM-based products and therapies to repair damage after cardiovascular events. Ultimately, animal models must be used to test these therapies. Choosing the correct model is critical, and careful consideration of cost, equipment available, infrastructure, personnel, and the stage of the investigation can help guide the choice of animal model. However, ultimately no experiment will be successful without proper experimental design.

References

1. Creemers EE, Davis JN, Parkhurst AM, Leenders P, Dowdy KB, Hapke E, Hauet AM, Escobar PG, Cleutjens JP, Smits JF, Daemen MJ, Zile MR, Spinale FG. Deficiency of TIMP-1 exacerbates LV remodeling after myocardial infarction in mice. Am J Phys Heart Circ Phys. 2003;284:H364–71.
2. Eskandari MK, Vijungco JD, Flores A, Borensztajn J, Shively V, WH P. Enhanced abdominal aortic aneurysm in TIMP-1-deficient mice. J Surg Res. 2005;123:289–93.
3. Ikonomidis JS, Hendrick JW, Parkhurst AM, Herron AR, Escobar PG, Dowdy KB, Stroud RE, Hapke E, Zile MR, Spinale FG. Accelerated LV remodeling after myocardial infarction in TIMP-1-deficient mice: effects of exogenous MMP inhibition. Am J Phys Heart Circ Phys. 2005;288:H149–58.
4. Roten L, Nemoto S, Simsic J, Coker ML, Rao V, Baicu S, Defreyte G, Soloway PJ, Zile MR, Spinale FG. Effects of gene deletion of the tissue inhibitor of the matrix metalloproteinase-type 1 (TIMP-1) on left ventricular geometry and function in mice. J Mol Cell Cardiol. 2000;32:109–20.
5. Kandalam V, Basu R, Abraham T, Wang X, Soloway PD, Jaworski DM, Oudit GY, Kassiri Z. TIMP2 deficiency accelerates adverse post-myocardial infarction remodeling because of enhanced MT1-MMP activity despite lack of MMP2 activation. Circ Res. 2010;106:796–808.
6. Kandalam V, Basu R, Moore L, Fan D, Wang X, Jaworski DM, Oudit GY, Kassiri Z. Lack of tissue inhibitor of metalloproteinases 2 leads to exacerbated left ventricular dysfunction and adverse extracellular matrix remodeling in response to biomechanical stress. Circulation. 2011;124:2094–105.
7. Givvimani S, Kundu S, Narayanan N, Armaghan F, Qipshidze N, Pushpakumar S, Vacek TP, Tyagi SC. TIMP-2 mutant decreases MMP-2 activity and augments pressure overload induced LV dysfunction and heart failure. Arch Physiol Biochem. 2013;119:65–74.
8. Ramani R, Nilles K, Gibson G, Burkhead B, Mathier M, McNamara D, McTiernan CF. Tissue inhibitor of metalloproteinase-2 gene delivery ameliorates postinfarction cardiac remodeling. Clin Transl Sci. 2011;4:24–31.
9. Fedak PW, Smookler DS, Kassiri Z, Ohno N, Leco KJ, Verma S, Mickle DA, Watson KL, Hojilla CV, Cruz W, Weisel RD, Li RK, Khokha R. TIMP-3 deficiency leads to dilated cardiomyopathy. Circulation. 2004;110:2401–9.
10. Kandalam V, Basu R, Abraham T, Wang X, Awad A, Wang W, Lopaschuk GD, Maeda N, Oudit GY, Kassiri Z. Early activation of matrix metalloproteinases underlies the exacerbated systolic and diastolic dysfunction in mice lacking TIMP3 following myocardial infarction. Am J Phys Heart Circ Phys. 2010;299:H1012–23.

11. Kassiri Z, Defamie V, Hariri M, Oudit GY, Anthwal S, Dawood F, Liu P, Khokha R. Simultaneous transforming growth factor beta-tumor necrosis factor activation and cross-talk cause aberrant remodeling response and myocardial fibrosis in Timp3-deficient heart. J Biol Chem. 2009;284:29893–904.
12. Kassiri Z, Oudit GY, Sanchez O, Dawood F, Mohammed FF, Nuttall RK, Edwards DR, Liu PP, Backx PH, Khokha R. Combination of tumor necrosis factor-alpha ablation and matrix metalloproteinase inhibition prevents heart failure after pressure overload in tissue inhibitor of metalloproteinase-3 knock-out mice. Circ Res. 2005;97:380–90.
13. Tian H, Cimini M, Fedak PW, Altamentova S, Fazel S, Huang ML, Weisel RD, Li RK. TIMP-3 deficiency accelerates cardiac remodeling after myocardial infarction. J Mol Cell Cardiol. 2007;43:733–43.
14. Takawale A, Zhang P, Azad A, Wang W, Wang X, Murray AG, Kassiri Z. Myocardial overexpression of TIMP3 after myocardial infarction exerts beneficial effects by promoting angiogenesis and suppressing early proteolysis. Am J Phys Heart Circ Phys. 2017;313:H224–36.
15. Koskivirta I, Kassiri Z, Rahkonen O, Kiviranta R, Oudit GY, McKee TD, Kyto V, Saraste A, Jokinen E, Liu PP, Vuorio E, Khokha R. Mice with tissue inhibitor of metalloproteinases 4 (Timp4) deletion succumb to induced myocardial infarction but not to cardiac pressure overload. J Biol Chem. 2010;285:24487–93.
16. Yarbrough WM, Baicu C, Mukherjee R, Van Laer A, Rivers WT, McKinney RA, Prescott CB, Stroud RE, Freels PD, Zellars KN, Zile MR, Spinale FG. Cardiac-restricted overexpression or deletion of tissue inhibitor of matrix metalloproteinase-4: differential effects on left ventricular structure and function following pressure overload-induced hypertrophy. Am J Phys Heart Circ Phys. 2014;307:H752–61.
17. Zavadzkas JA, Stroud RE, Bouges S, Mukherjee R, Jones JR, Patel RK, McDermott PJ, Spinale FG. Targeted overexpression of tissue inhibitor of matrix metalloproteinase-4 modifies post-myocardial infarction remodeling in mice. Circ Res. 2014;114:1435–45.
18. Ramirez FD, Motazedian P, Jung RG, Di Santo P, MacDonald ZD, Moreland R, Simard T, Clancy AA, Russo JJ, Welch VA, Wells GA, Hibbert B. Methodological rigor in preclinical cardiovascular studies: targets to enhance reproducibility and promote research translation. Circ Res. 2017;120:1916–26.
19. Borst O, Ochmann C, Schonberger T, Jacoby C, Stellos K, Seizer P, Flogel U, Lang F, Gawaz M. Methods employed for induction and analysis of experimental myocardial infarction in mice. Cellular physiology and biochemistry : international journal of experimental cellular physiology. Biochem Pharmacol. 2011;28:1–12.
20. Goldman S, Raya TE. Rat infarct model of myocardial infarction and heart failure. J Card Fail. 1995;1:169–77.
21. Camacho P, Fan H, Liu Z, He JQ. Small mammalian animal models of heart disease. Am J Cardiovasc Dis. 2016;6:70–80.
22. Barnes J, Pat B, Chen YW, Powell PC, Bradley WE, Zheng J, Karki A, Cui X, Guichard J, Wei CC, Collawn J, Dell'Italia LJ. Whole-genome profiling highlights the molecular complexity underlying eccentric cardiac hypertrophy. Ther Adv Cardiovasc Dis. 2014;8:97–118.
23. Tarnavski O. Mouse surgical models in cardiovascular research. Methods Mol Biol. 2009;573:115–37.
24. Hamrell BB, Hultgren PB. Reduced isotonic sarcomere shortening in rabbit right ventricular pressure overload hypertrophy. J Mol Cell Cardiol. 1992;24:133–47.
25. Kapur NK, Paruchuri V, Aronovitz MJ, Qiao X, Mackey EE, Daly GH, Ughreja K, Levine J, Blanton R, Hill NS, Karas RH. Biventricular remodeling in murine models of right ventricular pressure overload. PLoS One. 2013;8:e70802.
26. Rain S, Andersen S, Najafi A, Gammelgaard Schultz J, da Silva Goncalves Bos D, Handoko ML, Bogaard HJ, Vonk-Noordegraaf A, Andersen A, van der Velden J, Ottenheijm CA, de Man FS. Right ventricular myocardial stiffness in experimental pulmonary arterial hypertension: relative contribution of fibrosis and myofibril stiffness. Circ Heart Fail. 2016;9:1–9.
27. Reddy S, Zhao M, Hu DQ, Fajardo G, Katznelson E, Punn R, Spin JM, Chan FP, Bernstein D. Physiologic and molecular characterization of a murine model of right ventricular volume overload. Am J Phys Heart Circ Phys. 2013;304:H1314–27.

28. Abassi Z, Goltsman I, Karram T, Winaver J, Hoffman A. Aortocaval fistula in rat: a unique model of volume-overload congestive heart failure and cardiac hypertrophy. J Biomed Biotechnol. 2011;2011:729497.
29. Karram T, Hoffman A, Bishara B, Brodsky S, Golomb E, Winaver J, Abassi Z. Induction of cardiac hypertrophy by a controlled reproducible sutureless aortocaval shunt in the mouse. J Invest Surg. 2005;18:325–34.
30. Miyamoto T, Takeishi Y, Shishido T, Takahashi H, Itoh M, Kubota I, Tomoike H. Role of nitric oxide in the progression of cardiovascular remodeling induced by carotid arterio-venous shunt in rabbits. Jpn Heart J. 2003;44:127–37.
31. Borer JS, Truter S, Herrold EM, Falcone DJ, Pena M, Carter JN, Dumlao TF, Lee JA, Supino PG. Myocardial fibrosis in chronic aortic regurgitation: molecular and cellular responses to volume overload. Circulation. 2002;105:1837–42.
32. Corno AF, Cai X, Jones CB, Mondani G, Boyett MR, Jarvis JC, Hart G. Congestive heart failure: experimental model. Front Pediatr. 2013;1:33.
33. Nogueira-Ferreira R, Vitorino R, Ferreira R, Henriques-Coelho T. Exploring the monocrotaline animal model for the study of pulmonary arterial hypertension: a network approach. Pulm Pharmacol Ther. 2015;35:8–16.
34. Bai P, Mabley JG, Liaudet L, Virag L, Szabo C, Pacher P. Matrix metalloproteinase activation is an early event in doxorubicin-induced cardiotoxicity. Oncol Rep. 2004;11:505–8.
35. Carvalho FS, Burgeiro A, Garcia R, Moreno AJ, Carvalho RA, Oliveira PJ. Doxorubicin-induced cardiotoxicity: from bioenergetic failure and cell death to cardiomyopathy. Med Res Rev. 2014;34:106–35.
36. Hayward R, Hydock DS. Doxorubicin cardiotoxicity in the rat: an in vivo characterization. J Am Assoc Lab Anim Sci .: JAALAS. 2007;46:20–32.
37. Wang JJ, Rau C, Avetisyan R, Ren S, Romay MC, Stolin G, Gong KW, Wang Y, Lusis AJ. Genetic dissection of cardiac remodeling in an isoproterenol-induced heart failure mouse model. PLoS Genet. 2016;12:e1006038.
38. Carll AP, Willis MS, Lust RM, Costa DL, Farraj AK. Merits of non-invasive rat models of left ventricular heart failure. Cardiovasc Toxicol. 2011;11:91–112.
39. Wang Z, Schreier DA, Hacker TA, Chesler NC. Progressive right ventricular functional and structural changes in a mouse model of pulmonary arterial hypertension. Physiol Rep. 2013;1:e00184.
40. Liu A, Philip J, Vinnakota KC, Van den Bergh F, Tabima DM, Hacker T, Beard DA, Chesler NC. Estrogen maintains mitochondrial content and function in the right ventricle of rats with pulmonary hypertension. Physiol Rep. 2017;5:e13157.
41. Horn MA, Trafford AW. Aging and the cardiac collagen matrix: novel mediators of fibrotic remodelling. J Mol Cell Cardiol. 2016;93:175–85.
42. Hacker TA, McKiernan SH, Douglas PS, Wanagat J, Aiken JM. Age-related changes in cardiac structure and function in Fischer 344 x Brown Norway hybrid rats. Am J Phys Heart Circ Phys. 2006;290:H304–11.
43. Shiomi T, Tsutsui H, Ikeuchi M, Matsusaka H, Hayashidani S, Suematsu N, Wen J, Kubota T, Takeshita A. Streptozotocin-induced hyperglycemia exacerbates left ventricular remodeling and failure after experimental myocardial infarction. J Am Coll Cardiol. 2003;42:165–72.
44. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, Diamant M. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. Diabetologia. 2005;48:1229–37.
45. van Dokkum RP, Eijkelkamp WB, Kluppel AC, Henning RH, van Goor H, Citgez M, Windt WA, van Veldhuisen DJ, de Graeff PA, de Zeeuw D. Myocardial infarction enhances progressive renal damage in an experimental model for cardio-renal interaction. J Am Soc Nephrol. 2004;15:3103–10.
46. Lu D, Wang K, Wang S, Zhang B, Liu Q, Zhang Q, Geng J, Shan Q. Beneficial effects of renal denervation on cardiac angiogenesis in rats with prolonged pressure overload. Acta Physiol. 2017;220:47–57.

47. Windt WA, Henning RH, Kluppel AC, Xu Y, de Zeeuw D, van Dokkum RP. Myocardial infarction does not further impair renal damage in 5/6 nephrectomized rats. Nephrol Dial Transplant. 2008;23:3103–10.
48. Gavras H, Brunner HR, Laragh JH, Vaughan ED Jr, Koss M, Cote LJ, Gavras I. Malignant hypertension resulting from deoxycorticosterone acetate and salt excess: role of renin and sodium in vascular changes. Circ Res. 1975;36:300–9.
49. O'Brien D, Chunduri P, Iyer A, Brown L. L-carnitine attenuates cardiac remodelling rather than vascular remodelling in deoxycorticosterone acetate-salt hypertensive rats. Basic Clin Pharmacol Toxicol. 2010;106:296–301.
50. Dixon JA, Spinale FG. Large animal models of heart failure: a critical link in the translation of basic science to clinical practice. Circ Heart Fail. 2009;2:262–71.
51. Dubi S, Arbel Y. Large animal models for diastolic dysfunction and diastolic heart failure-a review of the literature. Cardiovasc Pathol. 2010;19:147–52.
52. Power JM, Tonkin AM. Large animal models of heart failure. Aust NZ J Med. 1999;29:395–402.
53. Yarbrough WM, Spinale FG. Large animal models of congestive heart failure: a critical step in translating basic observations into clinical applications. J Nucl Cardiol. 2003;10:77–86.
54. Recchia FA, Lionetti V. Animal models of dilated cardiomyopathy for translational research. Vet Res Commun. 2007;31(Suppl 1):35–41.
55. Jugdutt BI. The dog model of left ventricular remodeling after myocardial infarction. J Card Fail. 2002;8:S472–5.
56. Wayman NS, McDonald MC, Chatterjee PK, Thiemermann C. Models of coronary artery occlusion and reperfusion for the discovery of novel antiischemic and antiinflammatory drugs for the heart. Methods Mol Biol. 2003;225:199–208.
57. Schmuck EG, Koch JM, Hacker TA, Hatt CR, Tomkowiak MT, Vigen KK, Hendren N, Leitzke C, Zhao YQ, Li Z, Centanni JM, Hei DJ, Schwahn D, Kim J, Hematti P, Raval AN. Intravenous Followed by X-ray Fused with MRI-Guided Transendocardial Mesenchymal Stem Cell Injection Improves Contractility Reserve in a Swine Model of Myocardial Infarction. J Cardiovasc Transl Res. 2015;8:438–48.
58. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, van Belle E, Gyongyosi M, Sluijter JP, Cramer MJ, Doevendans PA, Chamuleau SA. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. Cardiovasc Res. 2011;91:649–58.
59. Harper J, Harper E, Covell JW. Collagen characterization in volume-overload- and pressure-overload-induced cardiac hypertrophy in minipigs. Am J Phys. 1993;265:H434–8.
60. Wittnich C, Belanger MP, Oh BS, Salerno TA. Surgical model of volume overload-induced ventricular myocardial hypertrophy (VHvo) to study a clinical problem in humans. J Invest Surg. 1991;4:333–8.
61. Kawase Y, Ly HQ, Prunier F, Lebeche D, Shi Y, Jin H, Hadri L, Yoneyama R, Hoshino K, Takewa Y, Sakata S, Peluso R, Zsebo K, Gwathmey JK, Tardif JC, Tanguay JF, Hajjar RJ. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. J Am Coll Cardiol. 2008;51:1112–9.
62. Hyldebrandt JA, Siven E, Agger P, Frederiksen CA, Heiberg J, Wemmelund KB, Ravn HB. Effects of milrinone and epinephrine or dopamine on biventricular function and hemodynamics in an animal model with right ventricular failure after pulmonary artery banding. Am J Phys Heart Circ Phys. 2015;309:H206–12.
63. Lambert V, Capderou A, Le Bret E, Rucker-Martin C, Deroubaix E, Gouadon E, Raymond N, Stos B, Serraf A, Renaud JF. Right ventricular failure secondary to chronic overload in congenital heart disease: an experimental model for therapeutic innovation. J Thorac Cardiovasc Surg. 2010;139:1197–1204, 1204 e1191.
64. Mazumder R, Schroeder S, Mo X, Clymer BD, White RD, Kolipaka A. In vivo quantification of myocardial stiffness in hypertensive porcine hearts using MR elastography. J Magn Reson Imaging. 2017;45:813–20.

65. Feld Y, Dubi S, Reisner Y, Schwammenthal E, Shofti R, Pinhasi A, Carasso S, Elami A. Energy transfer from systole to diastole: a novel device-based approach for the treatment of diastolic heart failure. Acute Card Care. 2011;13:232–42.
66. Li Y, Fuchimoto D, Sudo M, Haruta H, Lin QF, Takayama T, Morita S, Nochi T, Suzuki S, Sembon S, Nakai M, Kojima M, Iwamoto M, Hashimoto M, Yoda S, Kunimoto S, Hiro T, Matsumoto T, Mitsumata M, Sugitani M, Saito S, Hirayama A, Onishi A. Development of Human-Like Advanced Coronary Plaques in Low-Density Lipoprotein Receptor Knockout Pigs and Justification for Statin Treatment Before Formation of Atherosclerotic Plaques. J Am Heart Assoc. 2016;5:e002779.
67. Wei J, Ouyang H, Wang Y, Pang D, Cong NX, Wang T, Leng B, Li D, Li X, Wu R, Ding Y, Gao F, Deng Y, Liu B, Li Z, Lai L, Feng H, Liu G, Deng X. Characterization of a hypertriglyceridemic transgenic miniature pig model expressing human apolipoprotein CIII. FEBS J. 2012;279:91–9.

Chapter 4
Applications of Cardiac Extracellular Matrix in Tissue Engineering and Regenerative Medicine

Mark C. Daley, Spencer L. Fenn, and Lauren D. Black III

Abstract The role of the cardiac extracellular matrix (cECM) in providing biophysical and biochemical cues to the cells housed within during disease and development has become increasingly apparent. These signals have been shown to influence many fundamental cardiac cell behaviors including contractility, proliferation, migration, and differentiation. Consequently, alterations to cell phenotype result in directed remodeling of the cECM. This bidirectional communication means that the cECM can be envisioned as a medium for information storage. As a result, the reprogramming of the cECM is increasingly being employed in tissue engineering and regenerative medicine as a method with which to treat disease. In this chapter, an overview of the composition and structure of the cECM as well as its role in cardiac development and disease will be provided. Additionally, therapeutic modulation of cECM for cardiac regeneration as well as bottom-up and top-down approaches to ECM-based cardiac tissue engineering is discussed. Finally, lingering questions regarding the role of cECM in tissue engineering and regenerative medicine are offered as a catalyst for future research.

Keywords 3D bioprinting · Biomaterials · Cardiac development · Decellularization · Extracellular matrix · Matrix metalloproteases · Recellularization · Cardiac regenerative medicine · Cardiac tissue engineering · Whole-organ engineering

M. C. Daley
Department of Biomedical Engineering, Tufts University, Medford, MA, USA

S. L. Fenn
Department of Biomedical Engineering, Tufts University, Medford, MA, USA

Center for Biomedical Career Development, University of Massachusetts Medical School, Worcester, MA, USA

L. D. Black III (✉)
Department of Biomedical Engineering, Tufts University, Medford, MA, USA

Cellular, Molecular and Developmental Biology Program, Sackler School for Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA
e-mail: Lauren.Black@tufts.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_4

4.1 Introduction

It is well understood that embryonic development, cell fate determination and tissue morphogenesis are highly influenced by genetic controls and soluble factor release and signaling [1–6]. While the extracellular matrix (ECM) was previously viewed as a passive structural support for cells, its importance in directing organ and tissue development as well as maintaining organ homeostasis has become increasingly apparent [7–10]. The specific protein constituents of the mammalian ECM have been shown to be well conserved across species [8, 11], though the specific composition and proportions vary dramatically between individual tissues and organ systems within the same species. This suggests that the health and function of cells, tissues, and organs is directly related to the milieu of mechanical and biochemical cues provided to them by the surrounding ECM [12]. Indeed, the ECM can be thought of as an information storage medium – allowing cells to access information about tissue age, status, and their appropriate phenotype. Studies related to the heart show that aging and disease can lead to compositional and structural changes to the ECM and that these changes can subsequently influence cell phenotype and function [13–22], clarifying the impact that ECM composition and organization can have on cardiac tissue status. It is hoped that by gaining a better understanding of the complex and dynamic relationship between the ECM and cell and tissue function, the ECM can be used strategically in the treatment of disease and injury and as a potential biomarker for early stages of various cardiac diseases.

In this chapter we will explore the composition of the cardiac ECM (cECM), how specific components change during development and disease, and how these changes influence tissue health and function. Lastly, we will discuss how the cECM can be and has been leveraged in cardiac tissue engineering and regenerative medicine applications both as a therapeutic and via its incorporation within biomaterial constructs.

4.2 Cardiac Extracellular Matrix

The cECM is both complex and highly variable, with this variation dependent on location and health. Its primary components include protein fibers, glycoproteins, glycosaminoglycans, proteoglycans, and polysaccharides [8]. Many of these components serve structural and mechanical roles [23], influencing bulk material mechanics as well as microenvironmental features. The chemical and mechanical properties imparted by cECM components in turn act as cues influencing cytoskeletal organization [8] as well as specific gene and protein expression related to a variety of cell functions including proliferation [24, 25], migration [26], and differentiation [18, 27–29]. Indeed, the cells encapsulated within the cECM are not only responsible for its initial synthesis and organization but are capable of remodeling the cECM in response to aging, disease, or injury [30, 31], highlighting the

bidirectional nature of the signaling between the cECM and the cells that reside within. Additionally, the cECM has also been shown to signal cells via the sequestration of growth factors, presenting them to cells in a spatiotemporal manner that is dependent on cECM composition and organization [8]. Moreover, the degradation of the cECM during remodeling leads to the generation of bioactive ECM fragments, known as matrikines or matricryptins, that can also signal cells [32–35]. The following sections will discuss the composition of the cECM and its contribution to cardiac development and function.

4.2.1 Composition and Role of the Cardiac Extracellular Matrix

The cECM resembles a complex network mesh comprised of both structural and nonstructural components that house myocytes, fibroblasts, vascular endothelial, and immune cells such as leukocytes (Table 4.1) [36]. Traditionally, the protein makeup of the cECM was assessed by assaying for specific ECM components via gene expression or western blot [21, 22]. From these and other studies, the primary structural components of the cECM have been determined to be collagen and elastin fibers as well as other critical proteins that form the basement membrane that surrounds cardiomyocytes (e.g., laminins) [36]. The nonstructural components are mainly comprised of matricellular proteins such as tenascin, thrombospondin, and periostin, among others. In addition, a significant number of proteins, as in the case of fibronectin, contribute both structurally and nonstructurally [36, 37]. The diversity of cECM components is further enhanced by a variety of alternative splicing and posttranslational modification, such as calcium binding and glycosylation [38].

Importantly, the bidirectional signaling provided between cells and the cECM means its composition is highly dynamic [8, 31]. Recently, mass spectrometry-based proteomics has provided greater insight into the true diversity of and variation in cECM proteins [39]. Specifically, proteomic techniques have identified changes in cECM between individual patients [40, 41], at different developmental ages [14], and in the context of cardiac disease [15, 42]. This type of analysis has successfully been utilized to identify the regenerative potential of individual proteins, like agrin, through the comparison of hearts at different stages of development [43]. As methods such as these are continued to be developed for the analysis of the cECM, a better appreciation of its diversity will be cultivated at the omics scale, leading to broader insights into its diversity and interconnectedness.

Table 4.1 The cECM is a complex network of structural and nonstructural proteins that are further subdivided into glycoproteins, glycosaminoglycans (GAGs), and proteoglycans

Glycoproteins			GAGs	Proteoglycans			
Prototypical matricellular proteins	Fibers	Others	Hyaluronan	Hyalectans	Basement membrane	Cell surface	Small leucine rich
Thrombospondin	Collagens	Fibronectin		Versican	Perlecan	Syndecan	Class I – biglycan, decorin, asporin
SPARC	Elastins	Laminin		Neurocan	Collagen XVIII	Glypican	Class II – lumican, fibromodulin, PRELP, keratocan, osteoadherin
Tenascin				Brevican	Agrin		Class III – osteoglycin, epiphycan, optican
Osteopontin				Aggrecan			Class IV – chondroadherin, nyctalopin, tsukushi
Periostin							Class V – podocan, podocan-like protein 1
CCN							

Some proteins serve a structural (blue) function in the cECM (e.g., collagens), whereas others have nonstructural (pink) roles, such as matricellular proteins. In addition, there are glycoproteins such as fibronectin that are able to do both (purple). The reservoir of glycosylated proteins allows for flexibility and diversity in how cECM functions in health and disease
Adapted from [36]

4.2.2 The Role of ECM in Cardiac Development

The cECM plays a critical role in tissue and cell morphological development and begins to be synthesized and excreted by embryonic cells at very early stages [8]. The matrix microenvironment supports cell proliferation, encourages survival of maturing cells, and directs cell morphogenesis and differentiation using a complex blend of mechanical, biophysical, and biochemical cues [9, 10]. Intuitively, these processes play essential roles in early development, with knockout or mutation of key cECM components resulting in severe cardiac defects.

For example, precursor cardiac cells must migrate to the midline for heart tube formation during initial vertebrate heart formation [44]. Experiments in zebrafish have shown that either loss or knockdown of fibronectin expression disrupts or delays this process, preventing successful heart tube development [45, 46]. Precursor cell migration can be partially recovered in mutants via direct injection of fibronectin into the midline region of tube formation, highlighting therapeutic potential [46]. Similarly, the cECM component periostin has been implicated in facilitating ECM organization and cardiac fibroblast migration during heart development [47]. Periostin knockout mice have been shown to possess disorganized collagen fibers that are smaller and less cross-linked than those found in normal cardiac tissue. In this manner, loss of periostin further alters cECM composition, signaling, and gross mechanical properties, disrupting normal development.

The dynamic nature of the cECM does not end after birth but continues to change through adulthood as part of normal aging (Fig. 4.1) [14]. Examination of decellularized hearts procured from fetal, neonatal, and adult rats by LC-MS has identified substantial changes with age. Notably, the fraction of the cECM observed to be fibronectin or periostin, both critical for proper development, decreases with developmental age. Conversely, the proportion of collagen I, an important contributor to the cardiac mechanical environment, increases substantially during aging [23].

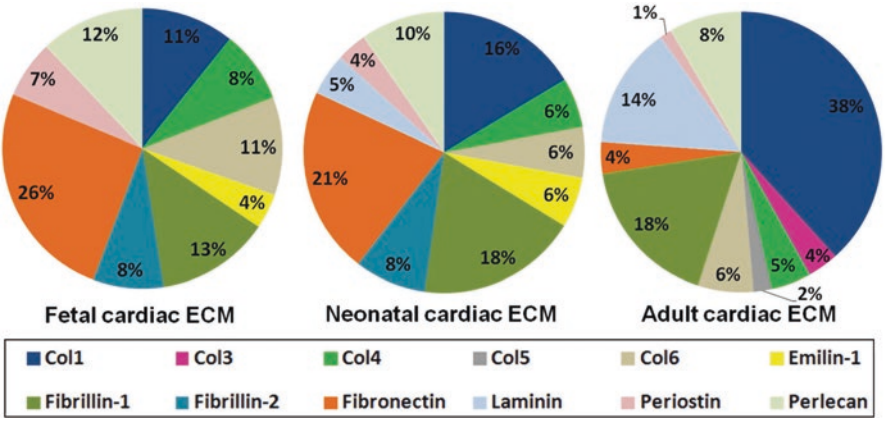

Fig. 4.1 Composition of rat cECM at three developmental ages represented by percent abundance of 15 most common proteins as measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). (Adapted from [14])

These compositional shifts are of particular interest in the heart since it fails to exhibit a significant ability to regenerate after injury in adult mammals but possesses this ability during fetal and neonatal development [14, 48–51].

Studies have already begun leveraging this information to alter cardiac progenitor and cardiomyocyte proliferation and maturation [15, 24]. Further elucidation of the compositional and structural changes within the cECM during development may identify new microenvironmental triggers critical for these shifts in cell behavior. Ultimately, these types of analysis provide researchers the ability to better study and engineer cardiac morphogenesis and regeneration for therapeutic purposes.

4.2.3 Alterations to ECM Composition and Structure from Injury and Disease

In addition to understanding how the cECM is structured in healthy, developing tissue, there is also a need to understand how the matrix changes in response to injury and disease. For example, it is well established that myocardial infarction leads to increased fibrosis of the affected area [16, 52]. This scar continues to remodel over time, leading to thinning of the ventricular wall, excacerbating the injury. Compared to healthy heart tissue, the cECM from an infarcted region of the ventricle demonstrates increasing proportions of many components, especially collagens, over time (Fig. 4.2) [17]. This newly remodeled tissue has different mechanical properties, primarily related to local stiffening, which results in decreased heart contractility [23, 30]. Recent evidence, however, suggests that the increased collagen deposited post-infarct is weaker at the fiber level than that of healthy tissue [53]. The weaker collagen fibers likely result from a lack of cross-linking, highlighting the role of cECM structural organization, not just composition, in the diseased microenvironment.

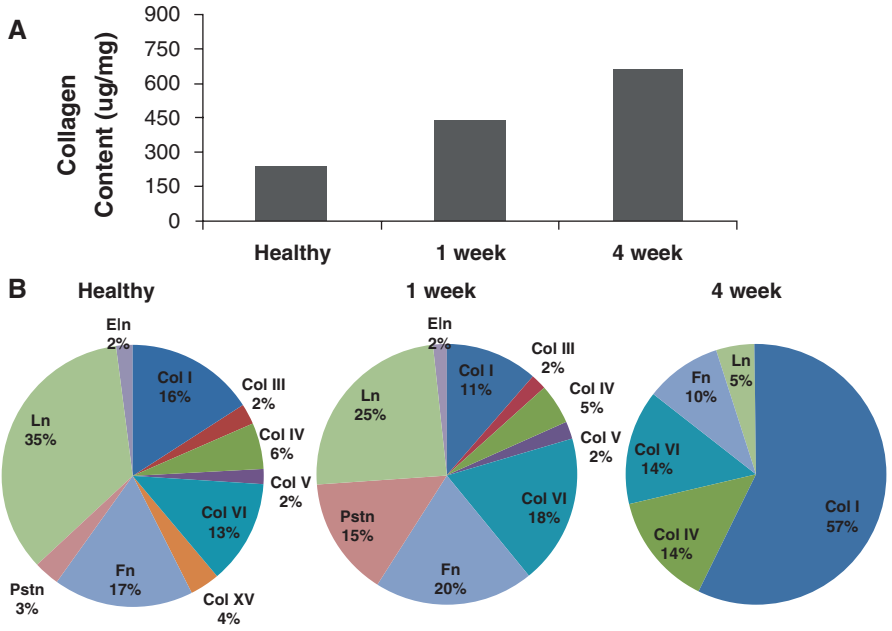

Fig. 4.2 Composition of rat cECM in response to myocardial infarction. (**a**) Total collagen content and (**b**) percent abundance of cECM components as measured by LC-MS/MS in decellularized healthy, 1-week post-infarct, and 4-week post-infarct hearts. (Adapted from [17])

A number of other heart diseases have similarly been linked to changes in the composition of the cECM. Calcific aortic valve disease (CAVD), for example, is distinguished by significant organizational, compositional, and mechanical alterations to ECM [54, 55]. Among the most established remodeling is a dense collagen fibrosis in which fibers have become disorganized [54]. Progression of CAVD is linked to these changes via stiffness-mediated valve interstitial cell activation and can be considered a product of bidirectional feedback between cells and cECM [56, 57]. Murine models of right ventricular pressure overload (RVPO) have been similarly shown to display significant collagen deposition and hypertrophy versus healthy hearts in both ventricles [58]. Interestingly, treatment with soluble endoglin, a matrisomal form of the transforming growth factor beta-1 co-receptor endoglin, mitigates fibrosis in these same animal models [59].

Adult-onset diseases, however, are not the sole examples of cECM remodeling in response to disease, and many congenital heart diseases have similarly been identified. Genetic defects in FBN1, the gene encoding fibrillin-1, has been linked to Marfan syndrome [20]. Fibrillin plays an important role in the formation of elastin fibers in multiple organs, including the heart. The most serious cardiovascular complications include dilation of the ascending aorta and mitral valve abnormalities. Comparison of the cECM composition of patients with hypoplastic left heart syndrome (HLHS) has also shown alterations including increased fibronectin and decreased collagen content [21]. Together these trends suggest HLHS results in a relatively immature cardiac microenvironment versus normally developed hearts.

As the interplay between disease and cECM is illuminated, a better understanding of disease pathogenesis can be achieved. These insights will lead to both identification of the effect of cECM defects on cell phenotype as well as potential targets for regenerative therapies.

4.3 Modulation of cECM in Regenerative Medicine Approaches in the Heart

Given that cECM composition and structure are influential in both development and disease, a logical next step is to explore how these changes can be leveraged as a tool to return the heart to a young, healthy, regenerative state. The goal of such work is to utilize parts of the cECM, and ECM in general, as therapeutic molecules [11]. Generally, the components identified as driving development or as part of healthy cardiac tissue are the most promising in terms of inducing regeneration. Therapies focus on eliciting cECM remodeling, cardiomyocyte proliferation, or progenitor cell differentiation to promote better overall cardiac function. The following sections will address some of the early successes that have employed these techniques.

4.3.1 The Role of cECM Proteins in Remodeling Post-Injury: Potential Strategies to Direct Cardiac Tissue Repair or Regeneration

Numerous studies have been carried out to assess the specific role of extracellular matrix proteins in the remodeling response following cardiac injury via myocardial infarction. These investigations often point to important roles for lesser known proteins in stabilizing scar tissue following the onset of remodeling. In studies employing murine models in which osteoglycin [60], syndecan-4 [61], and SPARC [62] had been downregulated post-myocardial infarction, increased mortality due to cardiac rupture was observed as a result of reduced matrix remodeling and maturation. Contrastingly, genetic knockdown of the cECM component fibulin-2 in mice resulted in significantly improved survival and preservation of left ventricular function [63]. Subsequent studies found that fibulin-2 is required for angiotensin II-induced cardiac remodeling, implicating it as a potential therapeutic target [64].

Another avenue of exploration is assessing the effects of ECM protein overexpression on cardiac remodeling. For instance, mice made to overexpress syndecan-1demonstrated reduced cardiac inflammation, dilation, and dysfunction in response to infarct [65]. Administration of elastin-overexpressing rat bone marrow stromal cells administered post-infarct improved tissue integrity and prevented scar expansion [66]. Finally, in vitro overexpression of cartilage intermediate layer protein 1 (CILP1) has been shown to interfere with fibrotic TGF-β signaling [67]. Evidence

also suggests that the extracellular protein periostin may promote cardiac regeneration via myocyte proliferation in both murine and porcine models [68, 69], but results are inconclusive. Other studies have observed that neither overexpression nor knockout of periostin in mice results in any difference in heart size or cardiomyocyte number at baseline or after myocardial infarction [70].

That said, research continues to identify promising therapeutic cECM components for direct delivery via the study of development and injury. The cardioregenerative properties of agrin, a neonatal extracellular protein, were recently elucidated via the comparison of the cECM of P1 and P7 mice via LC-MS [43]. Once identified, administration of agrin post-myocardial infarction was found to improve heart function in mice. Interestingly, agrin was observed to been enriched in P1 cECM that had been cleaved with matrix metalloproteinase 9 (MMP9), a member of the gelatinase family of MMPs. This suggests that the remodeling of cECM plays a crucial role in wound response and proper healing and may also provide future therapeutic potential.

4.3.2 Modulation of Protease Activity During Remodeling as a Tool for Cardiac Repair

While the importance of specific ECM proteins in cardiac health and disease has been explored in detail, another area of investigation that requires further exploration is the role of proteases responsible for ECM degradation (and their inhibitors) in the overall outcome of post-injury remodeling. A number of studies have focused on the effects of the major MMPs in cardiac remodeling, most notably MMP9. Specifically, genetic knockout of MMP9 in murine infarct models demonstrated reduced collagen deposition and increased cross-linking by lysyl oxidase (LOX), resulting in reduced ventricular dilation [71, 72]. MMP9 knockout has also been linked to improved neovascularization post-myocardial infarction in vivo [73]. Treatment with a broad MMP inhibitor has also demonstrated recovery of cardiac function after injury [74]. Similar studies have been carried out investigating the role of the tissue inhibitors of metalloproteases (TIMPs) and their role in remodeling. Genetic knockout of TIMP2 [75] and TIMP3 [76] in mice both result in worsened cardiac function post-myocardial infarction related to reduced collagen accumulation. Additionally, collagen that is deposited within the infarct is markedly less ordered than that found in wild type mice. Short-term treatment with broad spectrum MMP inhibitors was able to partially rescue cardiac function in TIMP3 knockout mice [76]. Knockout studies performed with TIMP4 identified increased mortality after infarct but not pressure overload, highlighting the injury-specific nature of cECM remodeling [77].

While MMP activity can affect ECM signaling to cells via altered collagen and LOX expression, an alternative mechanism is through the generation of ECM fragments following protease degradation that are themselves bioactive – so-called

matrikines or matricryptins [33]. Endostatin, an angiogenesis inhibitor, is derived from collagen XVIII and was among the first to be discovered in this class of molecules [35]. Given their relative abundance, additional collagen fragments have been found to be biologically active via the promotion of wound healing [32] and progenitor cell recruitment [34]. Similarly, injection of fibroblasts overexpressing elastin fragments postinfarction into rat hearts mitigated fibrosis and cardiac dysfunction [78]. With a myriad of potential cleavage targets, it is incredibly likely that numerous undiscovered MMP-liberated cECM fragments also influence the cellular microenvironment and cell phenotype in the heart. With both direct and indirect potential to influence disease state, MMPs provide a compelling option for cardiac repair and regeneration.

4.4 Bottom-Up Versus Top-Down Approaches in ECM–Based Cardiac Tissue Engineering

Bottom-up design is the process of building from the ground up, investigating and gaining an understanding of individual elements and adding them progressively to the final design. In tissue and organ engineering, this process often begins through the identification of a therapeutic protein, growth factor, or other ECM component which provides tissue specific instructions for regeneration and remodeling. Once identified, these molecules can be incorporated within a biomaterial scaffold in an attempt to mimic the conditions of native tissue. In this approach, a scaffold acts as the structural base material and the incorporated ECM guides biological remodeling by the cells [79]. The goal in using a bottom-up approach is to engineer and grow organs and tissues piece-by-piece, replicating innate structures and biophysical conditions to synthetically yield a functional tissue.

Alternatively, top-down approaches start with a whole-organ or tissue structure. Often this process begins with the complete decellularization of a tissue, leaving an ECM structure to be utilized as a scaffold for recellularization with patient-specific primary cells or differentiated stem and progenitor cells. Rather than building up, top-down approaches start with a complete material, in this case the cECM, and modify it into the desired product.

In the following sections, current research approaches to both bottom-up and top-down ECM-based cardiac tissue engineering will be explored, including 2D, 3D, and whole-organ techniques.

4.4.1 Bottom-Up

4.4.1.1 Isolation of ECM

Prior to assessing cell response and interaction with ECM-based cues, the proteins, glycans and sugars must be either synthesized or sourced from existing tissues. Certain molecules can be synthesized using microbial cultures, such as the glycosaminoglycan hyaluronic acid [80], while most others require isolation from a tissue source, such as collagen [11].

The most commonly used technique to extract matrix components is through a process of decellularization and subsequent purification [11]. These processes typically employ the use of various detergents, solvents, enzymes, and mechanical forces to lyse and remove cellular material and proteins, leaving the insoluble ECM intact, with numerous protocols having been developed in recent years as have been previously reviewed [11, 12, 81]. Critically, the method of decellularization has been shown to influence the quality of the resulting ECM. For example, soluble matrix components may be lost or altered via degradation from proteases or other molecular changes [11, 82]. With the variety of methods and techniques available to generate ECM components for use in tissue engineering approaches, it is important to consider the benefits and pitfalls of each technique, validating the maintenance of ECM components of interest for the desired application.

4.4.1.2 2D Platforms for Assessment of cECM on Cardiac Cells and Tissues

There are several two-dimensional (2D) platforms for assessing the therapeutic potential of ECM-based molecules in vitro. One of the simplest is to supplement cell culture medium with specific ECM components and monitor cell response. Outputs of interest include changes in proliferation, morphology, maturation, as well as gene or protein expression. While simple, this technique is not physiologically relevant to all types of ECM components. While matricellular proteins may be presented to cells in solution, structural ECM components require a more mechanically relevant presentation. When ECM is suspended in a fluid environment, cells fail to sense mechanical cues other than those provided by the plastic or glass culture substrate. To overcome this, others have cross-linked ECM components to culture substrates, seeding cells directly on top of the matrix-bound surface, allowing for mechanical interaction between the cells and bound ECM [83]. Ultimately, however, tissue culture-treated polystyrene and glass are not ideal substrates for cardiac tissue engineering as neither recapitulates the mechanics, primarily stiffness, of native the myocardium.

To address this concern, tunable, 2D hydrogel substrates are employed to match specific physiological mechanical properties. These systems can subsequently be supplemented with ECM components to more fully mimic natural environmental

signals as compared to stiff plastics and glass. For example, valve interstitial cells have been shown to be more highly activated when grown on normal tissue-culture plastic versus poly ethylene glycol (PEG) hydrogels in otherwise identical culture conditions [84]. These models are also useful in identifying the role of ECM-compliance on cell response [85]. Commonly, the substrate utilized is polyacrylamide-based, which allows for a broad range of achievable mechanics by altering ratios of monomers and crosslinkers [17, 28, 86]. Work from Engler et al. provides one of the chief examples of this work [28]. Culturing naïve mesenchymal stem cells (MSCs) on collagen-polyacrylamide gels demonstrated that substrate stiffness alone was able to direct differentiation toward multiple lineages. MSCs on gels that mimicked the stiffness of native myocardium, approximately 10 kPa, showed similar morphology and gene expression to C2C12 myoblasts. Analogous results were also achieved for neurogenic and osteogenic differentiation on softer and stiffer gels, respectively. Studies with neonatal rat cardiomyocytes on collagen-polyacrylamide gels demonstrated that myocytes cultured on gels with stiffness similar to native myocardium induce greater force generation and maturation [27, 87].

These 2D systems have further been utilized to ask questions regarding the interplay of substrate mechanics and composition. Specifically, the effect of both individual cECM components and full decellularized cECM from varying developmental stages and disease states has been examined [17, 86, 88]. For instance, the traction force exerted by MSCs has been shown to be dependent on both the developmental age of cECM and substrate stiffness [86]. MSCs exerted greater traction forces with increasing stiffness on gels incorporating fetal cECM but not adult cECM, where this trend disappeared. Despite their simplicity, 2D platforms have provided valuable insight into the role of varied mechanical microenvironments in cardiac regeneration.

4.4.1.3 Use of Biomaterial Scaffolds: 3D Platforms for Cardiac Tissue Engineering

In vivo, cardiac cells do not exist in 2D environments but interact with neighboring cells and ECM in three dimensions. To capture this, researchers frequently introduce three-dimensional (3D) biomaterial scaffolds to mimic normal tissue environments [89–91]. These systems not only provide more accurate in vitro systems but provide microenvironmental control for the delivery of cells and drugs in vivo. In principle, the goal of these scaffolds is to provide a structure which supports cells mechanically while promoting tissue development using biochemical (e.g., ECM and growth factor presentation) and biophysical (e.g., porosity, permeability) cues, with a degradation rate which inversely matches the rate of tissue growth. Thus, researchers must design scaffolds from synthetic polymers, isolated or synthesized extracellular matrix components, or a combination thereof to meet these design criteria [92].

PEG is among the most commonly utilized synthetic polymers for scaffold creation due to its non-immunogenicity, branched structure, modifiable functional groups, and variety of polymerization chemistries [93]. As such, it provides a highly modifiable platform on which to build biological function for cardiac tissue engineering. Not only can single biological molecules be incorporated, but full cECM-PEG hybrid hydrogels have been shown to successfully encapsulate cardiac cells during polymerization, demonstrating their potential for application as injectable therapies [94].

Synthetic polymers, however, do not provide the only platforms for scaffold design. Over the last several decades, researchers have begun to leverage natural proteins in manners similar to synthetic polymers but with the added benefit of being naturally derived. Given its ubiquity in the ECM, type I collagen is a commonly used scaffold for generating engineered cardiac tissues that improve cardiac function both in vitro [95] and in vivo [96]. Additionally, the immunoneutral ECM polysaccharide, hyaluronic acid (HA), has been employed extensively for 3D scaffold design due to its chemical flexibility [97]. Stiff, approximately 43 kPa, methacrylated HA hydrogels but not 8 kPa soft gels have also been shown to reduce infarct size and improve cardiac function compared to controls in ovine models [98]. The protein fibroin, derived from the cocoons of *Bombyx mori*, has proven to be a similarly flexible scaffold foundation both in vitro and in vivo as has been reviewed extensively [99–101]. In heart-based applications, anisotropic silk patches with incorporated cECM has also been shown to improve in vivo vascularization and maintain functional phenotypes in vitro [102].

Another natural alternative co-opts the polymerization of fibrin, a key component in clot formation, to encapsulate and deliver cells. Acellular fibrin delivery in vivo has itself been shown to improve post-myocardial infarct recovery [103]. As fibrin is known to stimulate ECM production in cells, the main goal of this biomaterial as used in tissue engineering approaches is to serve as a temporary scaffold that is eventually replaced by cell-derived ECM. Fibrin patches have further been shown to improve cardiac function via delivery of induced pluripotent stem cell-derived cardiomyocytes to both rats [104] and pigs [105] after myocardial infarction. The creation of fibrin scaffolds in vitro further permits the assessment of cardiomyocyte twitch force [106]. In similar in vitro systems, fibrin scaffold alignment [107] as well as the developmental age of co-cultured cardiac fibroblasts [108] has been shown to alter cardiomyocyte contractility. Incorporation of cECM within fibrin scaffolds of varied stiffnesses was also found to affect cardiac progenitor cell differentiation [109]. Thus, 3D synthetic, natural, and composite scaffolds provide highly customizable platforms with which to leverage and interrogate the cECM.

4.4.1.4 3D Printing

While strides have been made in studying the effects of the cECM in 3D, the manufacture of geometrically and structurally complex cardiac tissues has remained elusive. Recent advances in 3D printing technologies have revolutionized our capacity

for the bottom-up design of biological structures [110]. Unlike the mostly homogenous or simple geometries of conventional scaffold designs made by cast molds or other top-down approaches, 3D printing permits high-resolution precise deposition of multiple materials. For tissue engineering, these systems employ a multitude of biocompatible materials with and without cells. Given the variety of structures found within the cardiovascular system, including myocardium, valves, and vasculature, 3D bioprinting is uniquely situated to aid the manufacture of these structures [111–113].

Gaebel et al. utilized this high resolution when comparing randomly seeded and printed cardiac patches containing MSCs and human umbilical vein endothelial cells (HUVECs) [114]. Patterned patches demonstrated increased vessel formation and cardiac function when treating myocardial infarction in vivo. 3D printed myocardium is further augmented by the structured deposition of cECM components like HA and gelatin [115]. Specifically, cardiac-derived cardiomyocyte progenitor cells (CMPCs) printed in HA-gelatin patches demonstrated increased cell attachment and cell viability compared to a previous alginate-only system. Additionally, rats treated with printed HA-gelatin patches with incorporated CMPCs post-infarction showed a significant reduction in infarct size. More complex bioinks, consisting of whole decellularized ECM, have been shown to improve myocyte maturation (Fig. 4.3a, b) [116].

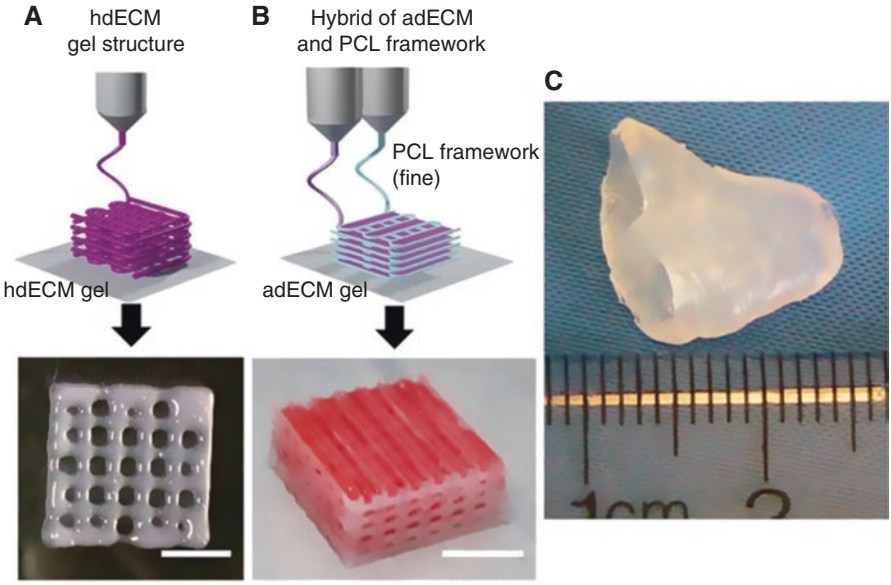

Fig. 4.3 Examples of 3D-printed structures of various ECM mimics. Geometries can be simples as demonstrated by (**a**) heart decellularized ECM (hdECM) only and (**b**) adipose decellularized ECM (adECM) in combination with polycaprolactone (PCL) or (**c**) more complex half-heart geometries. (Adapted from [116, 117])

Larger, non-patch geometries are also of interest to researchers with the goal of whole-organ printing. Cell-laden alginate-gelatin bioinks have previously been used to create “half-hearts” [117]. These structures could be subsequently bonded to form interconnected ventricle structures (Fig. 4.3c). The alginate-gelatin scaffold mimicked the natural stiffness of native tissue and supported cardiomyocyte beating, demonstrating the general principles of this technology for larger-scale cardiac tissue design. Soft biomaterials, however, are still challenging to 3D print due to their inability to maintain structural integrity. Recent advances have begun to tackle this challenge by printing directly into a viscous gelatin bath, which can subsequently be removed [118]. As these methods are developed, the range of bioinks available to clinicians and researchers will expand dramatically, furthering 3D bioprinting’s ability to create critically sized, complex geometries.

With the printing of larger tissues, supporting microvasculature is required to overcome the in vivo diffusion limit of 100–200 μm for oxygen, nutrient, and waste transport [119]. The resolution and multi-material nature of 3D printing techniques mean that it is possible to generate small-scale channels for media or blood perfusion throughout structurally complex engineered cardiac tissues. Successes have been achieved using multiple methods including sacrificial structures [120–122], direct printing of hollow vessels [123, 124], and spheroid self-assembly [125, 126]. The potential of bioprinting to combine cECM-like bioinks, multiple cell types, and high-resolution geometries grants it both the resolution and scalability needed for full bottom-up cardiac tissue design.

4.4.2 Top-Down

4.4.2.1 Directed Self-Assembly of Cardiac Tissue

In contrast to the bottom-up approaches described above, top-down tissue engineering focuses on the creation of large structures that can be sculpted into desired geometries and generate their own microenvironment [79]. Chamber organoids represent one such method given their self-assembled cECM and simple pump function. The unique shape of these chambers, such as those created in spherical molds, has permitted in vitro pressure and volume characterization with both neonatal rat cardiomyocytes [127] and human-induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) [128] in response to injury or drug exposure, respectively. Additionally, the utilization of cell sheets as larger building blocks with which to create full cardiac tissues of varying geometries has proven to be a fruitful technique [129–132]. The basic premise of this process is to culture confluent layers of cardiomyocytes on the thermo-responsive polymer poly(N-isopropylacrylamide) from which cells will detach in response to cooling. Afterward, sheets can be rolled or layered into pulsatile tubular structures [129, 130] or multilayer tissues [131, 132]. Both methods of manufacture have shown promise through the generation of contractile cardiac tissues in vivo from iPS-CMs [130, 131]. Free-standing tissues

are not the only option for cell-only cardiac tissues design as demonstrated by their incorporation onto patterned substrates [133]. Geometric confinement of both embryonic and induced pluripotent stem cells resulted in human cardiac microchambers, which could be assessed for cardiac differentiation and drug response.

Generally, the assembly of full cardiac tissues relies on cell-deposited cECM to create the appropriate microenvironment. Not only do these approaches still permit the creation of a variety of geometries via molds and physical assembly, but they better represent the normal ECM found in cardiac tissues as compared to minimally designed bottom-up approaches. Thus, the relative complexity of the cECM components present is greater without intricate manufacturing.

4.4.2.2 Decellularization of the Whole Heart

Among the most widely pursued top-down approaches to cardiac tissue engineering are the decellularization and subsequent recellularization of a whole heart. There are several key motivations for this method. One primary reason is the ability to preserve and utilize the overall geometry of the heart, including the atria, ventricles, and existing vasculature [12]. Additionally, the use of a decellularized whole organ retains its natural microenvironmental and matrisomal composition. This leaves an ideal scaffold for engineered organs that cannot currently be recreated via bottom-up approaches, such as 3D printing, despite their improving resolutions and multi-material abilities [110, 111]. This is principally because cECM proteins exist immediately on top of one another within a rich network structure. Thus, in contrast to decellularization methods employed for bottom-up design, whole-organ decellularization must be designed to preserve the overall shape of the heart. This can be achieved via the perfusion of detergents through existing vasculature, allowing for the removal of cellular material with minimal structural damage, the exact method of which varies [12, 41, 134].

A major secondary concern linked to the use of decellularized ECM scaffolds is the presence of residual cellular material which could trigger adverse biological or immune responses [11]. This is particularly concerning for those wishing to utilize allogenic tissue from either xenogeneic or non-autogeneic sources [82]. Thus, there has been significant research into optimizing the decellularization process to minimize or eliminate residual immunogenic cellular material while still retaining key matrisomal components [14, 135]. Moreover, care must be taken to prevent residual contaminants from the decellularization process itself to remain due to the possibility of adverse biological responses [11, 12, 136]. These substances are often removed using additional washing or dialysis techniques [136].

Given the need to preserve the cECM during decellularization, continued improvement and exploration of new methods are required. Expansion of available techniques will not only allow for the optimization for currently employed tissues but permit the expansion of this approach to novel organs and tissues.

4.4.2.3 Recellularization Techniques, Promise, and Pitfalls

Despite the opportunities provided by decellularized whole organs, this represents only the first half of whole-organ scaffolds. The process of recellularization, or seeding cells back into a decellularized scaffold, poses significant challenges in its own right [12]. Early success, however, has been achieved within the context of cardiac tissue. The first published full heart decellularization and recellularization was reported by Ott et al. in which cadaveric rat hearts were perfusion decellularized and subsequently recellularized with cardiomyocytes, fibrocytes, endothelial cells, and smooth muscle cells via intramural injection [134]. The recellularized hearts were subsequently maintained for 8–28 days within a perfusion bioreactor with macroscopic contractions observed by the fourth day in culture. While this work represents a significant step toward the generation of whole-organ tissue engineering, quantified pump function achieved only a small fraction of that of an adult heart.

Subsequent work has continued to optimize this type of system in a variety of ways including the use of human cell sources [41, 137], perfusion reendothelialization [138], and human-scale scaffolds [41]. Whole-heart scaffolds have also been employed to probe questions regarding the effects of different cECMs on cardiac cells [139]. As compared to adult whole-heart cECM, fetal scaffolds showed higher adherence of cardiac cells, suggesting that fetal microenvironments possess superior potential for whole-heart tissue engineering.

While advances continue, recellularized whole hearts and other organs are unable to achieve complete functionality and are limited to several hours in vivo [12]. Currently, the most widely employed methods of recellularization, injection and perfusion, rely on bulk introduction of cells. The expectation is that cells will then migrate to their appropriate niche. Given the heterogeneity of cardiac cell phenotypes (i.e., Purkinje cardiomyocytes) as well as their complex spatial distribution (i.e., interspersed fibroblasts), this intricacy is difficult, if not impossible, to recreate using existing approaches. Furthermore, when introduced as a bolus, only the outer fraction of cells is exposed to cECM signaling.

As these challenges are addressed, assessment of whole-organ function will be the final benchmark of success [12]. While previous attempts have only achieved a fraction of adult cardiac function [134, 138, 139], engineered whole hearts will need to mimic natural tissues in their contractility, conduction, pump function, and drug responsivity [12]. Ultimately, decellularized hearts represent the most physiologically relevant microenvironments for cardiac tissues available to researchers to date.

4.5 Open Questions for Engineering the Cardiac Extracellular Matrix

Despite the substantial progress made toward understanding and manipulating the cECM, numerous questions remain. Based on the review above, the most immediate and promising are outlined here as a stimulus for future research.

- What insights can be gleaned from advanced tools (e.g., LC-MS/MS, nonlinear microscopy) in elucidating the compositional and structural complexity of the cECM at various developmental stages and disease states?
- What is the interplay between changes in cECM composition, structure, and mechanics in the development and progression of cardiac disease? Can these insights be leveraged to promote healing and regeneration?
- How does the initial cECM microenvironment influence subsequent ECM production and cellular phenotype?
- For bottom-up approaches, what is the most appropriate cECM blend to induce a desired outcome (e.g., regeneration or maturation)? What is the minimum complexity required to achieve similar outcomes to whole cECM?
- What as-of-yet-untested methods may permit the reseeding of decellularized organs in a way that maintains the spatial heterogeneity of specialized cell types?
- How does the age and health status of donor matrix for whole-heart engineering alter clinical outcomes? Do certain conditions preclude transplantation?
- How do nonstructural components of cECM, including matrisomal proteins, MMPs, as well as matrikines and matricryptins, influence signaling in normal and pathological hearts?

4.6 Conclusion

Undoubtedly, the appreciation of cECM as an information storage medium that influences cardiac development, disease, and regeneration, has grown in recent decades. By answering the many still unresolved questions regarding cECM and enhancing the design of microenvironments, significant progress toward leveraging cECM therapeutically will be possible.

References

1. Nemer M. Genetic insights into normal and abnormal heart development. Cardiovasc Pathol. 2008;17(1):48–54. Epub 2007/12/28. https://doi.org/10.1016/j.carpath.2007.06.005. PubMed PMID: 18160060.
2. van Wijk B, Moorman AF, van den Hoff MJ. Role of bone morphogenetic proteins in cardiac differentiation. Cardiovasc Res. 2007;74(2):244–55. Epub 2006/12/26. https://doi.org/10.1016/j.cardiores.2006.11.022. PubMed PMID: 17187766.
3. Watanabe Y, Zaffran S, Kuroiwa A, Higuchi H, Ogura T, Harvey RP, Kelly RG, Buckingham M. Fibroblast growth factor 10 gene regulation in the second heart field by Tbx1, Nkx2-5, and Islet1 reveals a genetic switch for down-regulation in the myocardium. Proc Natl Acad Sci U S A. 2012;109(45):18273–80. Epub 2012/10/25. https://doi.org/10.1073/pnas.1215360109. PubMed PMID: 23093675; PMCID: PMC3494960.
4. Srivastava D, Olson EN. A genetic blueprint for cardiac development. Nature. 2000;407(6801):221–6. Epub 2000/09/23. https://doi.org/10.1038/35025190. PubMed PMID: 11001064.
5. de la Pompa JL. Notch signaling in cardiac development and disease. Pediatr Cardiol. 2009;30(5):643–50. Epub 2009/02/03. https://doi.org/10.1007/s00246-008-9368-z. PubMed PMID: 19184573.
6. Zaffran S, Frasch M. Early signals in cardiac development. Circ Res. 2002;91(6):457–69. Epub 2002/09/21. PubMed PMID: 12242263.
7. Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol. 2002;14(5):608–16.
8. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol. 2010;341(1):126–40. https://doi.org/10.1016/j.ydbio.2009.10.026. PubMed PMID: 19854168; PMCID: 2854274.
9. Lockhart M, Wirrig E, Phelps A, Wessels A. Extracellular matrix and heart development. Birth Defects Res A Clin Mol Teratol. 2011;91(6):535–50. https://doi.org/10.1002/bdra.20810. PubMed PMID: PMC3144859.
10. Mammoto T, Mammoto A, Ingber DE. Mechanobiology and developmental control. Annu Rev Cell Dev Biol. 2013;29:27–61. Epub 2013/10/09. https://doi.org/10.1146/annurev-cellbio-101512-122340. PubMed PMID: 24099083.
11. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. Biomaterials. 2006;27(19):3675–83. Epub 2006/03/08. https://doi.org/10.1016/j.biomaterials.2006.02.014. PubMed PMID: 16519932.
12. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. 2011;13:27–53. Epub 2011/03/23. https://doi.org/10.1146/annurev-bioeng-071910-124743. PubMed PMID: 21417722.
13. Bayomy AF, Bauer M, Qiu Y, Liao R. Regeneration in heart disease—Is ECM the key? Life Sci. 2012;91(17):823–7. https://doi.org/10.1016/j.lfs.2012.08.034.
14. Williams C, Quinn KP, Georgakoudi I, Black LD. Young developmental age cardiac extracellular matrix promotes the expansion of neonatal cardiomyocytes in vitro. Acta Biomater. 2014;10(1):194–204. https://doi.org/10.1016/j.actbio.2013.08.037. PubMed PMID: 24012606. PMCID: PMC3840040.
15. Williams C, Sullivan K, Black LD. Partially digested adult cardiac extracellular matrix promotes cardiomyocyte proliferation In Vitro. Adv Healthc Mater. 2015;4(10):1545–54. https://doi.org/10.1002/adhm.201500035. PubMed PMID: 25988681; PMCID: 4504755.
16. Sullivan KE, Black LD. The role of cardiac fibroblasts in extracellular matrix-mediated signaling during normal and pathological cardiac development. J Biomech Eng. 2013;135(7):71001. https://doi.org/10.1115/1.4024349. PubMed PMID: 23720014.

17. Sullivan KE, Quinn KP, Tang KM, Georgakoudi I, Black LD. Extracellular matrix remodeling following myocardial infarction influences the therapeutic potential of mesenchymal stem cells. Stem Cell Res Ther. 2014;5(1):14. https://doi.org/10.1186/scrt403. PubMed PMID: 24460869; PMCID: PMC4055039.
18. Jacot JG, Martin JC, Hunt DL. Mechanobiology of cardiomyocyte development. J Biomech. 2010;43(1):93–8.
19. Farhadian F, Contard F, Corbier A, Barrieux A, Rappaport L, Samuel JL. Fibronectin expression during physiological and pathological cardiac growth. J Mol Cell Cardiol. 1995;27(4):981–90.
20. Milewicz DM. Identification of defects in the fibrillin gene and protein in individuals with the Marfan syndrome and related disorders. Tex Heart Inst J. 1994;21(1):22.
21. Davies B, d'Udekem Y, Ukoumunne OC, Algar EM, Newgreen DF, Brizard CP. Differences in extra-cellular matrix and myocyte homeostasis between the neonatal right ventricle in hypoplastic left heart syndrome and truncus arteriosus. Eur J Cardiothorac Surg. 2008;34(4):738–44.
22. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, Li F, Ingram DA, Menick D, Field L. Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. Circ Res. 2008;102(7):752–60.
23. Fomovsky GM, Thomopoulos S, Holmes JW. Contribution of extracellular matrix to the mechanical properties of the heart. J Mol Cell Cardiol. 2010;48(3):490–6. Epub 2009/08/19. https://doi.org/10.1016/j.yjmcc.2009.08.003. PubMed PMID: 19686759; PMCID: PMC2823835.
24. French KM, Boopathy AV, DeQuach JA, Chingozha L, Lu H, Christman KL, Davis ME. A naturally derived cardiac extracellular matrix enhances cardiac progenitor cell behavior in vitro. Acta Biomater. 2012;8(12):4357–64.
25. Ahmann KA, Weinbaum JS, Johnson SL, Tranquillo RT. Fibrin degradation enhances vascular smooth muscle cell proliferation and matrix deposition in fibrin-based tissue constructs fabricated in vitro. Tissue Eng Part A. 2010;16(10):3261–70.
26. Loftis MJ, Sexton D, Carver W. Effects of collagen density on cardiac fibroblast behavior and gene expression. J Cell Physiol. 2003;196(3):504–11.
27. Jacot JG, Kita-Matsuo H, Wei KA, Vincent Chen H, Omens JH, Mercola M, McCulloch AD. Cardiac myocyte force development during differentiation and maturation. Ann N Y Acad Sci. 2010;1188(1):121–7.
28. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126(4):677–89.
29. Miskon A, Mahara A, Uyama H, Yamaoka T. A suspension induction for myocardial differentiation of rat mesenchymal stem cells on various extracellular matrix proteins. Tissue Eng Part C Methods. 2010;16(5):979–87.
30. Fomovsky GM, Holmes JW. Evolution of scar structure, mechanics, and ventricular function after myocardial infarction in the rat. Am J Physiol. 2010;298(1):H221–8. PubMed PMID: 19897714.
31. Gjorevski N, Nelson CM. Bidirectional extracellular matrix signaling during tissue morphogenesis. Cytokine Growth Factor Rev. 2009;20(5–6):459–65. https://doi.org/10.1016/j.cytogfr.2009.10.013. PubMed PMID: PMC2787686.
32. Lindsey ML, Iyer RP, Zamilpa R, Yabluchanskiy A, DeLeon-Pennell KY, Hall ME, Kaplan A, Zouein FA, Bratton D, Flynn ER. A novel collagen matricryptin reduces left ventricular dilation post-myocardial infarction by promoting scar formation and angiogenesis. J Am Coll Cardiol. 2015;66(12):1364–74.
33. Ricard-Blum S, Salza R. Matricryptins and matrikines: biologically active fragments of the extracellular matrix. Exp Dermatol. 2014;23(7):457–63.
34. Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. Tissue Eng Part A. 2011;17(19–20):2435–43.

35. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997;88(2):277–85.
36. Rienks M, Papageorgiou A-P, Frangogiannis NG, Heymans S. Myocardial extracellular matrix. an ever-changing and diverse entity. Circ Res. 2014;114(5):872–88. https://doi.org/10.1161/circresaha.114.302533.
37. George EL, Baldwin HS, Hynes RO. Fibronectins are essential for heart and blood vessel morphogenesis but are dispensable for initial specification of precursor cells. Blood. 1997;90(8):3073–81.
38. Adams JC, Watt FM. Regulation of development and differentiation by the extracellular matrix. Development. 1993;117(4):1183–98. Epub 1993/04/01.
39. Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. The extracellular matrix: tools and insights for the "omics" era. Matrix Biol. 2016;49:10–24.
40. Johnson TD, Hill RC, Dzieciatkowska M, Nigam V, Behfar A, Christman KL, Hansen KC. Quantification of decellularized human myocardial matrix: a comparison of six patients. Proteomics Clin Appl. 2016;10(1):75–83. https://doi.org/10.1002/prca.201500048.
41. Guyette JP, Charest JM, Mills RW, Jank BJ, Moser PT, Gilpin SE, Gershlak JR, Okamoto T, Gonzalez G, Milan DJ, Gaudette GR, Ott HC. Bioengineering human myocardium on native extracellular matrix. Circ Res. 2016;118(1):56–72. https://doi.org/10.1161/circresaha.115.306874.
42. Merna N, Fung KM, Wang JJ, King CR, Hansen KC, Christman KL, George SC. Differential β3 Integrin Expression Regulates the Response of Human Lung and Cardiac Fibroblasts to Extracellular Matrix and Its Components. Tissue Eng Part A. 2015;21(15–16):2195–205. https://doi.org/10.1089/ten.tea.2014.0337.
43. Bassat E, Mutlak YE, Genzelinakh A, Shadrin IY, Umansky KB, Yifa O, Kain D, Rajchman D, Leach J, Bassat DR. The extracellular matrix protein agrin promotes heart regeneration in mice. Nature. 2017;547(7662):179.
44. Glickman NS, Yelon D, editors. Cardiac development in zebrafish: coordination of form and function. Seminars in cell & developmental biology: Elsevier; 2002.
45. Trinh LA, Stainier DY. Fibronectin regulates epithelial organization during myocardial migration in zebrafish. Dev Cell. 2004;6(3):371–82.
46. Matsui T, Raya Á, Callol-Massot C, Kawakami Y, Oishi I, Rodriguez-Esteban C. Belmonte JCI. miles-apart-Mediated regulation of cell–fibronectin interaction and myocardial migration in zebrafish. Nat Rev Cardiol. 2007;4(S1):S77.
47. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. Circ Res. 2009;105(10):934–47.
48. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res. 1998;83(1):15–26.
49. Ieda M, Tsuchihashi T, Ivey KN, Ross RS, Hong T-T, Shaw RM, Srivastava D. Cardiac fibroblasts regulate myocardial proliferation through β1 integrin signaling. Dev Cell. 2009;16(2):233–44.
50. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. Science. 2011;331(6020):1078–80.
51. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, Sadek HA, Olson EN. Macrophages are required for neonatal heart regeneration. J Clin Invest. 2014;124(3):1382–92.
52. Brown RD, Ambler SK, Mitchell MD, Long CS. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. Annu Rev Pharmacol Toxicol. 2005;45(1):657–87. https://doi.org/10.1146/annurev.pharmtox.45.120403.095802. PubMed PMID: 15822192.
53. Quinn KP, Sullivan KE, Liu Z, Ballard Z, Siokatas C, Georgakoudi I, Black LD. Optical metrics of the extracellular matrix predict compositional and mechanical changes after myocardial infarction. Sci Rep. 2016;6:35823.

54. Chen J-H, Simmons CA. Cell–matrix interactions in the pathobiology of calcific aortic valve disease: critical roles for matricellular, matricrine, and matrix mechanics cues. Circ Res. 2011;108(12):1510–24.
55. Yip CYY, Simmons CA. The aortic valve microenvironment and its role in calcific aortic valve disease. Cardiovasc Pathol. 2011;20(3):177–82.
56. Kloxin AM, Benton JA, Anseth KS. In situ elasticity modulation with dynamic substrates to direct cell phenotype. Biomaterials. 2010;31(1):1–8.
57. Quinlan AM, Billiar KL. Investigating the role of substrate stiffness in the persistence of valvular interstitial cell activation. J Biomed Mater Res A. 2012;100(9):2474–82.
58. Kapur NK, Paruchuri V, Aronovitz MJ, Qiao X, Mackey EE, Daly GH, Ughreja K, Levine J, Blanton R, Hill NS. Biventricular remodeling in murine models of right ventricular pressure overload. PLoS One. 2013;8(7):e70802.
59. Kapur NK, Wilson S, Yunis AA, Qiao X, Mackey E, Paruchuri V, Baker C, Aronovitz MJ, Karumanchi SA, Letarte M. Reduced endoglin activity limits cardiac fibrosis and improves survival in heart failure. Circulation. 2012;125:2728. CIRCULATIONAHA. 111.080002
60. Van Aelst LN, Voss S, Carai P, Van Leeuwen R, Vanhoutte D, Sanders-van Wijk S, Eurlings L, Swinnen M, Verheyen FA, Verbeken EK. Osteoglycin prevents cardiac dilatation and dysfunction after myocardial infarction through infarct collagen strengthening. Circ Res. 2014;116:425. CIRCRESAHA. 114.304599
61. Matsui Y, Ikesue M, Danzaki K, Morimoto J, Sato M, Tanaka S, Kojima T, Tsutsui H, Uede T. Syndecan-4 prevents cardiac rupture and dysfunction after myocardial infarction. Circ Res. 2011;108:1328. CIRCRESAHA. 110.235689
62. Schellings MW, Vanhoutte D, Swinnen M, Cleutjens JP, Debets J, van Leeuwen RE, d'Hooge J, Van de Werf F, Carmeliet P, Pinto YM. Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. J Exp Med. 2009;206(1):113–23.
63. Tsuda T, Wu J, Gao E, Joyce J, Markova D, Dong H, Liu Y, Zhang H, Zou Y, Gao F. Loss of fibulin-2 protects against progressive ventricular dysfunction after myocardial infarction. J Mol Cell Cardiol. 2012;52(1):273–82.
64. Zhang H, Wu J, Dong H, Khan SA, Chu M-L, Tsuda T. Fibulin-2 deficiency attenuates angiotensin II-induced cardiac hypertrophy by reducing transforming growth factor-β signalling. Clin Sci. 2014;126(4):275–88.
65. Vanhoutte D, Schellings MW, Götte M, Swinnen M, Herias V, Wild MK, Vestweber D, Chorianopoulos E, Cortés V, Rigotti A. Increased expression of syndecan-1 protects against cardiac dilatation and dysfunction after myocardial infarction. Circulation. 2007;115(4):475–82.
66. Li SH, Sun Z, Guo L, Han M, Wood MF, Ghosh N, Alex Vitkin I, Weisel RD, Li RK. Elastin overexpression by cell-based gene therapy preserves matrix and prevents cardiac dilation. J Cell Mol Med. 2012;16(10):2429–39.
67. van Nieuwenhoven FA, Munts C, op't Veld RC, González A, Díez J, Heymans S, Schroen B, van Bilsen M. Cartilage intermediate layer protein 1 (CILP1): a novel mediator of cardiac extracellular matrix remodelling. Sci Rep. 2017;7(1):16042.
68. Kühn B, Del Monte F, Hajjar RJ, Chang Y-S, Lebeche D, Arab S, Keating MT. Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. Nat Med. 2007;13(8):962.
69. Polizzotti BD, Arab S, Kühn B. Intrapericardial delivery of gelfoam enables the targeted delivery of Periostin peptide after myocardial infarction by inducing fibrin clot formation. PLoS One. 2012;7(5):e36788.
70. Lorts A, Schwanekamp JA, Elrod JW, Sargent MA, Molkentin JD. Genetic manipulation of periostin expression in the heart does not affect myocyte content, cell cycle activity, or cardiac repair. Circ Res. 2009;104(1):e1–7.
71. Voorhees AP, DeLeon-Pennell KY, Ma Y, Halade GV, Yabluchanskiy A, Iyer RP, Flynn E, Cates CA, Lindsey ML, Han H-C. Building a better infarct: modulation of collagen cross-

linking to increase infarct stiffness and reduce left ventricular dilation post-myocardial infarction. J Mol Cell Cardiol. 2015;85:229–39.
72. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest. 2000;106(1):55–62.
73. Lindsey ML, Escobar GP, Dobrucki LW, Goshorn DK, Bouges S, Mingoia JT, McClister DM Jr, Su H, Gannon J, MacGillivray C. Matrix metalloproteinase-9 gene deletion facilitates angiogenesis after myocardial infarction. American Journal of Physiology-Heart and Circulatory Physiology. 2006;290(1):H232–H9.
74. Krishnamurthy P, Peterson JT, Subramanian V, Singh M, Singh K. Inhibition of matrix metalloproteinases improves left ventricular function in mice lacking osteopontin after myocardial infarction. Mol Cell Biochem. 2009;322(1–2):53–62.
75. Kandalam V, Basu R, Abraham T, Wang X, Soloway PD, Jaworski DM, Oudit GY, Kassiri Z. TIMP2 deficiency accelerates adverse post–myocardial infarction remodeling because of enhanced MT1-MMP activity despite lack of MMP2 activation. Circ Res. 2010;106(4):796–808.
76. Kandalam V, Basu R, Abraham T, Wang X, Awad A, Wang W, Lopaschuk GD, Maeda N, Oudit GY, Kassiri Z. Early activation of matrix metalloproteinases underlies the exacerbated systolic and diastolic dysfunction in mice lacking TIMP3 following myocardial infarction. Am J Physiol Heart Circ Physiol. 2010;299(4):H1012–H23.
77. Koskivirta I, Kassiri Z, Rahkonen O, Kiviranta R, Oudit GY, McKee TD, Kytö V, Saraste A, Jokinen E, Liu PP. Mice with tissue inhibitor of metalloproteinases 4 (Timp4) deletion succumb to induced myocardial infarction but not to cardiac pressure overload. J Biol Chem. 2010;285(32):24487–93.
78. Mizuno T, Mickle DA, Kiani CG, Li R-K. Overexpression of elastin fragments in infarcted myocardium attenuates scar expansion and heart dysfunction. American Journal of Physiology-Heart and Circulatory Physiology. 2005;288(6):H2819–H27.
79. Elbert DL. Bottom-up tissue engineering. Curr Opin Biotechnol. 2011;22(5):674–80. https://doi.org/10.1016/j.copbio.2011.04.001. PubMed PMID: 21524904; PMCID: PMC3153565.
80. Liu L, Liu Y, Li J, Du G, Chen J. Microbial production of hyaluronic acid: current state, challenges, and perspectives. Microb Cell Fact. 2011;10(1):99. https://doi.org/10.1186/1475-2859-10-99.
81. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods. 2015;84:25–34.
82. Schenke-Layland K, Vasilevski O, Opitz F, König K, Riemann I, Halbhuber KJ, Wahlers T, Stock UA. Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. J Struct Biol. 2003;143(3):201–8. https://doi.org/10.1016/j.jsb.2003.08.002.
83. Daley WP, Yamada KM. ECM-modulated cellular dynamics as a driving force for tissue morphogenesis. Curr Opin Genet Dev. 2013;23(4):408–14. https://doi.org/10.1016/j.gde.2013.05.005.
84. Mabry KM, Payne SZ, Anseth KS. Microarray analyses to quantify advantages of 2D and 3D hydrogel culture systems in maintaining the native valvular interstitial cell phenotype. Biomaterials. 2016;74:31–41.
85. Fischer RS, Myers KA, Gardel ML, Waterman CM. Stiffness-controlled three-dimensional extracellular matrices for high-resolution imaging of cell behavior. Nat Protoc. 2012;7(11):2056. https://doi.org/10.1038/nprot.2012.127.
86. Gershlak JR, Resnikoff JI, Sullivan KE, Williams C, Wang RM, Black LD. Mesenchymal stem cells ability to generate traction stress in response to substrate stiffness is modulated by the changing extracellular matrix composition of the heart during development. Biochem Biophys Res Commun. 2013;439(2):161–6. https://doi.org/10.1016/j.bbrc.2013.08.074. PubMed PMID: 23994333; PMCID: PMC3815602.

87. Jacot JG, McCulloch AD, Omens JH. Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. Biophys J. 2008;95(7):3479–87.
88. Gershlak JR, Black LD. Beta 1 integrin binding plays a role in the constant traction force generation in response to varying stiffness for cells grown on mature cardiac extracellular matrix. Exp Cell Res. 2015;330(2):311–24. https://doi.org/10.1016/j.yexcr.2014.09.007. PubMed PMID: 25220424.
89. Jia X, Kiick KL. Hybrid multicomponent hydrogels for tissue engineering. Macromol Biosci. 2009;9(2):140–56.
90. Baker BM, Chen CS. Deconstructing the third dimension–how 3D culture microenvironments alter cellular cues. J Cell Sci. 2012;125(13):3015–24.
91. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater. 2010;9(6):518.
92. Ye KY, Black LD. Strategies for tissue engineering cardiac constructs to affect functional repair following myocardial infarction. J Cardiovasc Transl Res. 2011;4(5):575.
93. Zhu J. Bioactive modification of poly (ethylene glycol) hydrogels for tissue engineering. Biomaterials. 2010;31(17):4639–56.
94. Grover GN, Rao N, Christman KL. Myocardial matrix–polyethylene glycol hybrid hydrogels for tissue engineering. Nanotechnology. 2013;25(1):014011.
95. Tiburcy M, Hudson JE, Balfanz P, Schlick S, Meyer T, Liao M-LC, Levent E, Raad F, Zeidler S, Wingender E. Defined engineered human myocardium with advanced maturation for applications in heart failure modeling and repairclinical perspective. Circulation. 2017;135(19):1832–47.
96. Zimmermann W-H, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat Med. 2006;12(4):452.
97. Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. Adv Mater. 2011;23(12):H41.
98. Ifkovits JL, Tous E, Minakawa M, Morita M, Robb JD, Koomalsingh KJ, Gorman JH, Gorman RC, Burdick JA. Injectable hydrogel properties influence infarct expansion and extent of postinfarction left ventricular remodeling in an ovine model. Proc Natl Acad Sci. 2010;107(25):11507–12.
99. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL. Silk-based biomaterials. Biomaterials. 2003;24(3):401–16.
100. Wang Y, Kim H-J, Vunjak-Novakovic G, Kaplan DL. Stem cell-based tissue engineering with silk biomaterials. Biomaterials. 2006;27(36):6064–82.
101. Thurber AE, Omenetto FG, Kaplan DL. In vivo bioresponses to silk proteins. Biomaterials. 2015;71:145–57.
102. Stoppel WL, Hu D, Domian IJ, Kaplan DL, Black LD III. Anisotropic silk biomaterials containing cardiac extracellular matrix for cardiac tissue engineering. Biomed Mater. 2015;10(3):034105.
103. Christman KL, Vardanian AJ, Fang Q, Sievers RE, Fok HH, Lee RJ. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. J Am Coll Cardiol. 2004;44(3):654–60.
104. Wendel JS, Ye L, Tao R, Zhang J, Zhang J, Kamp TJ, Tranquillo RT. Functional effects of a tissue-engineered cardiac patch from human induced pluripotent stem cell-derived cardiomyocytes in a Rat Infarct model. Stem Cells Transl Med. 2015;4(11):1324–32.
105. Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X, Kannappan R, Borovjagin AV, Walcott GP, Pollard AE. Large cardiac muscle patches engineered from human induced-pluripotent stem cell–derived cardiac cells improve recovery from myocardial infarction in swine. Circulation. 2018;137(16):1712–30.

106. Breckwoldt K, Letuffe-Brenière D, Mannhardt I, Schulze T, Ulmer B, Werner T, Benzin A, Klampe B, Reinsch MC, Laufer S. Differentiation of cardiomyocytes and generation of human engineered heart tissue. Nat Protoc. 2017;12(6):1177.
107. Black LD III, Meyers JD, Weinbaum JS, Shvelidze YA, Tranquillo RT. Cell-induced alignment augments twitch force in fibrin gel–based engineered myocardium via gap junction modification. Tissue Eng Part A. 2009;15(10):3099–108.
108. Liau B, Jackman CP, Li Y, Bursac N. Developmental stage-dependent effects of cardiac fibroblasts on function of stem cell-derived engineered cardiac tissues. Sci Rep. 2017;7:42290.
109. Williams C, Budina E, Stoppel WL, Sullivan KE, Emani S, Emani SM, Black LD. Cardiac extracellular matrix-fibrin hybrid scaffolds with tunable properties for cardiovascular tissue engineering. Acta Biomater. 2015;14:84–95. Epub 2014/12/03. https://doi.org/10.1016/j.actbio.2014.11.035. PubMed PMID: 25463503; PMCID: PMC4308538.
110. Sears NA, Seshadri DR, Dhavalikar PS, Cosgriff-Hernandez E. A review of three-dimensional printing in tissue engineering. Tissue Eng Part B Rev. 2016;22(4):298–310.
111. Duan B. State-of-the-art review of 3D bioprinting for cardiovascular tissue engineering. Ann Biomed Eng. 2017;45(1):195–209.
112. Mosadegh B, Xiong G, Dunham S, Min JK. Current progress in 3D printing for cardiovascular tissue engineering. Biomed Mater. 2015;10(3):034002.
113. Hockaday LA, Duan B, Kang KH, Butcher JT. 3D-printed hydrogel technologies for tissue-engineered heart valves. 3D Print Addit Manuf. 2014;1(3):122–36.
114. Gaebel R, Ma N, Liu J, Guan J, Koch L, Klopsch C, Gruene M, Toelk A, Wang W, Mark P. Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. Biomaterials. 2011;32(35):9218–30.
115. Gaetani R, Feyen DA, Verhage V, Slaats R, Messina E, Christman KL, Giacomello A, Doevendans PA, Sluijter JP. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. Biomaterials. 2015;61:339–48.
116. Pati F, Jang J, Ha D-H, Won Kim S, Rhie J-W, Shim J-H, Kim D-H, Cho D-W. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935. https://doi.org/10.1038/ncomms4935. https://www.nature.com/articles/ncomms4935#supplementary-information.
117. Xu T, Baicu C, Aho M, Zile M, Boland T. Fabrication and characterization of bio-engineered cardiac pseudo tissues. Biofabrication. 2009;1(3):035001.
118. Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue H-J, Ramadan MH, Hudson AR, Feinberg AW. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv. 2015;1(9):e1500758.
119. Lovett M, Lee K, Edwards A, Kaplan DL. Vascularization strategies for tissue engineering. Tissue Eng Part B Rev. 2009;15(3):353–70.
120. Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen D-HT, Cohen DM, Toro E, Chen AA, Galie PA, Yu X. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater. 2012;11(9):768.
121. Bellan LM, Singh SP, Henderson PW, Porri TJ, Craighead HG, Spector JA. Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. Soft Matter. 2009;5(7):1354–7.
122. Kolesky DB, Truby RL, Gladman AS, Busbee TA, Homan KA, Lewis JA. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater. 2014;26(19):3124–30.
123. Gao Q, He Y, J-z F, Liu A, Ma L. Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery. Biomaterials. 2015;61:203–15.
124. Cui X, Boland T. Human microvasculature fabrication using thermal inkjet printing technology. Biomaterials. 2009;30(31):6221–7.
125. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials. 2009;30(30):5910–7.

126. Jakab K, Norotte C, Damon B, Marga F, Neagu A, Besch-Williford CL, Kachurin A, Church KH, Park H, Mironov V. Tissue engineering by self-assembly of cells printed into topologically defined structures. Tissue Eng Part A. 2008;14(3):413–21.
127. Lee EJ, Kim DE, Azeloglu EU, Costa KD. Engineered cardiac organoid chambers: toward a functional biological model ventricle. Tissue Eng Part A. 2008;14(2):215–25.
128. Li RA, Keung W, Cashman TJ, Backeris PC, Johnson BV, Bardot ES, Wong AOT, Chan PKW, Chan CWY, Costa KD. Bioengineering an electro-mechanically functional miniature ventricular heart chamber from human pluripotent stem cells. Biomaterials. 2018;163:116–27. Epub 2018/02/21. https://doi.org/10.1016/j.biomaterials.2018.02.024. PubMed PMID: 29459321.
129. Kubo H, Shimizu T, Yamato M, Fujimoto T, Okano T. Creation of myocardial tubes using cardiomyocyte sheets and an in vitro cell sheet-wrapping device. Biomaterials. 2007;28(24):3508–16. Epub 2007/05/08. https://doi.org/10.1016/j.biomaterials.2007.04.016. PubMed PMID: 17482255.
130. Seta H, Matsuura K, Sekine H, Yamazaki K, Shimizu T. Tubular cardiac tissues derived from human induced pluripotent stem cells generate pulse pressure In Vivo. Sci Rep. 2017;7:45499. Epub 2017/03/31. https://doi.org/10.1038/srep45499. PubMed PMID: 28358136; PMCID: PMC5371992.
131. Komae H, Sekine H, Dobashi I, Matsuura K, Ono M, Okano T, Shimizu T. Three-dimensional functional human myocardial tissues fabricated from induced pluripotent stem cells. J Tissue Eng Regen Med. 2017;11(3):926–35. Epub 2015/01/30. https://doi.org/10.1002/term.1995. PubMed PMID: 25628251.
132. Shimizu T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res. 2002;90(3):40e–8. https://doi.org/10.1161/hh0302.105722.
133. Ma Z, Wang J, Loskill P, Huebsch N, Koo S, Svedlund FL, Marks NC, Hua EW, Grigoropoulos CP, Conklin BR. Self-organizing human cardiac microchambers mediated by geometric confinement. Nat Commun. 2015;6:7413.
134. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 2008;14(2):213–21. Epub 2008/01/15. doi: https://doi.org/10.1038/nm1684. PubMed PMID: 18193059.
135. Reing JE, Brown BN, Daly KA, Freund JM, Gilbert TW, Hsiong SX, Huber A, Kullas KE, Tottey S, Wolf MT. The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. Biomaterials. 2010;31(33):8626–33.
136. Kitahara H, Yagi H, Tajima K, Okamoto K, Yoshitake A, Aeba R, Kudo M, Kashima I, Kawaguchi S, Hirano A, Kasai M, Akamatsu Y, Oka H, Kitagawa Y, Shimizu H. Heterotopic transplantation of a decellularized and recellularized whole porcine heart. Interact Cardiovasc Thorac Surg 2016;22(5):571–9. Epub 2016/02/24. https://doi.org/10.1093/icvts/ivw022. PubMed PMID: 26902852; PMCID: PMC4892160.
137. Lu T-Y, Lin B, Kim J, Sullivan M, Tobita K, Salama G, Yang L. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat Commun. 2013;4:2307.
138. Robertson MJ, Dries-Devlin JL, Kren SM, Burchfield JS, Taylor DA. Optimizing recellularization of whole decellularized heart extracellular matrix. PLoS One. 2014;9(2):e90406.
139. Silva A, Rodrigues S, Caldeira J, Nunes A, Sampaio-Pinto V, Resende T, Oliveira M, Barbosa M, Thorsteinsdóttir S, Nascimento D. Three-dimensional scaffolds of fetal decellularized hearts exhibit enhanced potential to support cardiac cells in comparison to the adult. Biomaterials. 2016;104:52–64.

Chapter 5
Whole Cardiac Tissue Bioscaffolds

Karis R. Tang-Quan, Nicole A. Mehta, Luiz C. Sampaio, and Doris A. Taylor

Abstract Bioscaffolds serve as structures for cells in building complex tissues and full organs including heart. Decellularizing cardiac tissue results in cell-free extracellular matrix (ECM) that can be used as a cardiac tissue bioscaffold. The field of whole-heart tissue engineering has been revolutionized since the 2008 publication of the first perfusion-decellularized whole heart, and since then, studies have shown how decellularized cardiac tissue retains its native architecture and biochemistry following recellularization. Chemical, enzymatic, and physical decellularization methods preserve the ECM to varying degrees with the widely accepted standard of less than 50 ng/mg of double-stranded DNA present in decellularized ECM. Following decellularization, replacement of cells occurs via recellularization: seeding cells into the decellularized ECM structure either via perfusion of cells into the vascular conduits, injection into parenchyma, or a combination of perfusion and injection. Endothelial cells are often perfused through existing vessel conduits to provide an endothelial lining of the vasculature, with cardiomyocytes and other parenchymal cells injected into the myocardium of decellularized ECM bioscaffolds. Uniform

Authors contributed equally: Karis R. Tang-Quan and Nicole A. Mehta

K. R. Tang-Quan · D. A. Taylor (✉)
College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA
e-mail: dtaylor@texasheart.org

N. A. Mehta
College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

L. C. Sampaio
Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_5

cell density and cell retention throughout the bioscaffold still needs to be addressed in larger animal models of the whole heart. Generating the necessary cell numbers and types remains a challenge. Still, recellularized cardiac tissue bioscaffolds offer therapeutic solutions to heart failure, heart valve replacement, and acute myocardial infarction. New technologies allow for decellularized ECM to be bioprinted into cardiac bioscaffolds or formed into a cardiac hydrogel patch. This chapter reviews the advances made in decellularization and recellularization of cardiac ECM bioscaffolds with a discussion of the potential clinical applications of ECM bioscaffolds.

Keywords Decellularization · Recellularization · Cardiac extracellular matrix · Heart valves · Cardiac patches · Hydrogels

5.1 Introduction

Cardiovascular disease continues to be the leading cause of death worldwide, with more patients progressing to heart failure each year [1–3]. Current treatments for heart failure are targeted at symptomatic improvements, whereas newer investigative strategies are aimed at repairing injured myocardium or regenerating healthy myocardium, often via regenerative medicine approaches. Despite recent advancements in the field, few patients regain full cardiac function. Currently, the most effective treatment for patients with end-stage heart failure is cardiac allotransplantation [4, 5]. However, the list of patients awaiting transplant far exceeds donor hearts available [6]. Therefore, developing alternative treatments for heart failure remains a top priority.

One potential therapy is the use of a bioscaffold to replace or support damaged cardiac tissue. An acellular scaffold could be applied to the surface of the heart to prevent, or even reverse, dilatation or could be dosed with cells and delivered at the site of injury to aid in the restoration of lost cardiac cells and promote healing. Ideal scaffold candidates should be compatible with all cell types found in the heart, provide mechanical strength as location demands, guide cells to organize properly, and deliver biochemical cues for appropriate cell function within the heart [7]. These scaffolds may be sourced from biologic or synthetic materials, each of which has advantages and disadvantages. Synthetic materials are not always biodegradable and often lack the characteristics required for vascular and parenchymal cell attachment and infiltration [8] but can easily be crafted into virtually any size or shape. In contrast, biologic scaffolds – typically derived from extracellular matrix (ECM) – retain biologic cues necessary for cell migration, alignment, and differentiation but can be difficult to obtain in a sterile reproducible fashion and generally have low mechanical strength for cardiovascular application. The focus of this chapter is biologic scaffolds derived from whole hearts, typically via removal of cells, to yield the cardiac extracellular matrix (ECM).

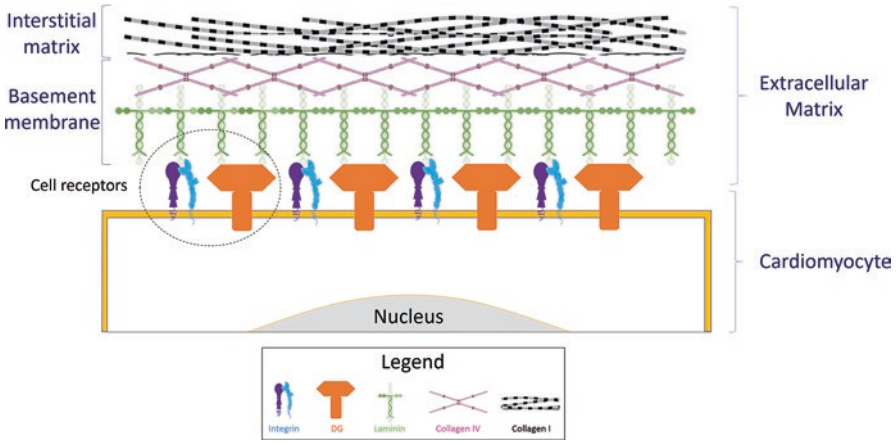

Fig. 5.1 Extracellular matrix compartments: (1) interstitial matrix with fibrillar collagens, elastin, proteoglycans, and glycoproteins; (2) basement membrane with non-fibrillar collagens and laminin. Cardiomyocytes attach to the basement membrane through integrins and dystroglycan (DG). Adapted from: [106]

In its intact state, the cardiac ECM is a complex system that contains a multitude of structural and nonstructural proteins organized as a meshwork to hold cardiac cells (myocytes, fibroblasts, cardiac vascular cells, etc.) and to provide specific biologic cues for their function. The ECM meshwork is comprised of collagens, elastin, laminin, fibronectin, proteoglycans, and glycoproteins [9–12] that are arranged into two specific compartments: basement membrane and interstitium (Fig. 5.1) [13–18]. The basement membrane plays a critical role in tissue function by facilitating cell-cell communication and organization [19]. It primarily consists of laminin and non-fibrillar type IV collagen that serves as an anchor for cells and is important for cell alignment. Proper cell alignment is especially critical in the heart, as it is necessary both for cell-cell electrical communication and for productive contraction required for adequate pumping [19]. In contrast with the basement membrane, the interstitium is comprised of fibrillar collagens, elastin, various proteoglycans, and glycoproteins. It underlies the basement membrane and provides mechanical rigidity to the tissue. The combined cardiac ECM creates a unique environment that supports multiple cell types including endothelial cells (ECs), smooth muscle cells (SMCs), cardiomyocytes, cardiac fibroblasts, neurons, and cardiac progenitor cells, in a structural arrangement sufficient for proper cardiac function.

The unique architecture of the cardiac ECM is difficult to recapitulate de novo. Not only is the macrostructure complex, but the microstructure varies with each cardiac chamber and with the valves. Furthermore, the entire myocardium is thoroughly vascularized and contains extensive neural and electrical circuits. Building a solid organ of this complexity via 3D printing, biomaterial chemistry, or other de novo methods remains difficult to date. Alternatively, some researchers have focused on deriving ECM-based scaffolds from whole hearts that are not suitable for transplantation.

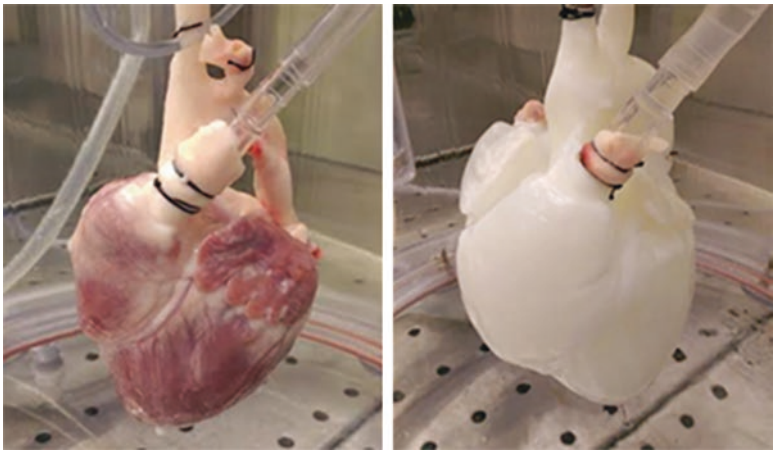

Fig. 5.2 Decellularized porcine heart (right) adjacent to a cadaveric porcine heart

One technique widely under investigation to generate a cell-free scaffold with native-like 3D architecture is perfusion decellularization of whole hearts where cells are removed, leaving only the cardiac ECM components. This usually involves the lysis of cells in some fashion (e.g., freezing, chemical perfusate) followed by vascular and chamber perfusion to wash out cell debris, leaving behind the ECM as a scaffold (Fig. 5.2). ECM bioscaffolds would be an ideal candidate for tissue engineering of a whole heart, in that they can be derived from both xenogeneic sources [20] and from hearts otherwise not suitable for transplant; they retain the native vascular conduits and the native ECM macro- and micro-architecture; they preserve intrinsic biochemical cues (e.g., stereochemistry of the matrix, surface ligand density) that guide the alignment and orientation of cells seeded onto the scaffold [21, 22]; and they can theoretically be repopulated using recipient's cells, allowing for creation of an autologous organ.

The first successful perfusion decellularization of a whole heart was achieved by our group in 2008 [23]. We successfully showed that a rat or pig heart could be decellularized and a rat heart could subsequently be partially recellularized, matured in vitro, or transplanted in vivo [23]. Since then, the decellularization of whole hearts has advanced, with new methods and techniques developed to decellularize and recellularize ECM bioscaffolds. This chapter will define the current approaches for decellularization and recellularization of whole hearts and discuss methodologies for utilizing cardiac tissue bioscaffolds.

5.2 Decellularization

Decellularization of heart is carried out by chemical [23–26], enzymatic [27–29], or physical [30] means with varying degrees of cell removal [31]. Chemical-based decellularization changes osmotic gradients to initiate cell membrane lysis and

removal. Enzymatic decellularization cleaves cell membranes, cell-cell attachments, cell-ECM attachments, or nucleic acid ECM attachments with specific enzymes to remove cells or cell remnants from the organ. Physical methods of decellularization use techniques such as tissue freeze-thawing cycles to lyse cell membranes. Each of these is followed by a cell debris washout either via immersion or perfusion. Immersion involves the submersion of the organ, with or without agitation, and then repeated solution changes to remove cellular debris. It can be viewed as an outside-in wash. Perfusion-based decellularization was developed in the Taylor lab. It is solution-agnostic, takes advantage of the native vasculature or other tissue conduits, and is the method of choice for solid, whole organs [23, 32, 33]. In the heart, perfusion is often performed via the aorta in a fashion that allows full perfusion through the coronary tree.

With any method of decellularization, the primary goal remains preservation of the native ECM composition, stiffness, and overall structure. However, each method of decellularization disrupts the ECM to varying degrees, and care must be taken to minimize ECM damage while also eliminating cellular content. The standard for determining complete decellularization has been established as follows: (1) less than 50 ng of double-stranded DNA (dsDNA) per mg dry weight of ECM, (2) less than 200 base pair DNA length, and (3) no nuclei visible upon staining using either hematoxylin and eosin (H&E) or DAPI (4′,6-diamidino-2-phenylindole) staining [34, 35]. A summary of decellularization techniques is covered in Table 5.1.

5.2.1 Chemical Decellularization

Chemical-based decellularization reagents are primarily comprised of ionic and nonionic detergents, acids and bases, and hypertonic or hypotonic solutions. These chemicals lyse cell membranes and wash out cellular and nuclear materials by changing osmotic gradients [36]. Detergent-based decellularization has been proven to be the most effective method for removing cellular content from many tissues but must be used at low concentrations to reduce the disruption to the ECM ultrastructure and preserve glycosaminoglycan (GAG) concentrations [37–39]. Sodium dodecyl sulfate (SDS) is one of the most widely used anionic detergents for cardiac scaffold generation, as it can effectively wash out cytoplasmic proteins and nuclear debris from the thick myocardium to a greater degree than other detergents [23, 40]. However, SDS can be difficult to remove from the ECM since it is an anionic surfactant and remains bound to ECM proteins. This leads to further undesired alterations in the decellularized ECM scaffold biochemistry and structure. Another detergent often used in conjunction with SDS is Triton X-100, a nonionic detergent shown to remove cellular contents and to aid in the washout of residual SDS from the ECM [23, 41]. While Triton X-100 treatment results in a cell-free heart valve, it is less effective in clearing the myocardium and aortic wall of cellular remnants [29].

Our group previously compared four different chemical-based decellularization techniques, including SDS, Triton X-100, enzymes, and polyethylene glycol.

Table 5.1 Summary of decellularization techniques used for whole cardiac tissues

Decellularization method	Species	Perfusion through cardiac vasculature	Decellularization protocol used	Sterilization	Advantages/ disadvantages	Outcomes	Reference
Chemical	Rat	Ascending aorta	Compared three perfusion protocols: 1% SDS, 1% PEG, 1% Triton X-100	Pen/strep with amphotericin B – 124 h	SDS gave improved DNA results and yielded fully decellularized construct over 12 h with minimal matrix alterations	SDS perfusion for over 12 h gave fully decellularized constructs when used with Triton X-100; there were almost no detectable residual SDS levels	Ott et al. [23]
	Murine	Aorta	1% SDS overnight, Triton X-100 1 h	Pen/ strep – 72 h	IHC confirmed low DNA content; no DNA data to support conclusion	GAG/collagen structure was retained; could decellularize heart in ~24 h	Ng et al. [57]
	Rat	Aorta	1% SDS overnight, 1% Triton X-100	Pen/strep in PBS		Successfully decellularized rat hearts with low DNA content	Robertson et al. [32]
	Porcine	Aorta, 100 mmHg	Heart was immersed in PBS solution. 4% SDS – 12 h, PBS rinse – every 3 h	Pen/ strep – 24 h	GAG/collagen/elastin content remained high. Higher DNA content than acceptable range (82.6 ± 3.2 ng DNA/ mg tissue)	Successfully decellularized the porcine heart with high residual DNA content ~17.45%	Weymann et al. [61]
	Human	Aorta, 80–100 mmHg	1% SDS for 4 days and then rinsed with PBS. Finished with 20 L of water	Pen/strep		Successfully decellularized the human heart, with only 5% residual DNA	Sanchez et al. [100]
	Human	Aorta, 60 mmHg	Perfusion with 1% SDS, 168 h; DIW, 24 h; 1% Triton X-100, 24 h; final PBS wash, 168 h	Antibiotics and Antifungal solutions	Decellularized matrices contained high amounts of collagens, GAGs, laminins, and fibrillins	Could decellularize human hearts with only 0.95% DNA content remaining	Guyette et al. [24]

	Porcine	Aorta, 4–5 PSI	Perfusion with 1x PBS 1 h, DW 1 h, 5% SDS 1 h, DW 1 h Repeat with SDS 2 h and DW for 2 cycles; SDS 3 h and DW 4 h Perfused with 1% Triton X-100 – 2 h	None noted	Finished entire protocol in 24 h; however, GAG, collagen, and ECM concentrations were not reported	Could complete decellularization of whole porcine heart with 98% DNA removal and only 6 h of exposure to detergents	Hodgson et al. [25]
	Porcine	Aorta	Four different experimental groups: (1) 3% SDS, 3% Triton X-100, 70–80 mmHg (2) 3% SDS, 3% Triton X-100, 90 mmHg (3) 3% SDS, 3% Triton X-100, 10% CHAPS, 1% OGP, 120 mmHg (4) 3% SDS, 3% Triton X-100, 1% OGP, 10% CHAPS, 140 mmHg	None noted	Protocol 2 was found to be optimal. Protocol 1 did not complete decellularization in 5 days due to low pressures. Protocols 3 and 4 did not have complete decellularization, and pressure was so high that the matrix did not resemble native tissue	Final decellularization protocol: 3% SDS, 12 h, water rinse; 3% SDS, 24 h, water rinse; 3% Triton X-100 rinse, 24 h, water rinse; and PBS rinses at 90 mmHg	Ferng et al. [101]
Chemical and enzymatic	Murine	Aorta	Frozen at −80 °C Rinsed with 0.02% trypsin, 0.05% EDTA, 0.05% NaN3 – 20 min 1% SDS, 0.05% NaN3–10 min 3% Triton X-100, 0.05% EDTA, 0.05% NaN3 – 10 min 4% DCA – 5–10 min	0.1% peracetic acid and 4% ethanol for 5 min	Decreased the DNA content to be 3% residual DNA	Could decellularize murine hearts successfully, proved that DNA could be detected accurately with histologic evaluation	Lu et al. [58]

(continued)

Table 5.1 (continued)

Decellularization method	Species	Perfusion through cardiac vasculature	Decellularization protocol used	Sterilization	Advantages/ disadvantages	Outcomes	Reference
Chemical and physical	Rat	Aorta, 75–80 mmHg	1% SDS, 1% DCA, 0.05% sodium azide – 12 h 20% glycerol, 0.05% sodium azide, 25 mM EDTA in 0.9% NaCl – 12 h 1% saponin and 0.05% sodium azide – 12 h 20% glycerol, 0.05% sodium azide, 25 mM EDTA in 0.9% NaCl – 12 h 200 IU/ml DNase I with MgCl – 12 h	Pen/strep – 12 h Storage: in PBS in pen/ strep	Made a whole heart bioreactor to automate the whole heart decellularization and recellularization process; could successfully decellularize rat hearts with no contamination	Reported low DNA results and some preliminary recell success	Hulsmann et al. [56], Aubin et al. [102]
	Porcine	Aorta and pulmonary artery	Repeated eight times in this order – 4% SDS, agitation with SDS, 1% Triton X-100, perfusion with Triton X-100	0.1% peracetic acid and sterile PBS Storage: in antibiotics and antifungal solutions	Low DNA content achieved, GAG content decreased in LV	Decellularized the whole porcine heart, but GAG content in LV was significantly damaged	Methe et al. [48]
	Porcine	Aorta, 3–5 PSI	Alternating cycles of 1x PBS – 1 h, water 3 cycles of 0.5% SDS – 2 h, DW – 2 h Rinsed with H2O – 2 h (nonrecirculating), then recirculating H2O – 12 h Perfused with 1% Triton X-100 – 2 h	None noted	GAG content was significantly reduced in the RA and RV but remained similar to native in LV and LA. Collagen contents were similar throughout the heart	Reported 99–97% DNA removal in different areas	Momtahan et al. [103]

	Porcine	Ascending aorta, 100 mmHg	Frozen hearts, 80 °C – 24 h, followed by 1% SDS, 1% Triton X-100	None noted	Did not quantify DNA/gag/collagen content	Saw no DNA staining with DAPI after 12 h	Kitahara et al. [26]
	Porcine	1. Upright hearts: decescending aorta 2. Vented hearts: BA with hole in LV and canula 3. Inverted orientation of hearts: DA at 45° angle upside down – 60 mmHg	500 mM NaCl rinse – 4 h 20 mM NaCl rinse – 2 h 1% SDS perfusion – 60 h Final PBS wash	None noted, did not recellularize scaffolds	Inverted orientation of heart had lowest DNA content, ~7%, and could achieve this within 3 days without compromising GAG/collagen content	Improved aortic valve conditions with inverted perfusion orientation, better coronary perfusion, and heart shape retention	Lee et al. [33]
Chemical, enzymatic, physical	Porcine	Aorta	Frozen hearts, 80 °C – 24 h 0.2% trypsin, 0.05% EDTA, 0.05% NaN3 at 37C – 3 h Water followed by 2x PBS perfusion 3% Triton X-100, 0.05% EDTA, 0.05% NaN3 – 2.5 h room temp 4% SDC – 3 h	0.1% peracetic acid, 4% ethanol solution – 1.5 h at 2200 ml/min	Higher than acceptable DNA content in RV~ 55 ng DNA/mg tissue	Fast perfusion protocol and low DNA in LV but higher than acceptable DNA in RV	Remlinger et al. [47]

(continued)

Table 5.1 (continued)

Decellularization method	Species	Perfusion through cardiac vasculature	Decellularization protocol used	Sterilization	Advantages/ disadvantages	Outcomes	Reference
	Porcine	Aorta	Frozen hearts, 80 °C – 24 h in four solutions: 1. 0.02% Trypsin, 0.05% EDTA, 0.05% NaN3 – **7 days** 2. 0.02% Trypsin, 0.05% EDTA, 0.05% NaN3 – **1 day** – and 3% Triton X-100, 0.05% EDTA, 0.05% NaN3 **6 days** 3. 0.02% Trypsin, 0.05% EDTA, 0.05% NaN3 – **3 days** – and 3% Triton X-100, 0.05% EDTA, 0.05% NaN3 **4 days** 4. 3% Triton X-100, 0.05% EDTA, 0.05% NaN3 – **7 days**	None noted	Triton X-100-only treatment caused a 40% decrease in collagen content and 30% decrease in elastin but had high residual DNA	Trypsin only removed 59% DNA, while Triton X-100 only removed 40% DNA. The combination of protocols 2 and 3 resulted in a decrease of 90% and 91%, respectively	Merna et al. [46]

Abbreviations used: *SDS* sodium dodecyl sulfate, *PBS* phosphate-buffered saline, *h* hours, *DIW* deionized water, *DW* distilled water, *min* minutes, CHAPS (3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate), *OGP* octyl β-D-glucopyranoside, *EDTA* ethylenediaminetetraacetic acid, *DCA* deoxycholic acid, *SDC* sodium deoxycholate, *BA* brachiocephalic artery, *DA* descending aorta, *LV* left ventricle, *RV* right ventricle, *ECM* extracellular matrix, *GAG* glycosaminoglycan, *HUVEC* human umbilical vein endothelial cell, *dECM* decellularized ECM

At that time, we found that a combination of SDS and Triton X-100 was the most effective for removing cells while preserving the ECM. This technique has been successfully carried over into larger-sized hearts, such as a porcine and human hearts [23–26, 30, 42]. Newer methods incorporated alternative chemical approaches.

5.2.2 *Enzymatic Decellularization*

Enzyme-based decellularization breaks nucleic acid bonds or cell-matrix attachments, which can be washed out from the native tissue. Nucleases, such as RNase and DNase, are used to cleave RNA and DNA into shorter strands and render the nuclei indistinguishable [43]. Since these nucleases target intracellular contents, they are often used alongside another decellularization technique, such as high pressure used in physical decellularization, to be effective [34]. Another common enzyme used in decellularization is trypsin, a serine protease that hydrolyzes proteins at the C-terminus of lysine or arginine, except when either is followed by proline. Trypsin is often used in cell culture to remove adherent cells from culture plates [44, 45]. When used in a decellularization protocol, trypsin cleaves peptide bonds that hold cells to the ECM. Other solutions, such as Triton X-100 or sodium deoxycholate, then follow the trypsin step to wash out remaining cellular material from the scaffold [46, 47]. Less common enzymes used in decellularization include collagenase, dispase, and alpha-galactosidase [34]. Just as was seen with detergents, enzyme concentrations that are too high or applied for too long can disrupt the ECM ultrastructure, strip the ECM of GAGs, and remove important glycoproteins such as laminin and fibronectin [41]. The balance between matrix preservation and nuclear and cellular clearance must be achieved for the successful generation of a usable acellular ECM bioscaffold.

5.2.3 *Physical Decellularization*

Multiple physical methods of decellularizing tissues have been employed such as agitation, freeze/thawing, and pressure application with supercritical fluids. These physical methods are typically followed with washing steps to remove any residual cellular debris. Wainwright et al. published a study that utilized freeze/thaw cycles for lysing cell membranes as the first step in decellularization [30]. After freeze/thaw cycles, a combination of enzymes and detergents was used to accomplish full decellularization of a porcine heart after only 10 h [30]. The freeze/thaw method thus shortened the total decellularization time by lysing cell membranes prior to enzymatic and chemical decellularization. Another physical method is agitation, in which the whole heart is immersed in a decellularization reagent, followed by shaking on a mixer or a stir plate, to physically lyse cell membranes. Immersion decellularization of the whole heart often damages the external surface of the organ by the time full decellularization of inner constituents is achieved. Agitation helps diffuse

reagents deeper into thick tissues and, in combination with the perfusion of the aorta, has effectively decellularized cardiac scaffolds [48].

The application of pressure in conjunction with supercritical fluids such as CO_2 that has already been used for smaller tissue pieces could potentially be used to decellularize whole organs. Supercritical CO_2 has a critical temperature of 31.1 °C and a pressure of 7.40 MPa, making it a biocompatible solution that does not require copious washing steps [49]. The fluid and pressure burst open cell membranes and remove cellular contents. Supercritical CO_2 has not been applied to the whole heart yet, but its success in other tissues and smaller pieces of the heart makes it a promising decellularization agent for creating whole cardiac bioscaffolds [49, 50].

Another approach to physical-based decellularization was recently published by our group, where we found that inversion of a porcine heart during detergent perfusion led to lower DNA content, higher collagen and elastin content, and higher heart shape index (Fig. 5.3) [33]. The heart shape index was defined as the ratio of the horizontal length to the vertical length of the rectangle that fits the anterior view of the decellularized hearts. Since higher inflow rates were necessary to maintain a pressure of 60 mmHg in the upright perfusion position, the inverted orientation of the heart during decellularization led to less aortic valve damage and improved coronary artery perfusion. Therefore, this new physical decellularization method appears superior to previously described methods used for porcine hearts [33].

As demonstrated by the above discussion of various decellularization methods, none has been accepted as the field or industry standard. Currently, different groups have practices that differ slightly from each other. Along with different methodologies comes numerous values for the measure of decellularization efficacy. While most methods will leave the decellularized scaffold with less than 50 ng/mg of dsDNA per mg dry weight and no nuclei on histological analysis, the variation from heart to heart is still controversial [51]. What these differences mean for recellularization and eventual application of the scaffold in vivo has not been fully elucidated, but ongoing research is bringing about new insights into the recellularized ECM bioscaffold.

5.3 Recellularization

Recellularization involves the seeding of vascular, parenchymal, and support cells into a previously decellularized scaffold. Parameters important for recellularizing the heart include cell type, cell concentrations, and seeding strategies. The variable cell composition within the heart presents a challenge when establishing the ratio of each cell type needed to recellularize the scaffold [52–54]. Research groups have recellularized murine and porcine hearts with murine or human cells, and a handful of labs have published results from human hearts recellularized with human cells [24, 55]. These reports employed different recellularization techniques: perfusion, direct injection, and a combination of perfusion and direct injection. This section discusses each recellularization strategy and its application in engineering whole cardiac tissue from decellularized ECM (dECM). A summary of the recellularization techniques covered can be found in Table 5.2.

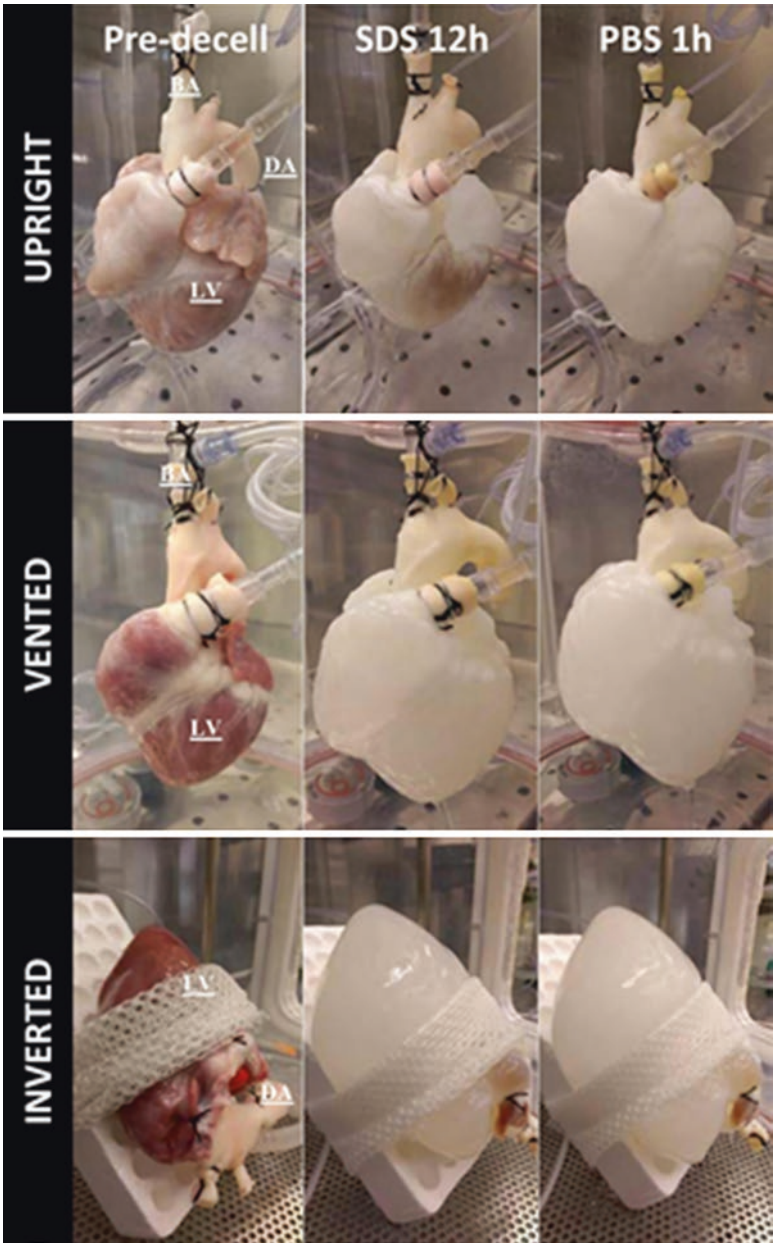

Fig. 5.3 Different orientations of the heart during perfusion decellularization affect the decellularization efficacy; inverted orientation (bottom) leads to higher collagen and elastin content, as well as aortic valve preservation. *LV* Left ventricle, *DA* descending aorta, *BA* brachiocephalic artery, *SDS* sodium dodecyl sulfate , *PBS* phosphate-buffered saline [33]

Table 5.2 Summary of recellularization techniques used for decellularized whole cardiac tissues

Recellularization method	Species	Cell type	Cell number	Culture conditions	Cell washout	Observations	Reference
Manual injection	Mouse	hESC and hMEC	3 million	Static culture for 14 days	Monitored by loss of cells from decellularized heart	Embryonic stem cells and progenitor cells expressed cardiac specific markers after 14 days of in vitro culture and after in vivo subcutaneous transplantation	Ng et al. [57]
Retrograde coronary perfusion via aorta	Mouse	Day 6 EBs	10 million	No continuous perfusion in first 5 days. 30-min media perfusion every 8 h	~10–15% repopulated cells preserved after 7 days of perfusion	New method of periodic perfusion saved time and medium and avoided contaminations during 14–16 days of perfusion. Periodic perfusion method could consistently make beating heart constructs	Lu et al. [58]
Aortic infusion	Rat	Rat aortic ECs	20 million	7 days of continuous perfusion via aorta with flow rate progressively increased from 1 to 3 ml/min over 3 days	Not measured	All infusion methods were effective in delivering cells into the dECM bioscaffold. Combination of IVC and BA infusion of ECs improved distribution of cells in the midventricular free wall vessels. Re-endothelialization before recellularization with neonatal cardiac cells improved contractility in vitro	Robertson et al. [32]
BA infusion			20 million				
BA infusion			40 million				
IVC and BA infusion			20 million IVC, 20 million BA				

5 injections in anterior left ventricle with 27 G needle	Rat	Rat neonatal CM, fibrocytes, EC, and smooth muscle cells	50–75 million	Injections performed during retrograde perfusion. No electrical stimulation in first 24 h, then 10 ms pulse of 5 V through epicardial leads	46% of cells lost within 20 min	Day 8: electric and contractile responses to single paces. Average recellularization was 33.8% proximally to injection sites. Areas of confluent cellularity were approximately 1 mm thick. Viability was >95% throughout thickness (0.5–1.1 mm)	Ott et al. [23]
Direct infusion into patent aorta		Rat aortic EC	20 million	45-min static period for attachment, then 1 week of perfusion	Not measured	Day 7: average cellularity was 550.7 endothelial cells per mm^2 on endocardial surface and 264.8 ECs per mm^2 within vascular tree	
Injected via cannula and directly through myocardium with an 18-gauge needle	Rat	Canine blood outgrowth EC	20 million	Static culture for 45 min before partially submerged in 500 ml of recirculating media	Not measured	Widespread adhesion of seeded cells following 9 days of culture. Observations: clusters of cells consistent with proliferation during culture; substantial cell spreading and multiple focal adhesions	Crawford et al. [104]
Aortic infusion	Porcine	HUVEC passage 2–6	5–6 million	12–14 days with electrodes pacing on left midventricular wall. 3 weeks of perfusion time	Not measured	Day 10: average recellularization per cross section of scaffold was >50% around injection sites. Multielectrode array demonstrated discrete foci with electric voltage undulations of up to 200 mV in time scale of ~500–1000 ms	Weymann et al. [61]
5 intramural injections (8–10 mm depth) in anterior left ventricle with a 27 G needle		Rat neonatal CM	8–9 million		Not measured		

(continued)

Table 5.2 (continued)

Recellularization method	Species	Cell type	Cell number	Culture conditions	Cell washout	Observations	Reference
10 segments of 0.1 ml suspension injected in left ventricular wall	Rat	Murine myoblast cell line (C2C12)	1 million/ml (100 k/0.1 ml)	After 24 h of seeding, 96 h with biomechanical conditioning: 10% longitudinal elongation of ECM	Not measured	Increased cellular viability was reported after 72 h of cultivation of pre-seeded scaffolds under stimulated vs. non-stimulated conditions	Hulsmann et al. [56]
5 intramyocardial injections	Human	BJ RiPS-derived cardiomyocytes	500 million	Static culture for 3–4 h, then perfusion culture started at 20 ml/min for 12 h, then 60 ml/min on day 2	Mattress sutures tied at each injection point before needle extraction to minimize cell loss	Dense regions of engrafted iPS-CM in left ventricular wall; tissue repopulation of <50% within target region of 5 cm^3. Coronary perfusion maintained >90% viability after 14 days in culture	Guyette et al. [24]
Antegrade coronary perfusion	Rat	Rat neonatal CM, fibroblasts, EC	100 million	Continuous perfusion in 5% CO_2 atmosphere. Medium changed at day 2 or 3 and then every 48–72 h following	Not measured.	Spontaneous contractions at days 2–30. Unsynchronized and well-synchronized beating was observed	Yasui et al. [59]

Abbreviations used: *EC* endothelial cells, *CM* cardiomyocyte, *hESC* human embryonic stem cell, *hMEC* human mammary epithelial cell, *EBs* embryoid bodies, *iPS* induced pluripotent stem cell, *IVC* inferior vena cava, *BA* brachiocephalic artery

5.3.1 Direct Injection

The direct injection of cells into the heart involves using a syringe and needle to inject cells suspended in media into the area of interest. The use of a needle presents a concern that the ECM is damaged during the injection process. Additionally, since cells are injected into one specific location, and migration is often limited, cell density is not uniform throughout the ECM. In the first published study of a recellularized human heart, 500 million cardiomyocytes derived from human BJ fibroblast RNA-induced pluripotent stem cells (BJ RiPS) were injected using five intramyocardial direct injections between the left anterior descending (LAD) artery and the left circumflex artery [24]. Upon histological analysis after 2 weeks, the 5 cm^3 injection region of the tissue showed approximately 50% cell repopulation, confirming that uniform cell density is still lacking after recellularization via direct injection [24].

A shortcoming of recellularizing a scaffold via direct injection is the loss of cells during the injection, contributing to low numbers of cells observed in the parenchyma of the cardiac dECM bioscaffold in various studies [24, 26, 56]. Steps can be taken to mitigate the loss of cells, such as adding sutures to the sites of injection, as was done in the recellularization of the human heart; however, full cellularity of the parenchyma was still not achieved in this study. As functional cardiac tissue requires enough viable cells for gap junction formation and cardiomyocyte contractility in the parenchyma, it is critical to have complete cellular coverage in the recellularized heart. Research is ongoing to develop improved injection techniques for complete cell coverage of whole heart bioscaffolds.

5.3.2 Perfusion

Perfusion-based recellularization utilizes the native vascular conduits in the heart as a pathway to deliver cells. Perfusion-based recellularization is accomplished by cannulating one of the major vessels leading to the heart, most often the aorta, which allows for access to coronary arteries. Perfusion usually involves two steps: delivery of the cells where flow occurs followed by a period of "rest" to allow cells to adhere. The adhesion of cells to the matrix is critical in all recellularization protocols but is particularly important when perfusion occurs shortly after delivery. If cells are not allowed sufficient time or provided sufficient conditions to adhere to the ECM, cells will be "washed out" of the ECM during reperfusion, and incomplete recellularization will occur. This loss of cells would therefore result in a need for larger cell numbers for any recellularization process.

In 2011, Ng et al. cannulated the aorta of a decellularized mouse heart to deliver human embryonic stem cells (hESCs) and human mesendodermal cells derived from hESCs to the vasculature of the heart via perfusion [57]. After the heart was in static culture for 14 days, researchers found that the stem cells expressed endothelial cell (EC) markers in the vasculature, suggesting that site-specific cues were retained in the matrix and contributed to progenitor cell differentiation. In a similar manner,

Lu et al. repopulated murine hearts with cells from an embryoid body, via retrograde coronary perfusion [58]. Cells then differentiated within the recellularized dECM into cardiomyocytes and smooth muscle cells (SMCs), resulting in spontaneous contractions, new vessel formation, and responsiveness to isoproterenol, a beta-adrenergic agonist, and E4031, an antiarrhythmic agent. Although contraction occurred, evidence of arrhythmias suggested the cells were immature and that gap junction formation between cardiomyocytes was incomplete. These studies provided further confirmation that the coronary vascular tree is intact after decellularization and can be used to deliver cells to various areas of the matrix.

Due to the unidirectional flow, perfusion recellularization can result in higher cell density in large vessels that are proximal to the infusion site – upstream of smaller vessels. Yasui et al. perfused a mixture of 100 million cardiomyocytes, fibroblasts, and EC antegrade through the coronary tree of a decellularized rat heart [59]. The variety of cells seeded into the matrix resulted in a nonhomogeneous distribution of cells, with a higher concentration of cells found closer to large vessels and near well-perfused vascular beds. However, spontaneous contractions of the heart started 2–3 days after recellularization and continued for the length of the 30-day culture period, suggesting that although cell distribution was uneven, enough cellularity was achieved through perfusion to allow the partial formation of gap junctions.

To compare injection and perfusion side by side, Kitahara et al. recellularized one group of porcine dECM bioscaffolds by injection and a second group by perfusion, using 1.5×10^7 porcine mesenchymal stem cells (pMSCs) in each [26]. Recellularized hearts were then heterotopically transplanted into recipient pigs. The perfusion-recellularized heart did not show patent coronary arteries during intraoperative coronary angiography, as was observed in the scaffold recellularized by injection. Interestingly, none of the perfused cells were observed in vessel lumens upon scaffold excision, while thrombi and inflammatory cells were evident in the parenchyma. Cells already present vs. those recruited into the parenchyma were not separately identified in the study. The injected pMSCs were seen in the parenchymal space in clusters and not homogeneously distributed. This observation confirmed that both methods could be used to revascularize and reseed portions of the cardiac dECM bioscaffolds, but both result in nonuniform distribution of cells throughout the matrix when compared directly.

5.3.3 Perfusion and Injection

Perfusion and injection is a combined approach to recellularizing the whole heart, delivering cells both with a needle into the parenchyma and to both parenchyma and vasculature via perfusion. Our group routinely uses a combination of perfusion and intramyocardial injections to recellularize rat and pig hearts [23, 32]. Previously, we established a closed-circuit retrograde perfusion system through the aorta to infuse rat aortic endothelial cells (RAECs) directly into the patent aorta of a decellularized rat heart [23]. Histological evaluation showed adhesion of RAECs on the

endocardial surface and within the vasculature of the heart. When five injections containing a mixture of neonatal cardiomyocytes, fibrocytes, ECs, and smooth muscle cells were delivered into the anterior left ventricle, a high degree of cell retention at injection sites was observed (>80%), which led to cell coupling and electrical activity propagation. By day 8, the areas of confluent cellularity were about 1 mm thick, and throughout the thickness of the ventricular wall, cell viability was greater than 95%. Although a high density of cells was maintained near the injection sites, density decreased with distance from the needle track.

In another study by our group, rat hearts were re-endothelialized via three different methods: direct aortic perfusion of cells, perfusion of cells into the brachiocephalic artery (BA), or a combination of venous and arterial cell perfusions through the inferior vena cava (IVC) and BA; the combination of venous and arterial perfusion resulted in enhanced distribution of endothelial cells within the vasculature. We found that re-endothelialization of the heart's vasculature by EC perfusion improved the contractility of cardiomyocytes injected into the myocardium [32]. This improved function is not surprising since endothelial cells have been previously shown to promote cardiomyocyte organization and survival [60]. Along with the method of delivery (injection or perfusion), the order in which cells are delivered into the matrix therefore plays a role in their survival and function.

Our findings of improved cell viability and contractility from a combination of perfusion and injection recellularization have been confirmed in the whole porcine heart as well. In a study by Weymann et al., $5–6 \times 10^6$ human umbilical vein endothelial cells (HUVECs) were first perfused through the aorta, and then five injections of $8–9 \times 10^6$ neonatal rat cardiomyocytes (NRCMs) were injected intramurally into the anterior left ventricle of the decellularized porcine heart [61]. The recellularized porcine hearts were found to have platelet endothelial cell adhesion molecule-1 (PECAM-1)-positive cells in the large and small coronary arteries, with minimal gaps in cell coverage. The seeded cardiomyocytes exhibited intrinsic electrical activity after 10 days in culture, but average recellularization of the scaffold was 50% around the sites of cell injection and significantly decreased farther away from the injection sites. The electrical activity of the injected cells, as measured by multielectrode array, demonstrated that areas of functionality could be achieved in a large whole organ, but a larger number of cells may be necessary to get complete cell coverage.

To summarize, successful recellularization of decellularized cardiac bioscaffolds will require essentially recapitulating a native heart by replacing cells in the vasculature, parenchyma, valves, etc. This will require achieving uniform cell density in the parenchyma while also promoting vascularization. High cell numbers are required to achieve complete cell coverage, and this is especially true with larger human-sized hearts. While direct injection requires the insertion of a needle with a diameter large enough for cells to pass into the scaffold, this technique allows cells to be delivered to a specific location in the myocardium. Perfusion-based recellularization allows cells to reach almost every part of the heart by taking advantage of the native vasculature and cavities. Order of cell delivery is also important, as re-endothelialization of the vasculature prior to parenchymal recellulariza-

tion increased retention of other cell types and improved function of the organ [23, 32]. Additional studies are required for conclusive statements on the order of recellularization, especially if a progenitor cell type is delivered to whole cardiac bioscaffolds.

5.4 Clinical Applications and Potential of Cardiac ECM Bioscaffolds

Clinical applications of decellularized whole heart scaffolds are numerous. Decellularized and/or recellularized matrices have the potential to replace heart valves [62], to create cardiac patches [63], or eventually to be used for whole heart transplantation. In fact, several acellular bioscaffolds have been FDA approved for use in vivo for cardiac repairs. However, the use of whole cardiac scaffolds is still being optimized, and several challenges must be addressed for in vivo applications. Figure 5.4 provides the workflow diagram for creating functional tissues from dECM matrices for various clinical applications.

Thrombus formation is one of the challenges of using whole cardiac dECM in vivo. Animal studies have shown that fully decellularized cardiac scaffolds transplanted into a pig retained blood vessel diameter and shape, including the right coronary artery [26]. Unfortunately, these dECM scaffolds induced platelet activation, which led to inflammation and thrombosis formation. Incomplete endothelialization of scaffolds also induces thrombus formation, so complete endothelial cell coverage must be achieved for surfaces in contact with blood. Remnants of cellular and nuclear content in these scaffolds also may induce thrombus formation and inflammation. In addition, decellularization solutions that have not been completely rinsed out of the scaffold can prevent successful recellularization and engraftment into the recipient. Thorough cleaning before transplantation must be accomplished and standardized for decellularized scaffolds to be used widely in vivo.

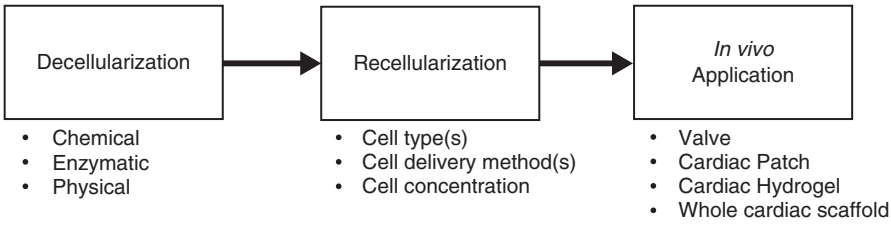

Fig. 5.4 Workflow for creating a functional cardiac tissue from decellularized bioscaffolds. Important considerations are method of decellularization, optimizing recellularization, and finally creating a product that matches the desired application

5.4.1 Heart Valves

A valve homograft from a human cadaver to a human recipient is ideal for biocompatibility, but the shortage of organ donors is still a problem worldwide. To address this issue, mechanical and bioprosthetic heart valves (BHVs) have been used. BHVs from porcine or bovine tissue are FDA-approved valves that have been sterilized and rendered biologically inactive by glutaraldehyde fixation [64]. One of the advantages of BHVs over mechanical valves is that anticoagulants are not required [65]. However, BHV calcification is commonly observed in patients, likely due to the glutaraldehyde fixation process, increased valve stiffness, and heightened immune competence of the recipient [66]. A non-fixed decellularized heart valve could be ideal for recipients to avoid failures associated with both BHVs and mechanical valves. In vivo studies of decellularized valves recellularized with endothelial cells and myofibroblasts showed no thrombus formation when implanted into juvenile sheep [67]. In some studies, decellularized valves implanted into sheep have also seen positive long-term outcomes, where animals were alive 9 months post implantation with functioning valves [68, 69].

Several decellularized allograft cardiac valves have already been FDA approved. CryoValve SG® (CryoLife Inc., Kennesaw, GA), the only approved human acellular pulmonary heart valve, and SynerGraft® (CryoLife Inc.) have both seen excellent long-term success when implanted in humans [70, 71].

5.4.2 Cardiac Patches

A cardiac patch provides necessary support and biochemical cues for restoring cardiac function for diseases ranging from atrial and ventricular defects to left ventricular dysfunction. Cardiac patches can be generated in different ways, whether it be a decellularized bioscaffold, injectable gel, or a printed bioscaffold. Additionally, these patches may be either acellular or recellularized, sourced from non-cardiac tissues, and can be formed into various shapes and sizes relevant to the designed study and therapeutic intervention. Patches may also be from xenogeneic sources, as these scaffolds have been shown to be biocompatible with human cells, which allows for further patient-specific customization due to variable wall thicknesses in different species [78]. Even without cells, an acellular scaffold, derived from cardiac or non-cardiac tissues, can provide mechanical support to the failing heart, repair major blood vessels, and promote intracardiac repair.

Bioscaffolds used for heart recovery and repair are not limited to cardiac tissues. In one study, acellular dermis was used to repair a left ventricular aneurysm [72]; however, cell engraftment was not studied in this patient. Using urinary bladder-derived ECM, researchers observed spindle-shaped cells in the matrix after 1 month,

showing a non-cardiac-derived tissue can support cardiac-shaped cells [73]. Furthermore, acellular scaffolds inactivated with chemical treatment still retained properties that made them a hospitable environment for cells [74].

In other studies, components of the decellularized cardiac ECM were sufficient to cause an increase in regenerative capacity in vivo. In a cross-species study, zebra fish hearts were decellularized and lyophilized into a cardiac ECM powder [75]. This powder was resuspended in saline and applied to a mouse heart with a permanent ligation of the left anterior descending (LAD) coronary artery. The results of the study were encouraging as the decellularized zebra fish ECM suspension could enable endogenous regeneration of the murine heart after acute myocardial infarction.

A few of these matrices have been FDA approved and are currently used in vivo with positive clinical outcomes. PhotoFix® (CryoLife Inc., Kennesaw, GA), a bovine pericardium patch, is used for patching vascular structures and intracardiac repair [76]. Similarly, CorMatrix® (Aziyo, Roswell, GA), derived from porcine small intestinal submucosa, is used for repairing atrial and ventricular septal defects, vascular abnormalities, and cardiac tumors [77]. Several other matrices are also in the pipeline for FDA approval for human use.

5.4.3 Hydrogels

Cardiac ECM hydrogels have recently emerged as a new technology in the field of regenerative medicine. These gels are created by using a novel method for retaining the native ECM components by turning a decellularized scaffold into a hydrogel.

5.4.3.1 Injectable Gel

The Christman Group pioneered the technique for forming a hydrogel from decellularized porcine left ventricular myocardium [78, 79]. The majority of the dECM myocardial gel protocols utilize chemical detergents such as SDS or Triton X-100, while some groups recently have used supercritical CO_2 as a method to decellularize myocardial pieces [80–84]. After the myocardium is decellularized, it is enzymatically digested down using either pepsin in hydrochloric acid (HCl) or acetic acid (AA) for a period of 48–72 h [85–89]. This digested dECM is then polymerized into a hydrogel by bringing the solution to physiologic pH and salt concentrations at 37 °C (Fig. 5.5). The mechanical strength of these ECM hydrogels, however, are not high (<1 kPa), and several researchers have explored methods to increase their mechanical strength.

To make the ECM hydrogels stiffer, different groups have added materials such as chitosan, polyethylene glycol (PEG), fibrin, and silk to make a hybrid hydrogel [89–93]. These materials often bind to the matrix using photo cross-linkers to polymerize gels. The addition of an inert biocompatible polymer, such as PEG, into the ECM hydrogel to provide mechanical stiffness has been researched by the

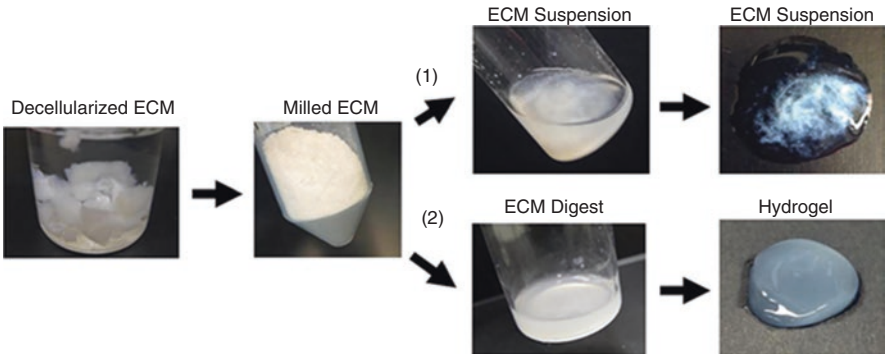

Fig. 5.5 Process for the development of extracellular matrix (ECM) hydrogels. After decellularization, resulting tissue is lyophilized and milled into a fine powder. This powder is then added to a solution to form a suspension (1, top right) or digested using enzymes to form a hydrogel (2, bottom right) [105]

Christman Group [90], where PEG cross-linked gels within 4 min and sustained a high cell viability. Addition of an inert biocompatible polymer is advantageous, in that the concentration of the ECM proteins is not disrupted, as would be for collagen or hyaluronic acid. Different groups have used other cross-linkers to increase the mechanical strength of these gels; for instance, genipin, a non-cytotoxic naturally derived chemical from gardenia fruit, has been used to render a hydrogel that has mechanical properties similar to native cardiac tissue [92]. The hydrogels can also be delivered in vivo using a catheter and cross-linked in situ with positive results of maintained cardiac function, no induced arrhythmias, and pro-angiogenic properties when applied to an area of myocardial infarction [85–88].

Currently there are no FDA-approved ECM hydrogel products, but VentriGel™ (Ventrix Inc., San Diego, CA), a myocardial ECM hydrogel derived from porcine left ventricles, is currently in a Phase 1 clinical trial and is set to complete in September 2018 [94]. Although there are limited clinical data on cardiac ECM hydrogels, this field has a high potential for growth, allowing researchers to take the lead in developing new applications for clinically viable therapies.

5.4.3.2 Bioprinting

Three-dimensional bioprinting involves the controlled construction of an object made layer by layer [95]. This allows the user to print objects of any shape and size for a desired application. Bioprinting is accomplished using different printing methods such as extrusion-based, ink-jet-based, and laser-based printing [96]. The inks used with these printers range from synthetic to biologic in origin, known as a "bioink." The candidate for a bioink must maintain the desired shape and be able to withstand the addition of layers during the print process. This ink must also foster an environment suitable for cellular growth and possibly differentiation.

ECM hydrogels have been used as bioinks for 3D printing [97, 98] and hold great promise, as these gels already retain in vivo ECM constituents. As a bioink, ECM hydrogels can support cell differentiation and survival better than traditional bioinks, encouraging differentiation to a specific cell type based on the tissue source [99]. In a comparative study, rat myoblasts showed higher expression of cardiogenic differentiation genes when cultured on bioprinted cardiac dECM than on collagen hydrogels, showing evidence that the bioink retains tissue-specific cues [99]. To cross-link dECM hydrogels for printing, different photo cross-linkers have been researched such as riboflavin (vitamin b2), a non-cytotoxic alternative to other photo cross-linking agents [92, 98]. Riboflavin with the ECM alone does not cross-link rapidly but can produce cytocompatible materials with a mechanical strength similar to native cardiac ECM [97, 98]. Three-dimensional bioprinting is thus a promising technology, as customizable structures may be created. However, an existing limitation is that these structures must be intricately vascularized to support complex tissues and organs. Further research and design development is necessary to bioprint whole organs with a vascular network out of ECM-derived hydrogels.

5.5 Conclusion

Cardiac tissue bioscaffolds continue to be of great interest in the field of regenerative medicine due to their ability to take various forms – a whole scaffold, cardiac patch, or hydrogel – along with their numerous therapeutic applications for cardiovascular disease. New technologies such as cardiac ECM hydrogels hold prospect as a potential bioink and as an in vivo patch; however, 3D printed structures do not innately contain vascular conduits, while decellularized matrices do. Decellularization of the whole heart in 2008 paved the way for whole bioscaffolds to be used in vivo and eventually in clinical trials. As recellularization and other bioengineering technologies improve, the uses and applications of cardiac bioscaffolds continue to grow. Furthermore, for standardized manufacturing of these recellularized dECM matrices, guidelines must be established to manufacture consistently safe and effective bioscaffolds for clinical applications. Although many unknowns exist before whole cardiac bioscaffolds become a clinical reality, current research shows tremendous potential. As new discoveries happen every day, the field moves closer to making whole cardiac bioscaffolds clinically feasible.

Disclosure Dr. Taylor holds a financial interest in Miromatrix Medical Inc. She is entitled to sales royalty through the University of Minnesota for products related to the research described in this paper. This relationship has been reviewed and managed by the University of Minnesota and Texas Heart Institute in accordance with its conflict of interest policies; the other authors have nothing to disclose.

References

1. GBD 2015 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1603–58.
2. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1459–544.
3. Cohn JN. Continue what we are doing to treat HF, but do it better. Nat Rev Cardiol. 2013;11:69–70.
4. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, et al. 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the Management of Heart Failure: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines and the Heart Failure Society of America. Circulation. 2017;136:e137–61.
5. Wilhelm MJ. Long-term outcome following heart transplantation: current perspective. J Thorac Dis. 2015;7:549–51.
6. Longnus SL, Mathys V, Dornbierer M, Dick F, Carrel TP, Tevaearai HT. Heart transplantation with donation after circulatory determination of death. Nat Rev Cardiol. 2014;11:354–63.
7. Wang B, Patnaik SS, Brazile B, Butler JR, Claude A, Zhang G, et al. Establishing early functional perfusion and structure in tissue engineered cardiac constructs. Crit Rev Biomed Eng. 2015;43:455–71.
8. Kaiser NJ, Coulombe KLK. Physiologically inspired cardiac scaffolds for tailored in vivo function and heart regeneration. Biomed Mater. 2015;10:034003.
9. Iozzo RV, Schaefer L. Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. Matrix Biol. 2015;42:11–55.
10. Engel J, Chiquet M. An overview of extracellular matrix structure and function. The extracellular matrix: an overview. Berlin/Heidelberg: Springer; 2011. p. 1–39.
11. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater. 2009;5:1–13.
12. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci. 2010;123:4195–200.
13. Halper J, Kjaer M. Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. Adv Exp Med Biol. 2014;802:31–47.
14. Rienks M, Papageorgiou A-P, Frangogiannis NG, Heymans S. Myocardial extracellular matrix: an ever-changing and diverse entity. Circ Res. 2014;114:872–88.
15. Kim S-H, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. J Endocrinol. 2011;209:139–51.
16. Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. Nat Rev Mol Cell Biol. 2014;15:771–85.
17. Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326:1216–9.
18. Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr Opin Cell Biol. 2012;24:645–51.
19. Bowers SLK, Banerjee I, Baudino TA. The extracellular matrix: at the center of it all. J Mol Cell Cardiol. 2010;48:474–82.
20. Rana D, Zreiqat H, Benkirane-Jessel N, Ramakrishna S, Ramalingam M. Development of decellularized scaffolds for stem cell-driven tissue engineering. J Tissue Eng Regen Med. 2017;11:942–65.
21. Zaman MH, Kamm RD, Matsudaira P, Lauffenburger DA. Computational model for cell migration in three-dimensional matrices. Biophys J. 2005;89:1389–97.

22. Gaudet C, Marganski WA, Kim S, Brown CT, Gunderia V, Dembo M, et al. Influence of type I collagen surface density on fibroblast spreading, motility, and contractility. Biophys J. 2003;85:3329–35.
23. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 2008;14:213–21.
24. Guyette JP, Charest JM, Mills RW, Jank BJ, Moser PT, Gilpin SE, et al. Bioengineering human myocardium on native extracellular matrix. Circ Res. 2016;118:56–72.
25. Hodgson MJ, Knutson CC, Momtahan N, Cook AD. Extracellular matrix from whole porcine heart Decellularization for cardiac tissue engineering. Methods Mol Biol. 2017; https://doi.org/10.1007/7651_2017_31.
26. Kitahara H, Yagi H, Tajima K, Okamoto K, Yoshitake A, Aeba R, et al. Heterotopic transplantation of a decellularized and recellularized whole porcine heart. Interact Cardiovasc Thorac Surg. 2016;22:571–9.
27. Rieder E, Kasimir M-T, Silberhumer G, Seebacher G, Wolner E, Simon P, et al. Decellularization protocols of porcine heart valves differ importantly in efficiency of cell removal and susceptibility of the matrix to recellularization with human vascular cells. J Thorac Cardiovasc Surg. 2004;127:399–405.
28. Courtman DW, Pereira CA, Kashef V, McComb D, Lee JM, Wilson GJ. Development of a pericardial acellular matrix biomaterial: biochemical and mechanical effects of cell extraction. J Biomed Mater Res. 1994;28:655–66.
29. Grauss R, Hazekamp M, Oppenhuizen F, Vanmunsteren C, Gittenbergerdegroot A, Deruiter M. Histological evaluation of decellularised porcine aortic valves: matrix changes due to different decellularisation methods. Eur J Cardiothorac Surg. 2005;27:566–71.
30. Wainwright JM, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, et al. Preparation of cardiac extracellular matrix from an intact porcine heart. Tissue Eng Part C Methods. 2010;16:525–32.
31. Akhyari P, Aubin H, Gwanmesia P, Barth M, Hoffmann S, Huelsmann J, et al. The quest for an optimized protocol for whole-heart decellularization: a comparison of three popular and a novel decellularization technique and their diverse effects on crucial extracellular matrix qualities. Tissue Eng Part C Methods. 2011;17:915–26.
32. Robertson MJ, Dries-Devlin JL, Kren SM, Burchfield JS, Taylor DA. Optimizing Recellularization of whole Decellularized heart extracellular matrix. PLoS One. 2014;9:e90406.
33. Lee P-F, Chau E, Cabello R, Yeh AT, Sampaio LC, Gobin AS, et al. Inverted orientation improves decellularization of whole porcine hearts. Acta Biomater. 2017;49:181–91.
34. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32:3233–43.
35. Garreta E, Oria R, Tarantino C, Pla-Roca M, Prado P, Fernández-Avilés F, et al. Tissue engineering by decellularization and 3D bioprinting. Mater Today. 2017;20:166–78.
36. Zahmati AHA, Alipoor R, Shahmirzadi AR, Khori V, Abolhasani MM. Chemical Decellularization methods and its effects on extracellular matrix. Int Med Med Investig J. 2017;2:76–83.
37. Gratzer PF, Harrison RD, Woods T. Matrix alteration and not residual sodium dodecyl sulfate cytotoxicity affects the cellular repopulation of a Decellularized matrix. Tissue Eng. 2006;12:2975.
38. Cebotari S, Tudorache I, Jaekel T, Hilfiker A, Dorfman S, Ternes W, et al. Detergent decellularization of heart valves for tissue engineering: toxicological effects of residual detergents on human endothelial cells. Artif Organs. 2010;34:206–10.
39. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods. 2015;84:25–34.
40. Sarig U, Au-Yeung GCT, Wang Y, Bronshtein T, Dahan N, Boey FYC, et al. Thick acellular heart extracellular matrix with inherent vasculature: a potential platform for myocardial tissue regeneration. Tissue Eng Part A. 2012;18:2125–37.

41. Gilpin A, Yang Y. Decellularization strategies for regenerative medicine: from processing techniques to applications. Biomed Res Int. 2017;2017:1–13.
42. University of Arizona College of Medicine, Department of Surgery, Division of Cardiothoracic Surgery, Tucson AZ, Khalpey Z. Acellular porcine heart matrices: whole organ decellularization with 3D-Bioscaffold & vascular preservation. Transl Res. 2017; 10.18053/jctres.03.201702.001.
43. Lynch AP, Ahearne M. Strategies for developing decellularized corneal scaffolds. Exp Eye Res. 2013;108:42–7.
44. Freshney RI, Ian Freshney R. Culture of specific cell types. Culture of animal cells; 2005.
45. Olsen JV, Ong S-E, Mann M. Trypsin cleaves exclusively C-terminal to arginine and lysine residues. Mol Cell Proteomics. 2004;3:608–14.
46. Merna N, Robertson C, La A, George SC. Optical imaging predicts mechanical properties during decellularization of cardiac tissue. Tissue Eng Part C Methods. 2013;19:802–9.
47. Remlinger NT, Wearden PD, Gilbert TW. Procedure for decellularization of porcine heart by retrograde coronary perfusion. J Vis Exp. 2012:e50059.Q4
48. Methe K, Bäckdahl H, Johansson BR, Nayakawde N, Dellgren G, Sumitran-Holgersson S. An alternative approach to decellularize whole porcine heart. Biores Open Access. 2014;3:327–38.
49. Seo Y, Jung Y, Kim SH. Decellularized heart ECM hydrogel using supercritical carbon dioxide for improved angiogenesis. Acta Biomater. 2017;67:270. https://doi.org/10.1016/j.actbio.2017.11.046.
50. Sawada K, Terada D, Yamaoka T, Kitamura S, Fujisato T. Cell removal with supercritical carbon dioxide for acellular artificial tissue. J Chem Technol Biotechnol. 2008;83:943–9.
51. Akhyari P, Oberle F, Huelsmann J, Heid H, Lehr S, Barbian A, et al. Characterization of the epicardial adipose tissue in decellularized human-scaled whole-hearts - implications for the whole-heart tissue engineering. Tissue Eng Part A. 2017; Available: https://www.ncbi.nlm.nih.gov/pubmed/28895502.
52. Zhou P, Pu WT. Recounting cardiac cellular composition. Circ Res. 2016;118:368–70.
53. Pinto AR, Ilinykh A, Ivey MJ, Kuwabara JT, D'Antoni ML, Debuque R, et al. Revisiting cardiac cellular composition. Circ Res. 2016;118:400–9.
54. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, et al. Dynamics of cell generation and turnover in the human heart. Cell. 2015;161:1566–75.
55. Garreta E, de Oñate L, Fernández-Santos ME, Oria R, Tarantino C, Climent AM, et al. Myocardial commitment from human pluripotent stem cells: rapid production of human heart grafts. Biomaterials. 2016;98:64–78.
56. Hülsmann J, Aubin H, Kranz A, Godehardt E, Munakata H, Kamiya H, et al. A novel customizable modular bioreactor system for whole-heart cultivation under controlled 3D biomechanical stimulation. J Artif Organs. 2013;16:294–304.
57. Ng SLJ, Narayanan K, Gao S, Wan ACA. Lineage restricted progenitors for the repopulation of decellularized heart. Biomaterials. 2011;32:7571–80.
58. Lu TY, Lin B, Kim J, Sullivan M, Tobita K, Salama G, et al. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat Commun. 2013;4:2307.
59. Yasui H, Lee J-K, Yoshida A, Yokoyama T, Nakanishi H, Miwa K, et al. Excitation propagation in three-dimensional engineered hearts using decellularized extracellular matrix. Biomaterials. 2014;35:7839–50.
60. Narmoneva DA, Vukmirovic R, Davis ME, Kamm RD, Lee RT. Endothelial cells promote cardiac myocyte survival and spatial reorganization: implications for cardiac regeneration. Circulation. 2004;110:962–8.
61. Weymann A, Patil NP, Sabashnikov A, Jungebluth P, Korkmaz S, Li S, et al. Bioartificial heart: a human-sized porcine model – the way ahead. PLoS One. 2014;9:e111591.
62. VeDepo MC, Detamore MS, Hopkins RA, Converse GL. Recellularization of decellularized heart valves: progress toward the tissue-engineered heart valve. J Tissue Eng. 2017;8:2041731417726327.

63. Wang B, Borazjani A, Tahai M, de Jongh Curry AL, Simionescu DT, Guan J, et al. Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. J Biomed Mater Res A. 2010;94A:1100–10.
64. Siddiqui RF, Abraham JR, Butany J. Bioprosthetic heart valves: modes of failure. Histopathology. 2009;55:135–44.
65. Bloomfield P. Choice of heart valve prosthesis. Heart. 2002;87:583–9.
66. Manji RA, Menkis AH, Ekser B, Cooper DKC. The future of bioprosthetic heart valves. Indian J Med Res. 2012;135:150–1.
67. Schmidt D, Stock UA, Hoerstrup SP. Tissue engineering of heart valves using decellularized xenogeneic or polymeric starter matrices. Philos Trans R Soc Lond Ser B Biol Sci. 2007;362:1505–12.
68. Dohmen PM, da Costa F, Holinski S, Lopes SV, Yoshi S, Reichert LH, et al. Is there a possibility for a glutaraldehyde-free porcine heart valve to grow? Eur Surg Res. 2006;38:54–61.
69. Baraki H, Tudorache I, Braun M, Höffler K, Görler A, Lichtenberg A, et al. Orthotopic replacement of the aortic valve with decellularized allograft in a sheep model. Biomaterials. 2009;30:6240–6.
70. Brown JW, Elkins RC, Clarke DR, Tweddell JS, Huddleston CB, Doty JR, et al. Performance of the CryoValve∗ SG human decellularized pulmonary valve in 342 patients relative to the conventional CryoValve at a mean follow-up of four years. J Thorac Cardiovasc Surg. 2010;139:339–48.
71. Brown JW, Ruzmetov M, Eltayeb O, Rodefeld MD, Turrentine MW. Performance of SynerGraft decellularized pulmonary homograft in patients undergoing a Ross procedure. Ann Thorac Surg. 2011;91:416–22. discussion 422–3
72. Lim PS, Bierowski N, Garrido M, Watson R, Balaratna A, Paul AV. Repair of left ventricular aneurysm with acellular dermis graft: a case report. J Cardiol Cases. 2012;6:e42–4.
73. Robinson KA, Li J, Mathison M, Redkar A, Cui J, Chronos NAF, et al. Extracellular matrix scaffold for cardiac repair. Circulation. 2005;112:I135–43.
74. Svystonyuk D, Turnbull J, Teng G, Belke D, Guzzardi D, Park D, et al. Acellular extracellular matrix SCAFFOLD reprograms cardiac fibroblasts and promotes adaptive cardiac remodeling and repair. Can J Cardiol. 2017;33:S49–50.
75. Chen WCW, Wang Z, Missinato MA, Park DW, Long DW, Liu H-J, et al. Decellularized zebrafish cardiac extracellular matrix induces mammalian heart regeneration. Sci Adv. 2016;2:e1600844.
76. Baird CW, Myers PO, Piekarski B, Borisuk M, Majeed A, Emani SM, et al. Photo-oxidized bovine pericardium in congenital cardiac surgery: single-Centre experience. Interact Cardiovasc Thorac Surg. 2017;24:240–4.
77. Woo JS, Fishbein MC, Reemtsen B. Histologic examination of decellularized porcine intestinal submucosa extracellular matrix (CorMatrix) in pediatric congenital heart surgery. Cardiovasc Pathol. 2016;25:12–7.
78. Singelyn JM, DeQuach JA, Seif-Naraghi SB, Littlefield RB, Schup-Magoffin PJ, Christman KL. Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. Biomaterials. 2009;30:5409–16.
79. Seif-Naraghi S, Singelyn J, Dequach J, Schup-Magoffin P, Christman K. Fabrication of biologically derived injectable materials for myocardial tissue engineering. J Vis Exp. 2010; https://doi.org/10.3791/2109.
80. Gaetani R, Ungerleider J, Christman KL. Acellular injectable biomaterials for treating cardiovascular disease. In: Stem cell and gene therapy for cardiovascular disease; 2016. p. 309–25.Q5
81. Wang RM, Christman KL. Decellularized myocardial matrix hydrogels: in basic research and preclinical studies. Adv Drug Deliv Rev. 2016;96:77–82.
82. Wassenaar JW, Boss GR, Christman KL. Decellularized skeletal muscle as an in vitro model for studying drug-extracellular matrix interactions. Biomaterials. 2015;64:108–14.
83. Suarez SL, Rane AA, Muñoz A, Wright AT, Zhang SX, Braden RL, et al. Intramyocardial injection of hydrogel with high interstitial spread does not impact action potential propagation. Acta Biomater. 2015;26:13–22.

84. Gaetani R, Yin C, Srikumar N, Braden R, Doevendans PA, Sluijter JPG, et al. Cardiac-derived extracellular matrix enhances cardiogenic properties of human cardiac progenitor cells. Cell Transplant. 2016;25:1653–63.
85. Singelyn JM, Sundaramurthy P, Johnson TD, Schup-Magoffin PJ, Hu DP, Faulk DM, et al. Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction. J Am Coll Cardiol. 2012;59:751–63.
86. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, et al. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med. 2013;5:173ra25.
87. Wassenaar JW, Gaetani R, Garcia JJ, Braden RL, Luo CG, Huang D, et al. Evidence for mechanisms underlying the functional benefits of a myocardial matrix hydrogel for post-MI treatment. J Am Coll Cardiol. 2016;67:1074–86.
88. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. Acta Biomater. 2017;49:1–15.
89. Stoppel WL, Gao AE, Greaney AM, Partlow BP, Bretherton RC, Kaplan DL, et al. Elastic, silk-cardiac extracellular matrix hydrogels exhibit time-dependent stiffening that modulates cardiac fibroblast response. J Biomed Mater Res A. 2016;104:3058–72.
90. Grover GN, Rao N, Christman KL. Myocardial matrix-polyethylene glycol hybrid hydrogels for tissue engineering. Nanotechnology. 2014;25:014011.
91. Williams C, Budina E, Stoppel WL, Sullivan KE, Emani S, Emani SM, et al. Cardiac extracellular matrix-fibrin hybrid scaffolds with tunable properties for cardiovascular tissue engineering. Acta Biomater. 2015;14:84–95.
92. Efraim Y, Sarig H, Cohen Anavy N, Sarig U, de Berardinis E, Chaw S-Y, et al. Biohybrid cardiac ECM-based hydrogels improve long term cardiac function post myocardial infarction. Acta Biomater. 2017;50:220–33.
93. Stoppel WL, Hu D, Domian IJ, Kaplan DL, Black LD 3rd. Anisotropic silk biomaterials containing cardiac extracellular matrix for cardiac tissue engineering. Biomed Mater. 2015;10:034105.
94. Identifier: NCT02305602; A Phase I, Open-label Study of the Effects of Percutaneous Administration of an Extracellular Matrix Hydrogel, VentriGel, Following Myocardial Infarction. In: Clinicaltrials.gov [Internet]. 2 Dec 2014 [cited Jan 2018]. Available: https://clinicaltrials.gov/ct2/show/NCT02305602.
95. Ferris CJ, Gilmore KG, Wallace GG, In het Panhuis M. Biofabrication: an overview of the approaches used for printing of living cells. Appl Microbiol Biotechnol. 2013;97:4243–58.
96. Kim BS, Kim H, Gao G, Jang J, Cho D-W. Decellularized extracellular matrix: a step towards the next generation source for bioink manufacturing. Biofabrication. 2017;9:034104.
97. Jang J, Park H-J, Kim S-W, Kim H, Park JY, Na SJ, et al. 3D printed complex tissue construct using stem cell-laden decellularized extracellular matrix bioinks for cardiac repair. Biomaterials. 2017;112:264–74.
98. Jang J, Kim TG, Kim BS, Kim S-W, Kwon S-M, Cho D-W. Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Acta Biomater. 2016;33:88–95.
99. Pati F, Jang J, Ha D-H, Won Kim S, Rhie J-W, Shim J-H, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935.
100. Sánchez PL, Fernández-Santos ME, Costanza S, Climent AM, Moscoso I, Gonzalez-Nicolas MA, Sanz-Ruiz R, Rodríguez H, Kren SM, Garrido G, Escalante JL, Bermejo J, Elizaga J, Menarguez J, Yotti R, Pérez del Villar C, Espinosa MA, Guillem MS, Willerson JT, Bernad A, Matesanz R, Taylor DA, Fernández-Avilés F. Acellular human heart matrix: A critical step toward whole heart grafts. Biomaterials. 2015;61:279–89.
101. Ferng AS, Connell AM, Marsh KM, Qu N, Medina AO, Bajaj N, Palomares D, Iwanski J, Tran PL, Lotun K, Johnson K, Khalpey Z. Acellular porcine heart matrices: whole organ decellularization with 3D-bioscaffold & vascular preservation. J Clin Trans Res. 2017;3(2):260–70.

102. Aubin H, Kranz A, Hülsmann J, Lichtenberg A, Akhyari P. Decellularized whole heart for bioartificial heart. In: Kao R, editor. Cellular Cardiomyoplasty. Methods in molecular biology (methods and protocols), vol. 1036. Totowa: Humana Press; 2013.
103. Momtahan N, Poornejad N, Struk JA, Castleton AA, Herrod BJ, Vance BR, Eatough JP, Roeder BL, Reynolds PR, Cook AD. Automation of pressure control improves whole porcine heart Decellularization. Tissue Eng Part C Methods. 2015;21(11):1148–61.
104. Crawford B, Koshy S, Jhamb G, Woodford C, Thompson CM, Levy AA, Rush JWE, Guillemette J, Lillicrap D, Jervis E. Cardiac decellularization with long-term storage and repopulation with canine peripheral blood progenitor cells. Can J Chem Eng. 2012;90(6):1457–64.
105. Spang MT, Christman KL. Extracellular matrix hydrogel therapies: in vivo applications and development. Acta Biomater. 2018;68:1–14.
106. Taylor DA, Sampaio LC, Ferdous Z, Gobin AS, Taite LJ. Decellularized matrices in regenerative medicine. Acta Biomater. 2018;74:74–89. PMID:29702289.

Chapter 6
Natural Sources of Extracellular Matrix for Cardiac Repair

Keith L. Spinali and Eric G. Schmuck

Abstract Tissue engineering and regenerative medicine have adopted the use of extracellular matrix (ECM) as a cell delivery device and bioactive regenerative agent. To this end, many ECMs have been investigated for cardiac tissue engineering and regenerative medicine applications with variable success. Many sources of natural ECMs have been tested for cardiac applications. Typically, natural ECMs have been made from decellularized organs or tissues and processed into either sheets or injectable hydrogels. This chapter will review natural sources of ECM materials that have been tested as therapeutic agents in models of heart failure.

Keywords Extracellular matrix · Biomaterial · Scaffolds · Acellular · Regenerative medicine · Small intestine submucosa · Urinary bladder matrix · Pericardium · Myocardium · Hydrogel

6.1 Introduction

The prevalence, mortality, and costs associated with heart failure (HF) remain a significant problem in the United States and worldwide. Despite modern therapies, it is expected that these statistics will continue increasing. Currently, HF affects an estimated six million Americans, with an incidence of 915,000 new cases in the United States every year [1]. By 2030, the prevalence of HF is expected to exceed eight million Americans [1, 2]. Approximately half of all patients with HF die within 5 years of receiving the diagnosis [1]. This high rate of mortality has led to HF being included on 1 out every 9 death certificates in the United States [1]. As of 2012, the annual cost for the direct treatment of HF was $21 billion in the United States alone [1]. This cost is predicted to increase to $53 billion by 2030 [1].

K. L. Spinali · E. G. Schmuck (✉)
Department of Medicine, Division of Cardiovascular Medicine, University of Wisconsin Madison, Madison, WI, USA
e-mail: egs@medicine.wisc.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_6

Therefore, finding an effective treatment for HF is important for both public health and economic reasons.

Current treatment options for HF focus on inhibiting maladaptive pathophysiological pathways with a combination of beta blockers and angiotensin-converting enzyme (ACE) inhibitors; however, these medications are only partially effective in preventing or reversing ventricular remodeling and are not universally well-tolerated [3]. Even with pharmacologic intervention, mature cardiac tissue in mammals is not capable of significant regeneration following injury [4]. Regenerative potential, therefore, must be provided to damaged myocardium by an external source.

Since 2002, stem cell-based therapies have been tested in clinical trials to treat heart failure [5]. It was initially thought that stem cells would differentiate into cardiomyocytes, integrate into the host tissue, and enhance cardiac function [5]. Unfortunately, further preclinical trials demonstrated that very few stem cells, if any, mature into cardiomyocytes [5]. Additional studies looked at the ability of stem cells to recruit endothelial progenitor cells in order to stimulate angiogenesis or vasculogenesis [5]. The findings of these studies suggested that there were not enough endothelial progenitor cells present in the adult myocardium to achieve measurable improvement in cardiac function [5]. Thus, despite a decade and a half of research, the FDA has yet to approve a stem cell-based therapy for the treatment of heart failure [5, 6].

In order to augment cell-based therapies, devices made from extracellular matrices (ECMs) and other biomaterials are being developed as delivery systems for therapeutic cells. These ECM-derived scaffolds, hydrogels, and particulate suspensions, in addition to acting as vehicles for cell delivery, are thought to enhance cell survival and retention in heart tissue. Frequently, however, significant therapeutic benefits were observed when these ECM devices were used without cells in post-ischemic models. These observations have expanded the field of cardiac regenerative medicine to now include therapeutic biomaterials and ECMs.

Historically, ECM was thought to be biologically inert, providing only a physical support to which cells attached but lacking intrinsic biological activity. It is now understood, however, that ECM—via biochemical and biomechanical signaling—is a potent mediator of cellular processes such as proliferation, differentiation, migration, and survival [7, 8]. ECM-based devices, used as stand-alone therapies or in conjunction with a variety of cell types, have demonstrated the ability to promote cardiac tissue repair in murine, leporine, and swine models [9, 10]. A variety of naturally occurring ECM sources have been investigated—with or without cells—as an epicardial patch or injected directly into the myocardium as a hydrogel or particulate suspension. Paracrine signaling occurs between the target tissue and the therapeutic ECM device that stimulates angiogenesis and myocyte proliferation and limits or reverses maladaptive postinfarction remodeling [11, 12]. An alternative to harvesting ECM from animal or human donor tissues is the manufacture of synthetic scaffolds or hydrogels using techniques such as electrospinning and 3D printing. These synthetic matrices can then be functionalized with specific growth factors or seeded with therapeutic cells. The application of these synthetic matrix-derived devices has similarly demonstrated an improvement in postinfarction ventricular function using animal models but will not be the focus of this chapter.

This chapter will provide an overview of natural source-based cell scaffolds and other ECM-based devices that have demonstrated the potential to stimulate cardiac tissue regeneration in the published literature. A systematic review was performed using PubMed in June 2017. Search terms included tissue scaffold, extracellular matrix, regenerative medicine, repair, and heart failure. Results of this search yielded 390 papers. Papers that discussed cell sheets, addressed therapies not specific to heart failure, or focused primarily on the mechanical properties of extracellular matrix were excluded. Forty-five papers met the criteria and will be presented in the following discussion.

6.2 Small Intestinal Submucosa (SIS)

The clinical use of submucosal tissue derived from the small intestines of pigs, referred to as small intestinal submucosa (SIS), dates back to the 1960s when Matsumoto et al. successfully grafted canine inferior and superior vena cava using various preparations of inverted jejunum [13]. In general, SIS devices are manufactured by harvesting porcine small intestine and mechanically separating the organ into its constituent layers (serosa, muscle layer, submucosa, and mucosa). Once isolated, the submucosa, a layer of connective tissue separating the muscle layer from the mucosa, is decellularized, lyophilized, and processed into multilayered patches or powdered and processed into a hydrogel [10, 14]. The main component of processed SIS extracellular matrix (ECM) is collagen type I, which makes up more than 90% by dry weight, as well as collagen types III, IV, V, and VI, which are present in much small amounts [15]. Glycosaminoglycans such as heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid are present in varying amounts, depending largely on the method used to decellularized the SIS ECM [15]. Additionally, SIS is reported to contain proteoglycans and growth factors including basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF) (Fig. 6.1) [16, 17].

SIS derivatives are now used in a variety of commercially available products: CuffPatch™ (Arthrotek, Warsaw, IN); Surgisis®, Durasis®, and Stratasis® (Cook Biotech, West Lafayette, IN); Restore® (DePuy, Warsaw, IN); and CorMatrix® and CorMatrix CanGaroo® (Aziyo Biologics, Roswell, GA). In general, patches made from SIS have a thickness ranging from 0.05 mm to 0.22 mm, with pores varying in diameter from 20 μm to 30 μm [18]. Clinical applications of these SIS-based technologies include the repair of blood vessels [19–22], skin [23], rotator cuffs [24], urinary tract [25–29], body wall [30], intestine [31], pericardium [32], and diaphragm [33, 34]. More recently, SIS has been investigated in cardiac applications such as the repair of right ventricular free wall defects [35], atrial septal defects [14], and ischemic injury [10, 36–38]. The use of SIS therapeutics to regenerate infarcted myocardium will be the focus for the remainder of this section.

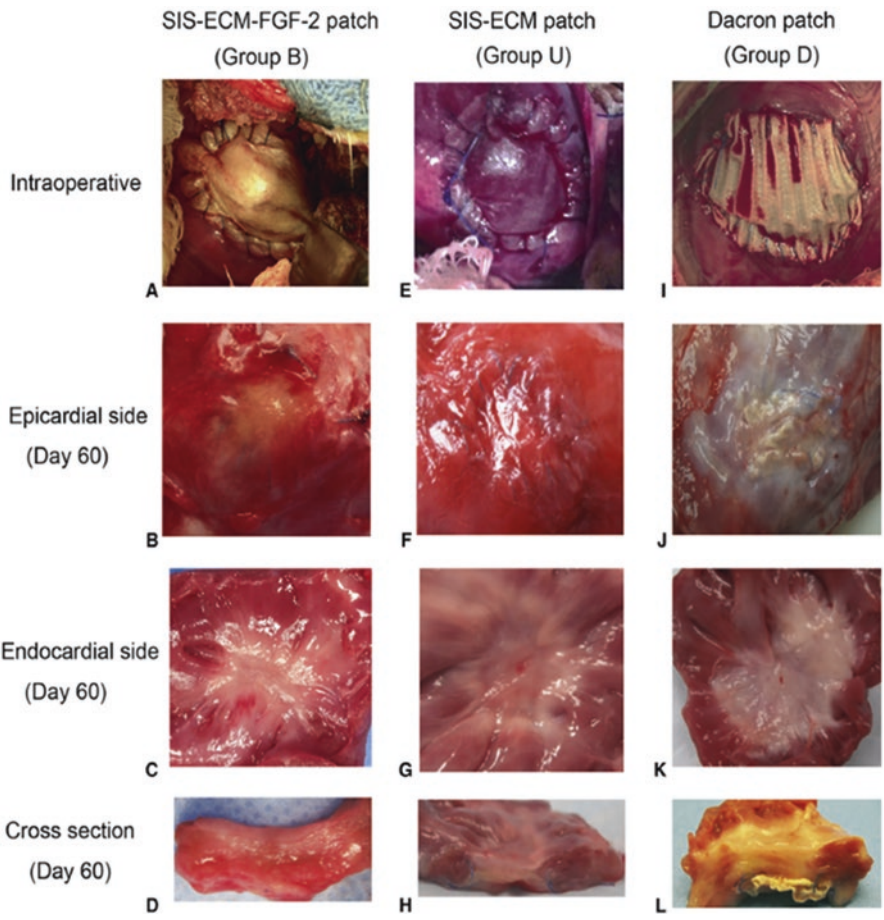

Fig. 6.1 SIS ECM with and without FGF-2 impregnation was tested against a Dacron patch for repair of a full-thickness myocardial defect repair. SIS-ECM-FGF-2 was showed that the graft was repopulated with host cells through the entire patch where SIS ECM and Dacron patches were not. Macro inspection. Intraoperative views (A, E, I), epicardial views at 60 days (B, F, J), endocardial views at 60 days (C, G, K), and cross-sectional views at 60 days (D, H, L) are demonstrated: A-D, SIS-ECM-FGF-2 patch. E-H, SIS ECM patch. I-L, Dacron patch. SIS-ECM-FGF-2, small intestine submucosa-extracellular matrix-basic fibroblast growth factor; SIS ECM, small intestine submucosa-extracellular matrix. (Figure by Tanaka et al. and reproduced with publisher permission)

In 2013, Toeg et al. used a mouse model of heart failure to test the therapeutic potential of an injectable SIS ECM to attenuate maladaptive remodeling following a myocardial infarction (MI) [10]. Following ligation of the left anterior descending (LAD) artery, mice were divided into four treatments and received injections of either phosphate-buffered saline (PBS), circulating angiogenic cells (CACs) which were harvested from bone marrow, SIS ECM, or SIS ECM with CACs [10]. After 3 weeks, mice that received injections of either the SIS ECM or SIS ECM with CACs demonstrated an

improvement in left ventricular ejection fraction, though the difference failed to achieve statistical significance ($P = 0.08$ for SIS ECM only and $P = 0.06$ for SIS ECM + CACs) [10]. A statistically significant ($P < 0.001$) improvement in the thickness of the posterior left ventricular wall was found in mice that received either the SIS ECM or SIS ECM + CACs injections [10]. Other notable findings that achieved statistical significance were reduced scar size after 21 days, increased arteriolar density in the peri-infarcted regions, and increased expression of β-catenin and GATA4, markers of cardiomyocytes, in the peri-infarcted regions [10]. It worth noting that the SIS ECM treatment was as efficacious as the SIS ECM with CACs.

The efficacy of SIS ECM, delivered as a patch, was evaluated in 2014 by Mewhort et al. [36] Using a rat model of heart failure, this study compared the application of either (i) a SIS ECM patch enhanced with bFGF, (ii) a SIS ECM patch without bFGF enhancement, or (iii) a sham treatment consisting of sutures onto the epicardial surface 3 weeks after inducing myocardial infarction via LAD artery ligation [36]. Sixteen weeks after treatment, echocardiography and pressure-volume analysis were used to demonstrate that treatment with a SIS ECM patch enhanced with bFGF resulted in improved ejection fraction ($P < 0.001$) [36]. Both the SIS ECM patch and the SIS ECM patch enhanced with bFGF resulted in reduced left ventricular end-diastolic volumes ($P < 0.01$) and improved left ventricular contractility ($P < 0.05$) [36]. The authors noted that immunohistochemistry failed to demonstrate an inflammatory reaction to the SIS ECM patches but showed that host cells had migrated into the patch, suggesting that new tissue was being formed in the infarcted region [36].

In 2016, Mewhort et al. conducted a similar study, this time using a porcine model of myocardial infarction [38]. After ligating the diagonal branches of the LAD for 75 min, pigs received either a SIS ECM patch sutured directly to the epicardium overlying the infarcted area or sutures were placed around the infarcted region in a sham treatment [38]. After 6 weeks, the SIS ECM treatment resulted in few intrathoracic adhesions ($P = 0.003$), increased ventricular wall thickness ($P = 0.021$), and increased density of capillary vessels within the infarcted region ($P < 0.001$) [38]. Significantly, there was no difference between the sham and SIS ECM treatments in terms of passive pressure distention of the left ventricle, demonstrating that the SIS ECM patch did not reduce compliance [38].

6.3 Urinary Bladder Matrix

Commercially available decellularized urinary bladder matrix (UBM) has been used in a number of clinical applications including reinforcement of cardiac [39–41], urological [42], abdominal wall [43], gastrointestinal tissues, [44] wounds [45–48], diabetic ulcers [49, 50], healing applications as well as management of burns [48], hernia repair [43, 51, 52], tissue reconstruction including breast [53], and skeletal muscle [54]. UBM is most frequently manufactured from porcine bladders but can also be harvested from the human bladder. To manufacture UBM,

bladders are excised and decontaminated with 10% iodine solution. The bladder is then mechanically delaminated, separating the detrusor muscle from the lamina propria and urothelium. The lamina propria is then further decellularized using established methods. Decellularized UBM has been reported to be composed of 98% collagen(s), 1% extracellular matrix glycoproteins, and 1% proteoglycans [46]. UBM can be manufactured in sheets for covering surfaces, particulates for suspension injection and as a hydrogel [55] [56, 57], and are a promising reagent for cardiovascular applications.

In 2003, Badylak et al. demonstrated the preclinical efficacy of UBM patches in the repair of heart defects using several animal models [35]. A full-thickness circular defect that measured 2.5 cm in diameter was created in the anterior wall of the right ventricle in both pigs and dogs. The defect was repaired using an acellular sheet of UBM. At 6 weeks, they were replaced by a mixture of vascularized connective tissue, cartilage, adipose connective tissue, and myocardial tissue [35]. Importantly, this remodeled tissue demonstrated spontaneous contractions with a peak contractile force equal to 70% of the adjacent native myocardium [35]. A similar study was conducted in 2005 by Kochupura et al., this time comparing UBM patches to Dacron patches for the repair of heart defects [41]. Again, circular defects were created in the anterior wall of the right ventricle of dogs and repaired with either a UBM patch or a Dacron patch. After 8 weeks, the UBM treatment was associated with significantly greater regional systolic and diastolic function [41].

In 2005, Robinson et al. compared the efficacy of a UBM-derived patch with a standard expanded polytetrafluoroethylene (ePTFE) patch in a porcine myocardial infarction (MI) model [58]. Briefly, a circular defect, 2–3 cm in diameter, was made in the infarcted region 6–8 weeks after ischemic injury (NCBI 40). Either a UBM or ePTFE patch was sutured over the defect on the endocardial surface, and either a UBM or latex patch was sutured over the epicardial surface [58]. After 3 months, the UBM patches had been replaced by a vascularized, collagen-rich tissue with numerous myofibroblasts. After 3 months, the ePTFE patch was found to have stimulated an intense foreign body reaction, necrosis, and calcification [58].

In 2013, Remlinger et al. compared the efficacy of a UBM patch to a patch made from decellularized cardiac tissue for closure of a defect in the right ventricle of rats [39]. Both patches preserved cardiac function, promoted cellular infiltration, and developed a continuous lining of endothelium on their endocardial surface [39]. Neither patch resulted in fibrotic encapsulation [39]. The authors concluded, however, that UBM may be a better material for cardiac applications for the following reasons: cellular infiltration occurred faster in the UBM patch than the decellularized cardiac tissue patch; the UBM demonstrated the presence of cardiomyocytes with appropriate sarcomeric development, the decellularized cardiac tissue patch did not; and, by the end of the study, the UBM patch had been completely replaced by host tissue, whereas the decellularized cardiac patch persisted and had been incorporated into the host tissue [39].

6.4 Pericardium

Pericardial tissue has also been investigated as a potential source of ECM for cardiac tissue engineering. The pericardium is a fibrous sac, composed primarily of collagen and elastin, that encloses the heart and is not essential for life [9]. Although FDA approval has been granted for the use of bovine pericardium in artificial valves [59] and dural grafts [60], a distinct advantage of using pericardium-derived ECM is that the donor tissue could be autologous, that is, a piece of pericardium could be harvested from a patient, processed, and then used in the same patient, thus avoiding problems associated with using xenogeneic or allogenic tissues [9].

In a 2010 study, Seif-Naraghi et al. demonstrated that both porcine and human pericardial tissue could be processed into an injectable matrix that gels shortly after injection into the myocardium [9]. The pericardial tissues were decellularized, characterized by electrophoresis and mass spectrometry, and then tested in mice. The composition of human pericardial ECM differed from porcine pericardium, but statistically equivalent results were obtained from in vivo experiments that looked at arteriole formation after 2 weeks [9]. In vitro migration assays, however, found that the porcine pericardial matrix was a significantly stronger chemoattractant for rat epicardial cells, human coronary artery endothelial cells, and rat aortic smooth muscle cells [9]. These two findings suggest that the porous structure of the ECM may mediate the observed in vivo effects of the pericardial matrix injections [9]. A follow-up study by Seif-Naraghi et al. found that variability in the composition of pericardial ECM between individual patients did not prevent successful processing of the ECM into an injectable scaffold [59].

A 2016 study conducted by Gálvez-Montón et al. used a pig model of MI to assess a patch made from decellularized human pericardium seeded with porcine adipose tissue-derived progenitor cells (pATPCs) [61]. Following ligation of the first marginal branch of the circumflex artery, pigs received either the pericardial patch seeded with pATPCs or acellular pericardial patches [61]. Both patches were fitted with an electrical impedance spectroscopy monitoring system that allowed for noninvasive assessment of scar formation, while cardiac function was measured using magnetic resonance imaging (MRI) [61]. After 1 month, the pigs that received the patch with pATPCs had larger left ventricular ejection fractions (7.5% ± 4.9% vs. 1.4% ± 3.7%; $P = 0.038$), larger stroke volumes (11.5 mL ± 5.9 mL vs. 3.0 ± 4.5 mL; $P = 0.019$), and smaller infarct size (3.4% ± 0.6% vs. 6.5% ± 1%; $P = 0.015$). Electrical impedance data showed statistically significant differences in both impendence magnitude ratio ($P = 0.002$) and phase angle slope ($P = 0.004$), which correlated with altered scar formation and inflammation between the two treatments [61]. When compared to the acellular patch, histological examination showed less CD25+ lymphocytes in the pATPCs treatment ($P = 0.006$) and a lower collagen I/III ratio (0.49 ± 0.06 vs. 1.66 ± 0.5; $P = 0.019$), demonstrating less inflammation in the pigs treated with the pATPC-seeded pericardial patch [61]. There was no difference between the patches in terms of blood vessel formation and density [61].

6.5 Myocardium

As highlighted in the previous sections, a variety of tissue types have been decellularized and processed into patches or injectable hydrogels and tested for their efficacy in repairing the damage caused by ischemic injury to the myocardium. It has been postulated, however, that because the composition and spatial organization of ECM components are tissue-specific, using ECM from non-cardiac tissues may fail to provide adequate or appropriate signaling to cells in the myocardium [62–64]. Characteristically, mammalian myocardial ECM is composed primarily of collagen type I (~80%) with lesser amounts of collagen type III (~10%), collagen type V (<5%), and small amounts of fibronectin, laminin, and elastin. These proteins are arranged into distinct tissue structures—i.e., endomysial, perimysial, and epimysial layers—that, in part, confer their biological function [65, 66]. Thus, in order to provide cardiac-specific biological and mechanical signals, some investigators have used decellularized myocardial tissue as the source of ECM for potential regenerative therapies.

In 2009, Singelyn et al. demonstrated that tissue harvested from the ventricles of pig hearts could be decellularized with detergents, lyophilized, and solubilized with pepsin in order to produce an injectable matrix that forms a fibrous gel at 37° C [64]. In vitro assays found that rat cardiomyocytes could be cultured on the matrix and that the matrix possessed chemoattractant properties that caused both human coronary artery endothelial cells and rat aortic smooth muscle cells to migrate toward the matrix [64]. In vivo studies determined that it took as little as 30 min for the matrix to form a gel after injection into the left ventricle free wall of rats [64]. Histological examination at 4 h and 11 days postinjection did not show signs of foreign body reactions, tissue necrosis, fibrosis, or chronic inflammation (Fig. 6.2) [64].

In 2013, Seif-Naraghi et al. published a study looking at the efficacy, biocompatibility, and safety of using an injectable matrix derived from decellularized porcine myocardium [67]. A pig model was used to assess potential therapeutic effects of the matrix: 2 weeks following an MI, the pigs received either an injection of the matrix, saline, or no injection. After 3 months, the group treated with the myocardial matrix had larger ejection fractions (73.7% ± 5.3% vs. 43.3% ± 7.6%; $P < 0.01$), smaller end-systolic volumes (8.1 mL ± 1.6 mL vs. 31.9 mL ± 5.0 mL; $P < 0.01$), and smaller end-diastolic volumes (33.5 mL ± 6.2 mL vs. 55.8 mL ± 2.8 mL; $P < 0.05$) [67]. However, the group treated with the myocardial matrix also had lower (i.e., better) global wall motion index scores and smaller fractional increases in infarct size compared to the control group [67]. To demonstrate biocompatibility, rats were given an intramyocardial injection of either the decellularized porcine myocardial matrix, a non-decellularized myocardial matrix, or saline. Histological examination of rat hearts took place at regular intervals from 1 to 112 days and showed that the decellularized hydrogel was completely degraded by day 28 and that inflammation had resolved by day 56 [67]. Tissue samples from the groups treated with saline and decellularized hydrogel both exhibited populations of fibroblasts, myofibroblasts, and cardiomyocytes at the site of injection, indicating a reparative response was taking place [67]. To demonstrate safety, the decellularized

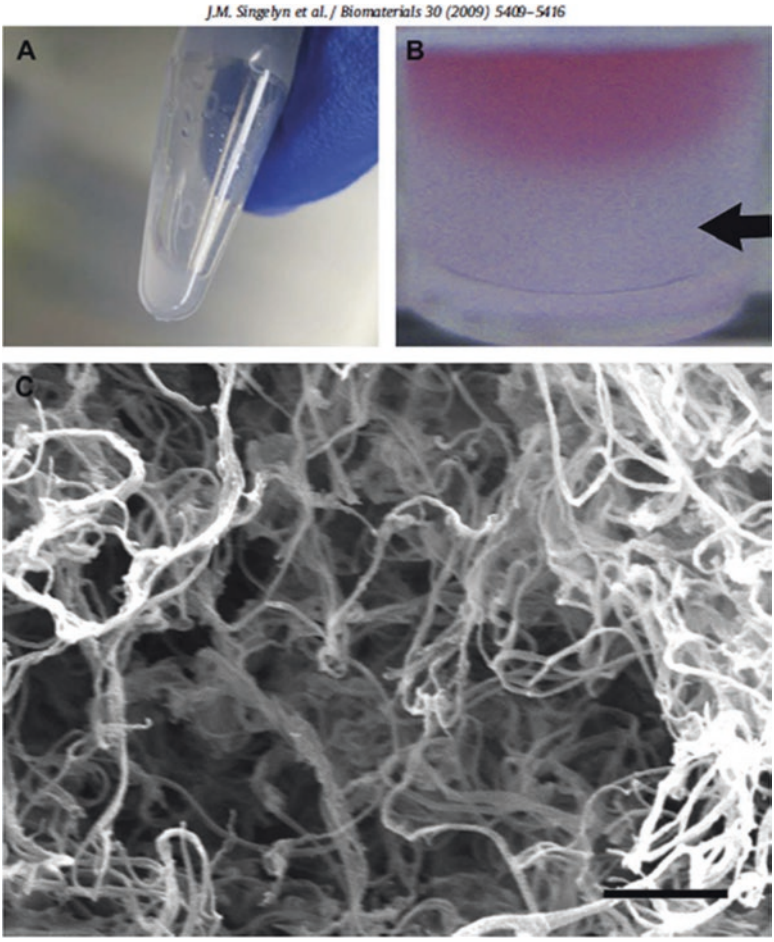

Fig. 6.2 Myocardial matrix gelation and characterization. (**a**) At room temperature, the solubilized matrix was a liquid. (**b**) At 37° C, the myocardial matrix self-assembled into a hydrogel, as indicated by arrow. Pink media is shown on top as a contrast to the solidified gel. (**c**) Scanning electron micrograph of a cross section of the myocardial matrix gel with nanofibers approximately 40–100 nm. Scale bar is 1 μm. (Figure by Singelyn et al. and reproduced with publisher permission)

hydrogel was injected directly into the lumen of the left ventricle in rats. Histological examination of various organs and tissues after 6 h failed to demonstrate signs of embolization or ischemia [67]. Finally, this study looked at the effects of mixing the myocardial matrix with human blood and platelet-rich plasma and found no effects on clotting times or platelet activation [67].

To identify potential mechanisms that underlie the observed therapeutic effects of myocardial matrix gels, Wassenaar et al. used a rat model of MI and transcriptome analysis to find cellular processes that are altered by matrix injections [68]. One week after ischemic injury, rats received injections of either saline or decellularized

porcine myocardial matrix. Whole transcriptome analysis of the infarcted region and border zone was then performed at 3 days and 1 week postinjection, with quantitative polymerase chain reaction and immunohistochemistry used to confirm findings [68]. At 3 days, the myocardial matrix injection was associated with downregulated apoptosis, upregulated blood vessel formation, and increased cell movement when compared to the saline treatment [68]. At 1 week, the myocardial matrix injection was associated with decreased apoptosis, decreased hypertrophy, and increased transcription of genes involved with fatty acid metabolism and mitochondrial biogenesis [68]. This study included an assessment of cardiac function at 6 weeks post-MI: hearts injected with the matrix had smaller percent changes in ejection fraction ($P = 0.028$) and end-diastolic volume ($P = 0.004$), higher peak left ventricular systolic pressure ($P = 0.002$), and better myocardial relaxation ($P = 0.003$) and contractility ($P = 0.002$) [68].

Another potential application of decellularized myocardium was demonstrated by Wang et al. in a study published in 2016. Instead of processing myocardial tissue into an injectable gel, whole rat hearts were decellularized and then cut into sheets with intact endocardial and epicardial surfaces [69]. The endocardial surface of each sheet was then seeded with human cardiomyocytes and CD90+ nonmyocytes, both of which were derived from induced pluripotent stem cells (iPSCs) [69]. Using a rat model of MI, the myocardial matrix sheets with cells were compared with myocardial matrix sheets that did not contain cells and with a control group that didn't receive treatment. Immediately following LAD ligation, the patches were applied to the infarcted region, with the cells facing the heart, using a fibrin-based glue [69]. After 2 weeks, rats treated with the cell-containing patch had significantly larger left ventricular ejection fractions, higher fractional shortening, and smaller left ventricular inner diameters in systole and diastole when compared to the acellular patch and no treatment groups [69]. Histological examination after 4 weeks demonstrated that the infract size was significantly smaller and vascular density significantly higher in the hearts treated with cell-seeded patch when compared to the other treatments (Fig. 6.3) [69].

A similar study published in 2016 by Sarig et al. looked at the efficacy of an acellular patch derived from porcine myocardium in both acute and chronic models of MI in rats [70]. In the acute model, the patches were either sutured or glued onto the infarcted surface immediately following ligation of the LAD. In the chronic model, the patches were applied 30 days post-MI, after scar tissue had formed. In both models, 30 days posttreatment, the patch reduced infarct size, decreased left ventricular internal diameter, and increased cardiac function as measured by fractional area change, ejection fraction, and fractional shortening [70]. Pathological evaluation of patches demonstrated a time-dependent increase in cells that stained positive for GATA4, c-kit, MYLC, and/or TrpI, suggesting that the patches were recruiting cardiac progenitor and muscle cells [70].

In addition to delivery modality (e.g., patch or injection) and cell content (e.g., used as a stand-alone therapy or in conjunction with therapeutic cells), the animal source of myocardial matrix appears to influence the therapeutic effects of myocardial matrix-based devices. In a study by Chen et al. published in 2016, myocardial tissue

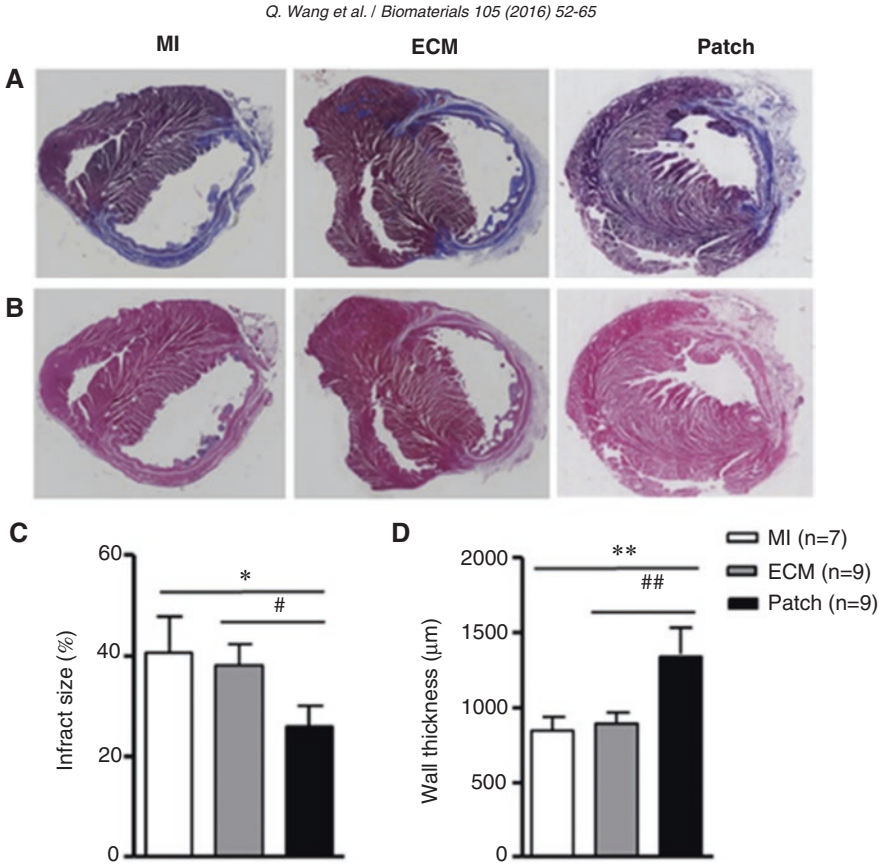

Fig. 6.3 Transplantation of the engineered human cardiac patches alleviated left ventricular remodeling in rat MI model. Representative Masson's trichrome staining (**a**) and H&E staining (**b**) graphs of dissected hearts in the MI, ECM, and patch group 4 weeks after MI and treatment. (**c**) Animals treated with the engineered human cardiac patches showed a significantly reduced infarct area at 4 weeks after MI (MI: 40.58%, $n = 7$; MI + ECM: 38.09%, $n = 9$; MI + Patch: 25.87%, $n = 9$). (**d**) Quantification of left ventricular wall thickness. Data represents mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the MI group; $^{\#\#}P < 0.001$, $^{\#}P < 0.05$ versus the ECM group. (Figure by Wang et al. reproduced with publisher permission)

was harvested from zebrafish, an animal whose heart possesses a high regenerative capacity [4]. Using a mouse model of MI, the efficacy of an injectable gel made from decellularized mouse myocardium was compared against an injectable gel made from either decellularized myocardium from injured zebrafish hearts (these hearts underwent ventricular resection and were thus in the process of regenerating) or non-injured zebrafish hearts; a saline injection was used as a control [4]. The mice received one of the injections immediately following an induced MI and were followed for 42 days. The matrix derived from injured zebrafish hearts demonstrated the greatest therapeutic efficacy as measured by left ventricular fractional area change and ejection fraction

($P < 0.001$); the matrix derived from non-injured zebrafish hearts was more efficacious than the matrix derived from mouse myocardium ($P < 0.005$); and the mouse myocardium matrix was more effective than the saline control ($P < 0.05$) [4]. Both zebrafish-derived matrices were more effective than the mouse-derived matrix in reducing left ventricle end-diastolic area and end-systolic area ($P < 0.005$) [4]. Pathological examination demonstrated that the zebrafish myocardial matrix, which contains neuregulin-1, increased proliferation of cardiac precursor cells and augmented the proliferation of cardiomyocytes, most likely through neuregulin-1-mediated activation of the ErbB2 signaling pathway [4].

6.6 Conclusion and Future Directions

As the field of tissue engineering and regenerative medicine continues to mature so does the understanding of the role of extracellular matrix in healing and regeneration. It has become clear that extracellular matrix is a key regulator of regeneration by modulating the immune system, directing stem/progenitor cell homing and differentiation, and modulating fibrosis. The use of extracellular matrix as a standalone therapy in cardiac regeneration is currently in clinical trials (NCT02305602), and one would expect that more will be soon to follow. In addition, exiting new and evolving technologies continue to grow the available ECM bioscaffolds for cardiac regeneration applications. These exciting technologies include cell-derived extracellular matrix scaffolds [71], three-dimensional bioprinting using decellularized ECM [72], and even using plant material such as the spinach leaf as a perfusable scaffold for tissue engineering [73]. While this review has largely focused on naturally sourced biomaterials for cardiac repair, there are a host of synthetic materials, many of which can be further functionalized with cytokines and growth factors to direct healing in the damaged myocardium. While tissue engineering and regenerative medicine have made great progress in the past decade, much work remains to deliver meaningful and durable solutions to repair damaged hearts. It seems highly likely that ECM, in some form, will be required to make regenerative therapies for the heart and other tissues a reality.

References

1. Writing Group M, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, DK MG, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB, American Heart Association Statistics C and Stroke Statistics S. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation. 2016;133:e38–360.

2. Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC, Ikonomidis JS, Khavjou O, Konstam MA, Maddox TM, Nichol G, Pham M, Pina IL, Trogdon JG, American Heart Association Advocacy Coordinating C, Council on Arteriosclerosis T, Vascular B, Council on Cardiovascular R, Intervention, Council on Clinical C, Council on E, Prevention and Stroke C. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. Circ Heart Fail. 2013;6:606–19.
3. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation. 2000;101:2981–8.
4. Chen WC, Wang Z, Missinato MA, Park DW, Long DW, Liu HJ, Zeng X, Yates NA, Kim K, Wang Y. Decellularized zebrafish cardiac extracellular matrix induces mammalian heart regeneration. Sci Adv. 2016;2:e1600844.
5. Nguyen PK, Rhee JW, Wu JC. Adult stem cell therapy and heart failure, 2000 to 2016: a systematic review. JAMA Cardiol. 2016;1:831–41.
6. Lader J, Stachel M, Bu L. Cardiac stem cells for myocardial regeneration: promising but not ready for prime time. Curr Opin Biotechnol. 2017;47:30–5.
7. Rao Pattabhi S, Martinez JS, Keller TC 3rd. Decellularized ECM effects on human mesenchymal stem cell stemness and differentiation. Differentiation. 2014;88:131–43.
8. Chen HY, Pan L, Yang HL, Xia P, Yu WC, Tang WQ, Zhang YX, Chen SF, Xue YZ, Wang LX. Integrin alpha5beta1 suppresses rBMSCs anoikis and promotes nitric oxide production. Biomed Pharmacother. 2018;99:1–8.
9. Seif-Naraghi SB, Salvatore MA, Schup-Magoffin PJ, Hu DP, Christman KL. Design and characterization of an injectable pericardial matrix gel: a potentially autologous scaffold for cardiac tissue engineering. Tissue Eng Part A. 2010;16:2017–27.
10. Toeg HD, Tiwari-Pandey R, Seymour R, Ahmadi A, Crowe S, Vulesevic B, Suuronen EJ, Ruel M. Injectable small intestine submucosal extracellular matrix in an acute myocardial infarction model. Ann Thorac Surg. 2013;96:1686–94. discussion 1694
11. Higuchi S, Lin Q, Wang J, Lim TK, Joshi SB, Anand GS, Chung MC, Sheetz MP, Fujita H. Heart extracellular matrix supports cardiomyocyte differentiation of mouse embryonic stem cells. J Biosci Bioeng. 2013;115:320–5.
12. Sottile J. Regulation of angiogenesis by extracellular matrix. Biochim Biophys Acta. 2004;1654:13–22.
13. Matsumoto T, Holmes RH, Burdick CO, Metzger JF, Heisterkamp CA 3rd, O'Connell TJ Jr. A study of inverted intestinal graft in the major veins. Angiology. 1966;17:842–50.
14. Rosen M, Roselli EE, Faber C, Ratliff NB, Ponsky JL, Smedira NG. Small intestinal submucosa intracardiac patch: an experimental study. Surg Innov. 2005;12:227–31.
15. Badylak SF, DO F, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater. 2009;5:1–13.
16. McDevitt CA, Wildey GM, Cutrone RM. Transforming growth factor-beta1 in a sterilized tissue derived from the pig small intestine submucosa. J Biomed Mater Res A. 2003;67:637–40.
17. Voytik-Harbin SL, Brightman AO, Kraine MR, Waisner B, Badylak SF. Identification of extractable growth factors from small intestinal submucosa. J Cell Biochem. 1997;67:478–91.
18. Shi L, Ronfard V. Biochemical and biomechanical characterization of porcine small intestinal submucosa (SIS): a mini review. Int J Burns Trauma. 2013;3:173–9.
19. Lantz GC, Badylak SF, Hiles MC, Coffey AC, Geddes LA, Kokini K, Sandusky GE, Morff RJ. Small intestinal submucosa as a vascular graft: a review. J Investig Surg. 1993;6:297–310.
20. Lantz GC, Badylak SF, Coffey AC, Geddes LA, Sandusky GE. Small intestinal submucosa as a superior vena cava graft in the dog. J Surg Res. 1992;53:175–81.
21. Robotin-Johnson MC, Swanson PE, Johnson DC, Schuessler RB, Cox JL. An experimental model of small intestinal submucosa as a growing vascular graft. J Thorac Cardiovasc Surg. 1998;116:805–11.
22. Sandusky GE Jr, Badylak SF, Morff RJ, Johnson WD, Lantz G. Histologic findings after in vivo placement of small intestine submucosal vascular grafts and saphenous vein grafts in the carotid artery in dogs. Am J Pathol. 1992;140:317–24.

23. MacLeod TM, Sarathchandra P, Williams G, Sanders R, Green CJ. Evaluation of a porcine origin acellular dermal matrix and small intestinal submucosa as dermal replacements in preventing secondary skin graft contraction. Burns. 2004;30:431–7.
24. Phipatanakul WP, Petersen SA. Porcine small intestine submucosa xenograft augmentation in repair of massive rotator cuff tears. Am J Orthop (Belle Mead NJ). 2009;38:572–5.
25. Alpert SA, Cheng EY, Kaplan WE, Snodgrass WT, Wilcox DT, Kropp BP. Bladder neck fistula after the complete primary repair of exstrophy: a multi-institutional experience. J Urol. 2005;174:1687–9. discussion 1689-90.
26. Jones JS, Vasavada SP, Abdelmalak JB, Liou L, Ahmed ES, Zippe CD, Rackley RR. Sling may hasten return of continence after radical prostatectomy. Urology. 2005;65:1163–7.
27. Jones JS, Rackley RR, Berglund R, Abdelmalak JB, DeOrco G, Vasavada SP. Porcine small intestinal submucosa as a percutaneous mid-urethral sling: 2-year results. BJU Int. 2005;96:103–6.
28. Misseri R, Cain MP, Casale AJ, Kaefer M, Meldrum KK, Rink RC. Small intestinal submucosa bladder neck slings for incontinence associated with neuropathic bladder. J Urol. 2005;174:1680–2. discussion 1682.
29. Mantovani F, Trinchieri A, Castelnuovo C, Romano AL, Pisani E. Reconstructive urethroplasty using porcine acellular matrix. Eur Urol. 2003;44:600–2.
30. Pu LL and Plastic Surgery Educational Foundation DC. Small intestinal submucosa (Surgisis) as a bioactive prosthetic material for repair of abdominal wall fascial defect. Plast Reconstr Surg. 2005;115:2127–31.
31. Sardeli C, SM A, Bek KM. Use of porcine small intestinal submucosa in the surgical treatment of recurrent rectocele in a patient with Ehlers-Danlos syndrome type III. Int Urogynecol J Pelvic Floor Dysfunct. 2005;16:504–5.
32. Stelly M, Stelly TC. Histology of CorMatrix bioscaffold 5 years after pericardial closure. Ann Thorac Surg. 2013;96:e127–9.
33. Oelschlager BK, Barreca M, Chang L, Pellegrini CA. The use of small intestine submucosa in the repair of paraesophageal hernias: initial observations of a new technique. Am J Surg. 2003;186:4–8.
34. Strange PS. Small intestinal submucosa for laparoscopic repair of large paraesophageal hiatal hernias: a preliminary report. Surg Technol Int. 2003;11:141–3.
35. Badylak S, Obermiller J, Geddes L, Matheny R. Extracellular matrix for myocardial repair. Heart Surg Forum. 2003;6:E20–6.
36. Mewhort HE, Turnbull JD, Meijndert HC, Ngu JM, Fedak PW. Epicardial infarct repair with basic fibroblast growth factor-enhanced CorMatrix-ECM biomaterial attenuates postischemic cardiac remodeling. J Thorac Cardiovasc Surg. 2014;147:1650–9.
37. Tanaka A, Kawaji K, Patel AR, Tabata Y, Burke MC, Gupta MP, Ota T. In situ constructive myocardial remodeling of extracellular matrix patch enhanced with controlled growth factor release. J Thorac Cardiovasc Surg. 2015;150:1280–90. e2
38. Mewhort HE, Turnbull JD, Satriano A, Chow K, Flewitt JA, Andrei AC, Guzzardi DG, Svystonyuk DA, White JA, Fedak PW. Epicardial infarct repair with bioinductive extracellular matrix promotes vasculogenesis and myocardial recovery. J Heart Lung Transplant. 2016;35:661–70.
39. Remlinger NT, Gilbert TW, Yoshida M, Guest BN, Hashizume R, Weaver ML, Wagner WR, Brown BN, Tobita K, Wearden PD. Urinary bladder matrix promotes site appropriate tissue formation following right ventricle outflow tract repair. Organogenesis. 2013;9:149–60.
40. Wainwright JM, Hashizume R, Fujimoto KL, Remlinger NT, Pesyna C, Wagner WR, Tobita K, Gilbert TW, Badylak SF. Right ventricular outflow tract repair with a cardiac biologic scaffold. Cells Tissues Organs. 2012;195:159–70.
41. Kochupura PV, Azeloglu EU, Kelly DJ, Doronin SV, Badylak SF, Krukenkamp IB, IS C, Gaudette GR. Tissue-engineered myocardial patch derived from extracellular matrix provides regional mechanical function. Circulation. 2005;112:I144–9.

42. Davis NF, Cunnane EM, O'Brien FJ, Mulvihill JJ, Walsh MT. Tissue engineered extracellular matrices (ECMs) in urology: evolution and future directions. Surgeon. 2018;16(1):55–65.
43. Eberli D, Atala A, Yoo JJ. One and four layer acellular bladder matrix for fascial tissue reconstruction. J Mater Sci Mater Med. 2011;22:741–51.
44. Afaneh C, Abelson J, Schattner M, Janjigian YY, Ilson D, Yoon SS, Strong VE. Esophageal reinforcement with an extracellular scaffold during total gastrectomy for gastric cancer. Ann Surg Oncol. 2015;22:1252–7.
45. Eweida AM, Marei MK. Naturally occurring extracellular matrix scaffolds for dermal regeneration: do they really need cells? Biomed Res Int. 2015;2015:839694.
46. Sadtler K, Sommerfeld SD, Wolf MT, Wang X, Majumdar S, Chung L, Kelkar DS, Pandey A, Elisseeff JH. Proteomic composition and immunomodulatory properties of urinary bladder matrix scaffolds in homeostasis and injury. Semin Immunol. 2017;29:14–23.
47. Rommer EA, Peric M, Wong A. Urinary bladder matrix for the treatment of recalcitrant nonhealing radiation wounds. Adv Skin Wound Care. 2013;26:450–5.
48. Lanteri Parcells A, Abernathie B, Datiashvili R. The use of urinary bladder matrix in the treatment of complicated open wounds. Wounds. 2014;26:189–96.
49. Alvarez OM, Smith T, Gilbert TW, Onumah NJ, Wendelken ME, Parker R, Markowitz L. Diabetic foot ulcers treated with porcine urinary bladder extracellular matrix and total contact cast: interim analysis of a randomized, controlled trial. Wounds. 2017. [Epub ahead of Print].
50. Frykberg RG, Cazzell SM, Arroyo-Rivera J, Tallis A, Reyzelman AM, Saba F, Warren L, Stouch BC, Gilbert TW. Evaluation of tissue engineering products for the management of neuropathic diabetic foot ulcers: an interim analysis. J Wound Care. 2016;25(Suppl 7):S18–25.
51. Riganti JM, Ciotola F, Amenabar A, Craiem D, Graf S, Badaloni A, Gilbert TW, Nieponice A. Urinary bladder matrix scaffolds strengthen esophageal hiatus repair. J Surg Res. 2016;204:344–50.
52. Sasse KC, Warner DL, Ackerman E, Brandt J. Hiatal hernia repair with novel biological graft reinforcement. JSLS. 2016;20:1–5.
53. Salzberg CA. Nonexpansive immediate breast reconstruction using human acellular tissue matrix graft (AlloDerm). Ann Plast Surg. 2006;57:1–5.
54. Teodori L, Costa A, Marzio R, Perniconi B, Coletti D, Adamo S, Gupta B, Tarnok A. Native extracellular matrix: a new scaffolding platform for repair of damaged muscle. Front Physiol. 2014;5:218.
55. Zhang L, Zhang F, Weng Z, Brown BN, Yan H, Ma XM, Vosler PS, Badylak SF, Dixon CE, Cui XT, Chen J. Effect of an inductive hydrogel composed of urinary bladder matrix upon functional recovery following traumatic brain injury. Tissue Eng Part A. 2013;19:1909–18.
56. Faulk DM, Londono R, Wolf MT, Ranallo CA, Carruthers CA, Wildemann JD, Dearth CL, Badylak SF. ECM hydrogel coating mitigates the chronic inflammatory response to polypropylene mesh. Biomaterials. 2014;35:8585–95.
57. Freytes DO, Martin J, Velankar SS, Lee AS, Badylak SF. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. Biomaterials. 2008;29:1630–7.
58. Robinson KA, Li J, Mathison M, Redkar A, Cui J, Chronos NA, Matheny RG, Badylak SF. Extracellular matrix scaffold for cardiac repair. Circulation. 2005;112:I135–43.
59. Bagno A, Aguiari P, Fiorese M, Iop L, Spina M, Gerosa G. Native bovine and porcine pericardia respond to load with additive recruitment of collagen fibers. Artif Organs. 2018;42(5):540–8.
60. Sekhar LN, Mai JC. Dural repair after craniotomy and the use of dural substitutes and dural sealants. World Neurosurg. 2013;79:440–2.
61. Galvez-Monton C, Bragos R, Soler-Botija C, Diaz-Guemes I, Prat-Vidal C, Crisostomo V, Sanchez-Margallo FM, Llucia-Valldeperas A, Bogonez-Franco P, Perea-Gil I, Roura S, Bayes-Genis A. Noninvasive assessment of an engineered bioactive graft in myocardial infarction: impact on cardiac function and scar healing. Stem Cells Transl Med. 2017;6:647–55.
62. Leor J, Amsalem Y, Cohen S. Cells, scaffolds, and molecules for myocardial tissue engineering. Pharmacol Ther. 2005;105:151–63.

63. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol. 2005;23:47–55.
64. Singelyn JM, DeQuach JA, Seif-Naraghi SB, Littlefield RB, Schup-Magoffin PJ, Christman KL. Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. Biomaterials. 2009;30:5409–16.
65. Brower GL, Gardner JD, Forman MF, Murray DB, Voloshenyuk T, Levick SP, Janicki JS. The relationship between myocardial extracellular matrix remodeling and ventricular function. Eur J Cardiothorac Surg. 2006;30:604–10.
66. Bruckner P. Suprastructures of extracellular matrices: paradigms of functions controlled by aggregates rather than molecules. Cell Tissue Res. 2010;339:7–18.
67. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, Kwan OL, Strachan GM, Wong J, Schup-Magoffin PJ, Braden RL, Bartels K, DeQuach JA, Preul M, Kinsey AM, DeMaria AN, Dib N, Christman KL. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med. 2013;5:173ra25.
68. Wassenaar JW, Gaetani R, Garcia JJ, Braden RL, Luo CG, Huang D, DeMaria AN, Omens JH, Christman KL. Evidence for mechanisms underlying the functional benefits of a myocardial matrix hydrogel for post-MI treatment. J Am Coll Cardiol. 2016;67:1074–86.
69. Wang Q, Yang H, Bai A, Jiang W, Li X, Wang X, Mao Y, Lu C, Qian R, Guo F, Ding T, Chen H, Chen S, Zhang J, Liu C, Sun N. Functional engineered human cardiac patches prepared from nature's platform improve heart function after acute myocardial infarction. Biomaterials. 2016;105:52–65.
70. Sarig U, Sarig H, de-Berardinis E, Chaw SY, Nguyen EB, Ramanujam VS, Thang VD, Al-Haddawi M, Liao S, Seliktar D, Kofidis T, Boey FY, Venkatraman SS, Machluf M. Natural myocardial ECM patch drives cardiac progenitor based restoration even after scarring. Acta Biomater. 2016;44:209–20.
71. Schmuck EG, Mulligan JD, Ertel RL, Kouris NA, Ogle BM, Raval AN, Saupe KW. Cardiac fibroblast-derived 3D extracellular matrix seeded with mesenchymal stem cells as a novel device to transfer cells to the ischemic myocardium. Cardiovasc Eng Technol. 2014;5:119–31.
72. Pati F, Cho DW. Bioprinting of 3D tissue models using decellularized extracellular matrix bioink. Methods Mol Biol. 2017;1612:381–90.
73. Gershlak JR, Hernandez S, Fontana G, Perreault LR, Hansen KJ, Larson SA, Binder BY, Dolivo DM, Yang T, Dominko T, Rolle MW, Weathers PJ, Medina-Bolivar F, Cramer CL, Murphy WL, Gaudette GR. Crossing kingdoms: using decellularized plants as perfusable tissue engineering scaffolds. Biomaterials. 2017;125:13–22.

Chapter 7
Cardiac Extracellular Matrix Modification as a Therapeutic Approach

Mikayla L. Hall and Brenda M. Ogle

Abstract The cardiac extracellular matrix (cECM) is comprised of proteins and polysaccharides secreted by cardiac cell types, which provide structural and biochemical support to cardiovascular tissue. The roles of cECM proteins and the associated family of cell surface receptor, integrins, have been explored in vivo via the generation of knockout experimental animal models. However, the complexity of tissues makes it difficult to isolate the effects of individual cECM proteins on a particular cell process or disease state. The desire to further dissect the role of cECM has led to the development of a variety of in vitro model systems, which are now being used not only for basic studies but also for testing drug efficacy and toxicity and for generating therapeutic scaffolds. These systems began with 2D coatings of cECM derived from tissue and have developed to include recombinant ECM proteins, ECM fragments, and ECM mimics. Most recently 3D model systems have emerged, made possible by several developing technologies including, and most notably, 3D bioprinting. This chapter will attempt to track the evolution of our understanding of the relationship between cECM and cell behavior from in vivo model to in vitro control systems. We end the chapter with a summary of how basic studies such as these have informed the use of cECM as a direct therapy.

M. L. Hall
Department of Biomedical Engineering, University of Minnesota – Twin Cities, Minneapolis, MN, USA

Stem Cell Institute, University of Minnesota – Twin Cities, Minneapolis, MN, USA

B. M. Ogle (✉)
Department of Biomedical Engineering, University of Minnesota – Twin Cities, Minneapolis, MN, USA

Stem Cell Institute, University of Minnesota – Twin Cities, Minneapolis, MN, USA

Masonic Cancer Center, University of Minnesota – Twin Cities, Minneapolis, MN, USA

Lillehei Heart Institute, University of Minnesota – Twin Cities, Minneapolis, MN, USA

Institute for Engineering in Medicine, University of Minnesota – Twin Cities, Minneapolis, MN, USA
e-mail: ogle@umn.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_7

Keywords ECM mimetic · Recombinant ECM · Cardiac therapy · Cardiac repair · Bioink · Bioprinting

7.1 In Vivo Models to Evaluate Macroscale Function of cECM

Collagen type I (Col I) is a fibril-forming collagen and the most abundant component of cECM with well-described composition-function relationships. Col I is secreted from cells as procollagen, which contains a triple-helical domain flanked by non-collagenous propeptides. When the propeptides are removed, the triple-helical domain can self-assemble into fibrils which are further stabilized by lysyl oxidase-mediated covalent cross-links between and within the triple helix [1]. The structure of Col I is the primary means by which strength and stiffness are conferred to the heart and vascular tissues. If one tracks the density and organization of Col I in the developing mouse embryo, it increases over time and reaches a peak at day 12.5 in all layers of heart tissue (i.e., epicardium, myocardium, and endocardium) [2]. The amount of Col I relative to other proteins then drops, allowing the heart to become more compliant and thus better able to respond to the contractile apparatus of maturing cardiomyocytes of cardiac muscle [3]. Col I is also important for repair of heart tissue following disease or injury especially when such a state is accompanied by loss of cardiomyocytes. Cardiac fibroblasts are recruited to compensate for lost muscle, and they do so primarily by secreting large amounts of Col I. It is not surprising therefore that mutations in the α2(I) chain impair cardiac development [4] and also restrict cardiac repair following myocardial infarction [5]. In the vasculature, changes in Col I synthesis can cause vascular abnormalities leading to aneurysms and cerebral artery dissections in patients with abnormal procollagen genes and to varicose veins where Col I synthesis is upregulated [6, 7].

Collagen type III (Col III) is another fibrillar collagen of cECM which is prevalent in tissues which require compliance [8]. Col III forms a more compliant network composed of finer fibers than fibers formed from Col I [9]. Col III is necessary for normal cardiovascular development, as it colocalizes with Col I and regulates Col I fibrillogenesis [10]. Knockout mice have shortened lifespans due to rupture of major blood vessels and abnormal formation of Col I fibrils in the blood vessels and heart [11]. Mutations on Col III have also been linked to the vascular phenotype of Ehlers-Danlos syndrome which causes spontaneous arterial dissections, primarily of medium-sized vessels [12]. The ratio of Col I to Col III is often referenced as a means to gauge the relative stiffness of cardiovascular tissue with age or disease. The ratio of Col I to Col III is high in comparably stiff neonatal hearts. The ratio then decreases for some time after birth until stabilization in adulthood, contributing to the relative compliance of the adult heart [3]. This ratio is also affected by blood volume overload as seen during pregnancy where the Col I/Col III ratio decreases to accommodate the movement of increased blood volume. The decreased Col I/Col

III ratio with pregnancy is reversible postpartum [13]. The reversibility of this change indicates that ECM homeostasis in the heart is active throughout the lifespan and that a healthy heart can repeatedly remodel to accommodate major changes in cardiovascular load.

Col IV is the most abundant component of arterial basement membranes of cECM [14] and plays a role in both cardiac tissue stabilization and angiogenesis. In contrast to Col I and Col III, Col IV is a non-fibrillar collagen composed of six distinct α-chains which assemble to form three heterotrimers α1α1α2, α3α4α5, and α5α5α6 [15]. The α1α1α2 and α3α4α5 heterotrimers are essential to cardiac development and angiogenesis and dominate in vascular basement membranes. However, Col IV is not essential to the deposition of proteins in the basement membrane, only to its integrity. Col IV knockout mice develop until embryonic day 9.5, and basement membrane proteins deposit appropriately in the developing embryos, and the embryos showed normal organ development and beating hearts. However, the knockout is lethal in the following days due to structural defects of the basement membrane, primarily in the vasculature. At embryonic days 10.5–11.5, the Col IV-deficient mice developed pericardial bleeding and dilated blood vessels, indicating that although development of the major organs and vasculature appeared normal at early stages, subtle, likely subcellular differences in the organization of the basement membrane are eventually lethal [16].

Fibronectin is a glycoprotein involved in the regulation of cell adhesion and which provides tissue mechanical properties by binding of cells to cECM components. It is composed of two subunits connected by disulfide bonds. The subunits contain repeating modules I, II, and III which comprise the functional domains of the protein [17]. Fibronectin is essential for development and repair of cardiac tissue. Mice that lack the fibronectin gene die in early embryonic development and exhibit a variety of defects of cardiac and vascular tissues [18]. *Mice that lack fibronectin also respond less favorably to cardiac injury due, at least in part, to decreased proliferation and survival of cardiac progenitor cells relative to wild type* [19]. *Cardiac progenitors that do survive in this model tend to localize to fibronectin synthesized prior to induction of the knockout* [19]. In vivo studies have also led to an understanding of fibronectin interactions with integrins. Knockouts of the main fibronectin receptor, integrin α5β1, led to defects similar to those of fibronectin knockouts, whereas other integrins which can bind fibronectin, α3β1, α4β1, α8β1, αvβ1, αvβ3, αvβ6, and αIIbβ3, lead to less severe defects, which indicates independent functions of the fibronectin receptor integrins during development as well as functional redundancy between fibronectin receptors other than α5β1 integrin [20]. While global knockdown of the α5 integrin subunit was embryonic lethal and showed severe cardiovascular defects, it was difficult to discern the exact implications for specific cell types. *Chen et al.* [21] *addressed this difficulty by generating conditional fibronectin knockouts specific to endothelial, smooth muscle, and pharyngeal mesodermal cells. Interestingly, they did not observe defects in vascular morphogenesis, suggesting that either there is redundancy of production of fibronectin by the cardiovascular cell types tested here or that an alternative cell type is a potent synthesizer of fibronectin. Indeed, cardiac fibroblasts are a major producer*

of fibronectin in the heart [22] *and a likely source of the observed production redundancy of fibronectin in cardiac tissues. Further, the study by Chen et al.* [21] *did not examine the effects of conditional knockouts on the development of cardiac muscle, though the results in the vasculature suggest multiple cell types are capable of contributing fibronectin to the cECM to thereby to support cardiac muscle*.

Elastin is the major component in elastic fibers of cECM, which provide elastic recoil to tissues subject to repetitive stress. It is assembled from its soluble, monomeric form, tropoelastin, which consists of alternating hydrophobic and hydrophilic regions. Elastin has been shown to be essential to proper development of the heart and vasculature [23]. Mice lacking the elastin gene die a few days after birth due to aortic stenosis, suggesting elastin in the heart is important not only for chamber and vessel recoil but also for regulating arterial smooth muscle growth. Artery sections from patients with supravalvular aortic stenosis caused by mutations in the elastin gene show an increased number of lamellar units and significant fibrosis, indicating that the role of elastin includes regulation of ECM synthesis in vessel lamellae [24]. Levels of elastin in the developing mouse heart are low early in development and peak at embryonic day 16.5 in the myocardium and endocardium, while elastin levels peak later in the epicardium [2]. The dynamic nature of elastin synthesis with development is likely essential to the changing elasticity of cardiac tissue, which changes to accommodate additional stresses as the heart grows and develops. Heterozygous mice which are deficient in elastin exhibit altered arterial morphology and cardiac hypertrophy [25]. Further studies in these heterozygous mice have shown that cardiovascular developmental defects begin to appear at embryonic day 18. The late appearance of cardiovascular defects in these heterozygous mice indicates that elastin is not essential until late in embryonic development, likely due to an increase in cardiac stresses near birth [23].

Laminins are a family of proteins, which are found in the basement membranes of vasculature and cardiac tissue. They are composed of one α, one β, and one γ chain that form a T-shaped heterotrimer. Laminins are named based on the subunits they contain. For example, laminin 511 contains the α5, β1, and γ1 subunits. These subunits share a common domain structure which contains rod-like domains but differ in their globular domains [26]. The laminins most commonly associated with cardiac and vascular tissue are 111, 411, and 511. Deletions of the α1 chain present in Laminin 111 are lethal at early embryonic stages, which have made discerning its exact functions in development difficult [27]. Laminins 411 and 511 are predominant in vascular basement membranes. Laminin 411 expression is essential to microvessel maturation and affects deposition of other basement membrane components, including Col IV [28]. Laminin 511 expression in vascular basement membranes begins later, about 3–4 weeks after birth, and likely has a role in vessel maturation [29].

In vivo experiments, primarily via the generation of transgenic mice, have established the critical utility of cECM proteins during development and with disease and their significance for the structural integrity of the heart and vasculature. In particular, it is clear that each of the primary ECM proteins of the cardiovascular system is unique in its function in terms of tissue type and developmental time point.

Conditional knockout experiments have added to that with information to further delineate cell types involved in matrix synthesis and have revealed details about the roles of ECM in cardiac development even when global mutations are lethal early in the embryonic period. These studies have led to a greater understanding of the major players in the cECM and have established motive for the use of cECM proteins as therapeutics for cardiovascular disease. However, in vivo studies face the unavoidable limitation that one must study the whole organism, which precludes isolation of the effects of a given ECM component on one organ, cell type, or process which is essential to understanding the mechanisms by which cECM affects cell behavior during development, health, disease, and therapy. Understanding these mechanisms is necessary to the development of more advanced cECM-based therapeutics for cardiac diseases and for improving patient outcomes. This has led to the advent of in vitro systems to study the cECM. The central advantage of in vitro studies is the ability to better distill the interplay of specific cell types with particular matrices to discern signaling pathways elicited and functional behaviors attained. In vitro studies allow uncoupling of competing or compensatory factors, which can mask critical interactions missed in in vivo studies.

7.2 In Vitro Control Systems to Evaluate Cell-cECM Interplay at the Microscale

First, efforts to break down the interplay between specific ECM proteins and individual cardiac cell types involved electrostatic adsorption of ECM proteins to cell culture plates. Plates coated with individual cECM were used to determine the attachment of adult and neonatal cardiomyocytes to several ECM proteins. Adult cardiomyocytes attached most efficiently to laminin and Col IV and bound weakly to fibronectin. Neonatal cardiomyocytes attached well to Cols I, II, III, IV, and V as well as to fibronectin and laminin [30]. The difference in adhesion with age is thought to reflect developmental regulation of extracellular matrix binding via changes in the affinity of cell surface receptor integrins for the ECM and through changes in the expression of Col I receptors during development. Fibronectin, laminin 111, and Col I were compared as substrates for the proliferation, attachment, and differentiation of late endothelial progenitor cells. They were found to attach more strongly to fibronectin and Col I than to laminin and had higher proliferation rates on these surfaces. However, significant differences in endothelial differentiation of the cells on different surfaces were not observed, indicating that either these ECM proteins are not essential for differentiation or that ECM is more important for differentiation that precedes the endothelial progenitor state or that multiple ECM-based signals are required in concert [31]. These simplistic studies of single ECM components led to the study of combinations of cECM proteins in 2D in order to discern how interactions between proteins influence cardiac cell behavior. When human pluripotent stem cells were seeded on combinatorial ECMs composed of Col

IV, Lam 111, and heparan sulfate or Col IV, gelatin, and heparan sulfate, a significant increase in CD31 expression was observed over single-factor ECMs [32]. The study implies that cell behavior is guided through a combination of signals from the ECM, several of which are required in order to enhance differentiation. Even greater complexity can be achieved in 2D tissue culture format via the use of cardiac tissue-derived ECM. A biomaterial sheet prepared from decellularized rat hearts spurred enhanced cardiac gene expression when compared to cells cultured without the ECM sheets. However, because the biomaterial was not compared to single ECM proteins, it is unclear whether the added complexity significantly affects cell behavior. The cardiomyocytes also had higher rates of proliferation and viability on the naturally derived cECM sheets compared to cells cultured without ECM [33]. Though intriguing, these types of studies need to be coupled to additional experiments designed to determine which element(s) of the ECM are most critical to trigger a desired cellular effect.

As a whole, 2D experiments have provided beneficial insight into the appropriate culture conditions for cardiac cell types. However, a 2D environment is not physiologically relevant, and in recent years, accumulating evidence has shown that three-dimensional engagement of ECM in vitro, akin to that experienced in vivo, gives rise to augmented and sometimes different cellular behaviors than in 2D [34–36]. Thus, to better recapitulate ECM exposure in tissues, both single and combinatorial protein approaches have been applied in three dimensions. A polymer platform, wherein 3D poly(ethylene glycol) hydrogels cross-linked via native chemical ligation, was used to entrap individual ECM and known combinations of ECM with induced pluripotent stem cells. The hydrogels formed were compatible with multiwell plate formats, and so the number of conditions (i.e., different combinations and concentrations of ECM) could be scaled. Then by applying a “design of experiments” statistical approach to the platform, systematic optimization of the ECM ratios led to the identification of a formulation of cECM proteins optimized for cardiomyocyte differentiation. The theoretical formulation was confirmed experimentally via immunophenotyping and functional analyses [37]. Natural multicomponent ECMs derived from tissues have also been used in 3D to influence cell differentiation toward cardiomyocytes. Cardiogel, which is derived from cardiac fibroblasts, has been shown to control differentiation of bone marrow-derived stem cells toward a cardiomyocyte phenotype [38]. The components of cardiogel have been characterized and contain laminins, fibronectin, Col I, Col III, and a variety of proteoglycans [39]. However, while multicomponent ECM extracted from tissue can effectively control cell behavior, it is important to note that the composition of these materials is not well defined and batch-to-batch variability can be difficult to resolve. Thus, there are plenty of advantages of using natural ECM extracted from tissue to generate 2D and 3D in vitro models of cardiac tissue, and these include (1) the inclusion of complex biologic functionalities, even when those functionalities are not fully understood, and (2) evolutionarily guided periodicity and coordinated interplay of multiple ECM domains and multiple cellular domains, with (3) resultant sophisticated intracellular signaling that guides remodeling of the ECM and associated cell behavior. However, it can be difficult to study natural ECM proteins

because they are difficult to reliably source and they do not all form hydrogels spontaneously in 3D necessitating inclusion of gelation platforms.

Advances of the last several decades have led to the identification of some of the critical motifs of cECM that affect cell behavior. For example, studies utilizing recombinant ECM have shown that the arginine-glycine-aspartic acid (RGD) binding domain on fibronectin is highly sensitive to the surrounding synergy residues and that the GFOGER and GLOGER sites on fibrillar collagens are selective for the α2β1 and α1β1 integrins, respectively [40, 41]. With this improved understanding of ECM activity in mind, we are now moving toward the development of artificial ECM that affects cell behavior. These artificial ECMs typically fall into three categories: "blank" polymer backbones with added functionality, ECM mimetics, and modified natural proteins.

Synthetic polymers, often referred to as "blank slates," can be modified to include ECM-like domains which add functionality such as cell-binding motifs and matrix metalloproteinase-binding motifs. One commonly used synthetic polymer is poly (ethylene glycol) (PEG). PEG hydrogels functionalized with RGD have been used to direct differentiation of embryoid bodies toward endothelial cells and cardiomyocytes. The addition of the RGD peptides to the PEG hydrogels decreased cell aggregation in the gels and also drove the cells toward endothelium. A reduction in RGD led to an increase in both cell aggregation and cardiomyocyte differentiation [42]. A polyurethane elastomer functionalized with RGD, another cell binding domain YIGSR, and heparin has been shown to reduce platelet adhesion and enhance attachment of endothelial cells to the surface [43]. An E8 fragment of laminin 411 which represents the C-terminal region of the protein has also been shown to increase differentiation of human iPSCs toward endothelial cells and could be combined with a blank slate to study endothelial differentiation [44]. The fragment improves endothelial differentiation over the entire laminin 411 protein, and endothelial cells created this way showed higher expression of genes associated with vascular development and angiogenesis. Studies using blank slates with added functionality allow for the isolation of the effects of specific domains within either a 2D or 3D system, which addresses several of the problems with using whole, natural ECM proteins by creating platforms which allow for gelation to form 3D structures containing well-defined protein domains. However, creation of these systems requires an understanding of which protein domains influence cell behavior in the desired way and can be limited in the inclusion of synergy sites and lack often necessary secondary, tertiary, and quaternary protein structures.

In an attempt to include synergy sequences or molecular structures of cECM, other groups have attempted to mimic a larger portion of or the entire protein structure with a peptide-based self-assembly approach. *These polymers are intended to recreate the structural, and sometimes biochemical, complexity of ECM and are often termed "ECM mimetics.*" An elastin-like polypeptide hydrogel was designed by combining RGD domains with structural, elastin-like domains that contain repeating hydrophobic residues. A QK peptide that mimics the activity of vascular endothelial growth factor (VEGF) was grafted to the elastin-like polypeptide to enhance endothelial cell proliferation. The material also showed outgrowth of the

endothelial cells from their original colonies, indicating the cells are able to remodel the material to begin forming 3D structures [45]. Heparin mimetics have also been used to include VEGF binding ability reflective of the functionality of heparan sulfate in the basement membrane [46, 47]. An electrospun poly(Ɛ-caprolactone) material with fiber diameters and arrangement designed to mimic the organization of healthy cECM was found to promote arrangement of cardiomyocytes and formation of organized actin/myosin bands compared to fibers arranged to mimic the ECM of a failing heart [48]. A Col I mimic which forms fibril-like structures and ultimately a hydrogel have also been created; however, to this point, the mimetic lacks cell-binding motifs and therefore is unlikely to be of much therapeutic benefit beyond a structural Band-Aid [49]. ECM mimetics provide well-defined materials with specific properties and functionalities. However, the majority of cECM mimetics replicate either specific functional domains (heparin-mimetic polymers) or the microscale structure and arrangement of the cECM (electrospun poly(Ɛ-caprolactone) and collagen mimetic). The elastin-like polypeptide goes one step further by replicating the structure of elastin and including functional domains. However, the domains included are nonnative to elastin. The creation of more complex ECM mimetics which incorporate native functional domains, with microscale structural motifs and associated mechanical attributes that match that of the native protein complex, is essential to the use of ECM mimetics to study ECM interactions and for moving these materials toward clinical therapies.

To this point we have discussed the impact of purified cECM components or synthetic mimics of cECM, but it can also be useful from a basic science and applications standpoint to couple cECM with synthetic modifications. cECM can be altered using synthetic components to alter cell or growth factor binding or change mechanical properties of a scaffold or tissue. Col I can be modified to add nonnative cysteines, which can interact with a PEG cross-linker to immobilize growth factors. When the growth factor TGF-β1 was immobilized within a Col I gel in this manner, the gel induced myofibroblast differentiation [50]. The growth factor immobilization is a synthetic way to replicate the natural growth factor binding of the ECM. These modifications can allow a single component system to harbor multiple functionalities of the ECM. Synthetic modifications to cECM can also be made to tune mechanical properties. A cell-derived ECM has been cross-linked with tunable density using the naturally derived cross-linker, genipin. The material could be tuned to have an elastic modulus between approximately 1 and 10 kPa, which brings the stiffness of the material into the range for the neonatal rat heart (4–11.4 kPa). This cross-linked ECM substrate was supportive of cardiomyocyte differentiation compared to the uncross-linked ECM [51]. The ability to tune the mechanical properties of ECM with synthetic modifications addresses one of the major concerns for the use of natural ECM to study cell-ECM interactions because these modifications could allow creation of 3D structures with appropriate mechanical properties from ECM which does not form a hydrogel on its own. This can aid in decoupling of mechanical stiffness from ECM interactions in ECM studies and provide a platform for the study of a larger number of natural ECM components.

The culmination of the basic in vitro studies described above lays the groundwork for developing ECM-based biomaterials for cardiac tissue engineering applications on the road to therapeutic tissue replacement. The earliest studies using ECM for cardiac regeneration in vivo involved the injection of ECM at the site of injury. Col I injected at the site of myocardial infarction has been shown to improve ejection fraction following treatment and to reduce scar tissue formation when compared to control animals [52]. A bone marrow-derived ECM has also been injected into the heart following myocardial infarction. The rationale for the use of bone marrow-derived ECM was that hematopoietic-derived progenitor cells contribute to angiogenesis following injury. The injection led to decreased apoptosis and lower macrophage counts at the infarct border after 7 days. A decrease in the fibrotic area and increased angiogenesis were also observed [53]. Additionally, several studies have used cECM as an injectable therapy following myocardial infarction. An injectable porcine pericardial matrix gel used as a therapeutic was analyzed by mass spectroscopy to determine the major matrix components in the material. The material contained a large number of ECM components, including Col I, Col IV, and elastin, as well as a variety of other proteins, primarily collagens. Interestingly, the matrix did not contain laminin or Col III, both of which are important components of the cECM. The material increased formation of vasculature and caused infiltration of c-kit+ stem cells into the infarct region [54]. Intramyocardial injection of decellularized cardiac ECM has also been shown to increase left ventricular wall thickness and improve heart function following myocardial infarction [55]. Injection of ECM for cardiac repair following myocardial infarction has shown the power of the ECM in cardiac regeneration. However, injection of ECM does not allow for the creation of organized ECM structures, and these therapies did not demonstrate recovery of cardiac muscle mass, which will be essential for full recovery following cardiac muscle damage.

One potential means to recover cardiac muscle mass is to incorporate cells into materials used for cardiac repair as a means to engineer new tissue. A decellularized cardiac ECM has been used as a scaffold for cardiac tissue repair. The scaffold was seeded with cardiomyocytes, fibroblasts, and mesenchymal stem cells in separate studies with good cell viability. Cells also showed elongated morphologies, and scaffolds seeded with cardiomyocytes began to beat synchronously after a few days of culture on the scaffold [56]. There can also be difficulties using only native ECM as a scaffold for tissue repair because natural cECM forms a soft hydrogel (storage modulus around 100 Pa) in comparison to native myocardium [57]. *This mismatch in mechanical properties can mean cells seeded in the material are receiving mixed signals from the ECM and material mechanics, which can alter cell behavior. The softness of the gel can also make it difficult to secure to the epicardial surface, and it is likely to break down under the stresses of cardiac muscle contraction or blood flow. The native ECM has also been combined with fibrin to enhance stiffness and associated manipulation of the scaffold for therapy. The resulting gel was shown to have mechanical stiffness spanning the range of native myocardium (32–46 kPa) with the capacity to promote differentiation of cardiac progenitor cells isolated from pediatric patients with heart defects toward cardiomyocytes* [58, 59].

Organization of cardiomyocytes prior to therapeutic transplantation might also be facilitated by cECM. One method, which has been used to attain this goal, is 3D bioprinting. Bioprinting allows for the controlled deposition of materials and has the potential to create more organized ECM-based structures. A decellularized ECM bioink has been used to print a cell-laden structure which supports cell viability and improves cardiac differentiation over a Col I control scaffold using an extrusion printer [60]. The viscosity and associated printability of decellularized cECM have been improved through vitamin B12-based photocross-linking. This approach allowed for the decellularized ECM to be printed with high fidelity using an extrusion bioprinter and for the final mechanical stiffness of the construct to match those of native myocardium. The final construct supported cardiac progenitor cell proliferation and improved cardiomyocyte differentiation [61]. However, most extrusion printers are not capable of creating structures with features at a scale that can be discriminated by cells (i.e., 1–100 microns). A multiphoton-based 3D printing system has been developed to print an ECM-based tissue patch for cardiac regeneration with submicron patterning. The scaffold was then seeded with cardiomyocytes, endothelial cells, and smooth muscle cells in a 2:1:1 ratio. The submicron scale patterning of the patch replicated the pattern of fibronectin expression in the native heart and created channels into which the cells quickly settled, providing organization for the tissue and smooth propagation of electromechanical signals across the patch. The patches were implanted in mice following myocardial infarction, and the treated mice showed improved cardiac function, smaller infarct size, and vascularization of the infarct region [62]. These bioprinted structures for myocardial repair show the potential of the method to create structures with improved organization of ECM-based materials and to improve outcomes following myocardial infarction. However, the majority of these structures are thin patches, which limit the efficacy of the structures in promoting full thickness repair of tissue.

Vascularization of tissue grafts for myocardial repair is a major challenge for moving from the creation of thin patches to larger structures. For the creation of large vascularized structures, acellular vessels have been used to create vascular grafts. These include a decellularized fibrin graft which has been implanted into sheep and showed graft endothelialization [63] and a decellularized vessel created by culturing human vascular smooth muscle cells on a biodegradable polymer which has reached clinical trials [64]. However, ECM- based vascular grafts continue to face challenges, particularly balancing the need for cross-linking with calcification in vivo. This calcification occurs partially due to the use of chemical cross-linkers like glutaraldehyde, which can have toxic effects. However, the use of natural cross-linking agents including genipin and procyanides has been shown to inhibit calcification of vascular grafts [65]. The creation of larger-scale vascular grafts using ECM has proven the potential of ECM as a material for the creation of vascular channels. However, it is important to note that the creation of thicker engineered cardiac tissues will not only require creation of larger channels for perfusion but also the creation of complex vascular networks. This subject is under development by several groups globally and has been well-reviewed [66–68]. Successful creation of vascularized thick tissues will promote the development of even larger

cardiac tissue structures, perhaps even a cardiac graft in the distant future. Though far off, efforts have already been initiated to use 3D printing to create complex biological tissues including cardiac tissues. An embryonic chick heart has been printed using a soft bioink (alginate) with the support of a gelatin slurry. The structure showed open internal chambers with complex architecture, an advantage of this printing method [69]. However, while the material used to print this structure is a soft material with a modulus of <100 kPa, the material is not able to support cell growth or differentiation and cannot be remodeled by cells. So, while the ability to print soft materials in complex structures is a major advance toward printing viable cardiac tissues, modifications will be required in order to incorporate cells into these complex structures.

7.3 Concluding Remarks

The essential role of cECM in supporting tissue structure and dictating cardiac cell behavior creates opportunities for a wide range of engineered ECMs, which might ultimately be used to therapeutic effect. Indeed the development of cECM-based clinical therapeutics for the treatment of cardiac and vascular disease is well underway. Proposed therapeutics range in complexity from cell-free scaffolds to thick, vascularized structures. Whether and to which extent these approaches will see significant clinical impact is yet unknown. There are many critical factors that, if not addressed, will limit clinical success, and most of these – including batch variation of natural cECM, compliance mismatch of engineered cECM with cardiovascular tissue, cellular disorganization in engineered cECM, and poor vascularization of thicker cECM-based tissues – have been addressed in this review. Many of these critical factors might be surmounted with a better understanding of the signaling pathways that link integrin engagement of cECM to effector outcomes. We know that focal adhesions or costameres (in the case of cardiomyocytes) are formed with integrin engagement and that these are primarily linked to functions of the cytoskeleton. But the mechanisms by which engagement of different integrins yields varied behavioral outcomes linked to the action of the cytoskeleton (e.g., adhesion, proliferation, migration) are underexplored. Also underexplored are cECM-triggered signaling pathways that may begin with integrin engagement but bypass cytoskeletal components; chief among these is stem cell differentiation.

There is now clear evidence from our group and the work of others that stem cell maturation and differentiation and their respective normal function are critically dependent on the temporal and spatial specification of cECM during developmental [70] and with ex vivo stem cell culture [71–77]. As one of many examples, mesoderm specification has been linked to α5β1 integrin activation. Engagement of this integrin by ECM (especially laminin 511/laminin 111 and fibronectin) modulates BMP4 expression, which together with Wnt, fibroblast growth factor, and transforming growth factor-β/nodal/activin signaling mediates this differentiation [77]. It has also been shown that peptide activation of this integrin can drive osteogenic

differentiation of mesenchymal stem cells via the Wnt/β-catenin pathway activated via PI3K/Akt signaling [78]. In addition, engagement of fibroblast-derived ECM via β1, α2, and α3 integrins in human embryonic stem cells has been shown to activate the Wnt/β-catenin pathway via the MEK-ERK pathway, which drives endoderm differentiation [79]. Finally, fibronectin/integrin β1/ beta-catenin signaling was shown to promote the emergence of mesoderm from induced pluripotent stem cells. This study was the first to establish a direct link between elements of the focal adhesion, namely, integrin-linked kinase (ILK), with GSK3β [80], the primary antagonist of β-catenin, and all of this seemingly untethered to cytoskeletal dynamics. We predict that cECM-based therapeutics that tap our growing understanding of cECM-linked signaling pathways will better control cell behavioral outcomes and lead to significant clinical benefit (Figs. 7.1, 7.2, and 7.3).

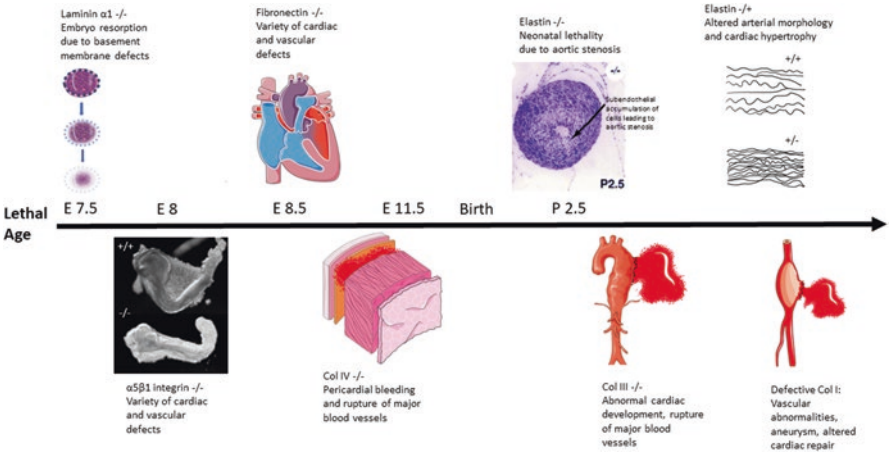

Fig. 7.1 Timeline showing the lethal age of cECM and integrin mutations. Laminin α1 −/− is lethal at embryonic day 7.5, and the embryo is resorbed due to defects of the basement membrane [81]. α5β1 integrin −/− is lethal at embryonic day 8, and embryos show a variety of cardiac and vascular defects. The image compares a wild-type embryo to a knockout embryo at embryonic day 8 [20]. Fibronectin −/− shows similar defects to the α5β1 integrin −/− and is lethal at embryonic day 8.5 [18]. Col IV −/− is lethal at embryonic day 10.5–11.5 due to pericardial bleeding (shown in the image) and rupture of major blood vessels [16]. Elastin −/− is lethal in the neonatal period due to aortic stenosis, which is shown in the image [24]. Col III −/− leads to abnormal cardiac development and the rupture of major blood vessels after birth [11]. The image shows a dissecting aortic aneurysm due to the defect. Elastin −/+ heterozygotes show less severe defects than the knockouts but still have altered arterial morphology and show an increased number of elastic lamellae compared to normal animals [82]. Defects in Col I lead to a variety of vascular abnormalities and can affect cardiac repair following injury [83]. (Image components from Servier Medical Art [84])

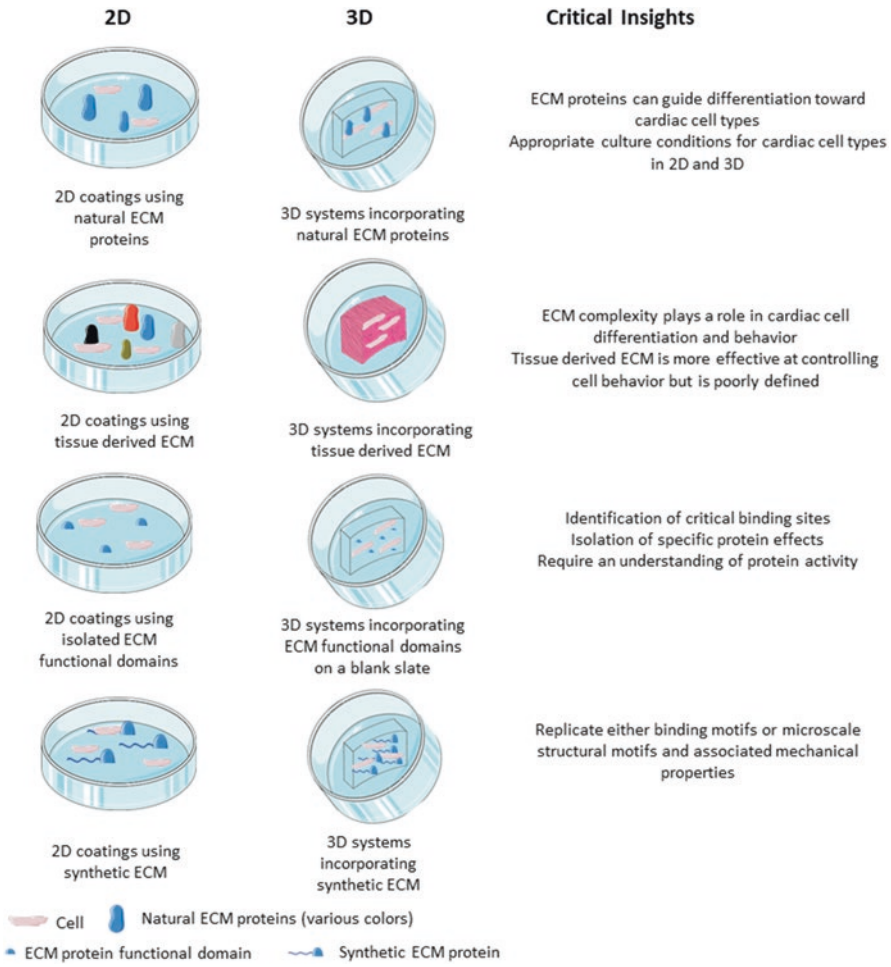

Fig. 7.2 Schematic of in vitro systems for studying the effects of cECM on cell behavior. 2D systems include coating with natural ECM, tissue-derived ECM, isolated ECM functional domains, and synthetic ECM. 3D systems have incorporated natural and tissue-derived ECM as well as functional domains and synthetic ECMs. Each of these systems has provided distinct insights into the functions of the cECM. (Image components from Servier Medical Art [84])

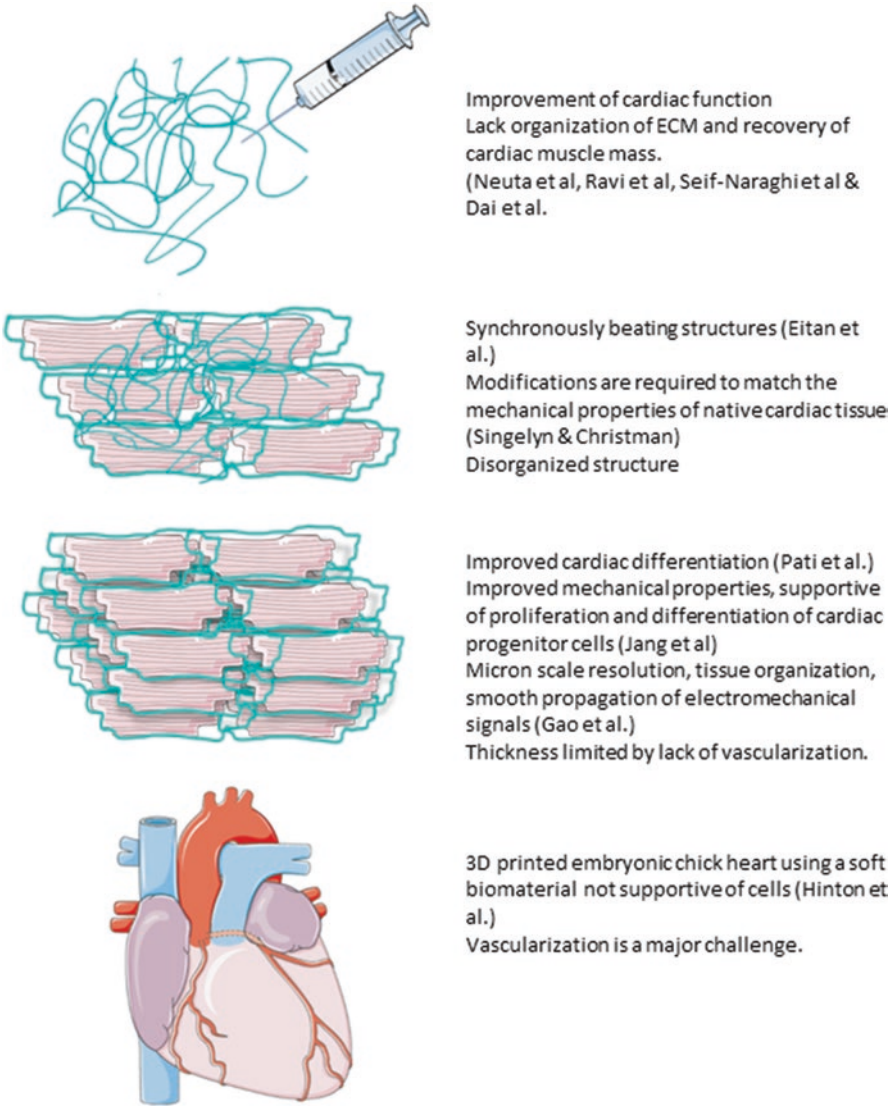

Fig. 7.3 Schematic of cECM used for cardiac therapy. Injected ECM has shown improvements in cardiac function but lacks organization and recovery of cardiac muscle mass [52–55, 85]. cECM-based cardiac patches incorporating cells have been shown to create synchronously beating structures [56]. However, modifications to natural ECM are typically required in order to match native tissue mechanical properties [57], and these structures still lack the organization of native tissue. Organized ECM structures have been created through 3D printing modalities and have led to improved cardiac differentiation [60] and mechanical properties [61]. 3D-printed tissues have also been created with micron-scale resolution and tissue organization [62]. Thickness of these tissues, as well as of more complex heart structures is limited by vascularization. However, a whole 3D printed embryonic chick heart has been printed with a soft biomaterial but is not yet able to support cells [69]. (Image components from Servier Medical Art [84])

References

1. Mienaltowski MJ, Birk DE. Structure, physiology, and biochemistry of collagens. Dordrecht: Springer; 2014. p. 5–29. Available from: http://link.springer.com/10.1007/978-94-007-7893-1_2
2. Hanson KP, Jung JP, Tran QA, Hsu S-PP, Iida R, Ajeti V, et al. Spatial and temporal analysis of extracellular matrix proteins in the developing murine heart: a blueprint for regeneration. Tissue Eng Part A. 2013;19(9–10):1132–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23273220
3. Marijianowski MMH, van der Loos CM, Mohrschladt MF, Becker AE. The neonatal heart has a relatively high content of total collagen and type I collagen, a condition that may explain the less compliant state. J Am Coll Cardiol. 1994;23(5):1204–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/0735109794906122
4. Omens JH, Fung YC, Becker KD, McBride DJ, Omens JH, McCulloch AD. Myocardial mechanics and collagen structure in the Osteogenesis Imperfecta murine (oim). Circ Res. 1990;66(1):37–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2295143
5. Hofmann U, Bonz A, Frantz S, Hu K, Waller C, Roemer K, et al. A collagen α2(I) mutation impairs healing after experimental myocardial infarction. Am J Pathol. 2012;180(1):113–22. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0002944011009266
6. Sansilvestri-Morel P, Rupin A, Jaisson S, Fabiani J-N, Verbeuren TJ, Vanhoutte PM. Synthesis of collagen is dysregulated in cultured fibroblasts derived from skin of subjects with varicose veins as it is in venous smooth muscle cells. Circulation. 2002;106(4):479–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12135949
7. Malfait F, Symoens S, Goemans N, Gyftodimou Y, Holmberg E, López-González V, et al. Helical mutations in type I collagen that affect the processing of the amino-propeptide result in an Osteogenesis Imperfecta/Ehlers-Danlos Syndrome overlap syndrome. Orphanet J Rare Dis. 2013;8:78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23692737
8. Cameron GJ, Alberts IL, Laing JH, Wess TJ. Structure of type I and type III heterotypic collagen fibrils: an X-ray diffraction study. J Struct Biol. 2002;137(1–2):15–22. Available from: https://ac.els-cdn.com/S104784770294459X/1-s2.0-S104784770294459X-main.pdf?_tid=e6fe778c-a483-11e7-a4f1-00000aab0f27&acdnat=1506627396_64c77199a25fa903418103d2fc666d66
9. Katz AM, Editor G, Weber KT, Chicago F. Basic concepts in cardiology cardiac interstitium in health and disease: the fibrillar collagen network. JACC. 1989;13(7):1637–52. Available from: https://ac.els-cdn.com/0735109789903604/1-s2.0-0735109789903604-main.pdf?_tid=589b9f52-a487-11e7-8073-00000aacb360&acdnat=1506628867_79e2ea81848b5dd69515ed5181463003
10. Akhtar S, Meek KM, James V. Immunolocalization of elastin, collagen type I and type III, fibronectin, and vitronectin in extracellular matrix components of normal and myxomatous mitral heart valve chordae tendineae. Cardiovasc Pathol. 1999;8(4):203–11. Available from: http://www.sciencedirect.com/science/article/pii/S1054880799000034?via%3Dihub
11. Liu X, Wu H, Byrne M, Krane S, Jaenisch R. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci U S A. 1997;94(5):1852–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9050868
12. Shalhub S, Black JH, Cecchi AC, Xu Z, Griswold BF, Safi HJ, et al. Molecular diagnosis in vascular Ehlers-Danlos syndrome predicts pattern of arterial involvement and outcomes. J Vasc Surg. 2014;60(1):160–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24650746
13. Limon-Miranda S, Salazar-Enriquez DG, Muñiz J, Ramirez-Archila MV, Sanchez-Pastor EA, Andrade F, et al. Pregnancy differentially regulates the collagens types I and III in left ventricle from rat heart. Biomed Res Int. 2014;2014:984785. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25147829
14. Germain S, Monnot C, Muller L, Eichmann A. Hypoxia-driven angiogenesis: role of tip cells and extracellular matrix scaffolding. Curr Opin Hematol. 2010;17(3):245–51. Available from: https://insights.ovid.com/pubmed?pmid=20308893

15. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. Microsc Res Tech. 2008;71(5):357–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18219669
16. Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development. 2004;131(7). Available from: http://dev.biologists.org/content/131/7/1619.long
17. Mao Y, Schwarzbauer JE. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. Matrix Biol. 2005;24(6):389–99. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0945053X05000855
18. George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development. 1993;119(4). Available from: http://dev.biologists.org/content/119/4/1079.long
19. Konstandin MH, Toko H, Gastelum GM, Quijada P, De La Torre A, Quintana M, et al. Fibronectin is essential for reparative cardiac progenitor cell response after myocardial infarction. Circ Res. 2013;113(2):115–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23652800
20. Yang JT, Bader BL, Kreidberg JA, Ullman-Culleré M, Trevithick JE, Hynes RO. Overlapping and independent functions of fibronectin receptor Integrins in early mesodermal development. Dev Biol. 1999;215(2):264–77. Available from: http://www.sciencedirect.com/science/article/pii/S0012160699994514
21. Chen D, Wang X, Liang D, Gordon J, Mittal A, Manley N, et al. Fibronectin signals through integrin α5β1 to regulate cardiovascular development in a cell type-specific manner. Dev Biol. 2015;407(2):195–210. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26434918
22. Turner NA. Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). J Mol Cell Cardiol. 2016;94:189–200. Available from: https://www.sciencedirect.com/science/article/pii/S0022282815301073?via%3Dihub#bb0005
23. Wagenseil JE, Ciliberto CH, Knutsen RH, Levy MA, Kovacs A, Mecham RP. The importance of elastin to aortic development in mice. Am J Physiol Heart Circ Physiol. 2010;299(2):H257–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20495146
24. Li DY, Brooke B, Davis EC, Mecham RP, Sorensen LK, Boak BB, et al. Elastin is an essential determinant of arterial morphogenesis. Nature. 1998;393(6682):276–80. Available from: http://www.nature.com/doifinder/10.1038/30522
25. Wagenseil JE, Knutsen RH, Li DY, Mecham RP. Elastin-insufficient mice show normal cardiovascular remodeling in 2K1C hypertension despite higher baseline pressure and unique cardiovascular architecture. Am J Physiol Heart Circ Physiol. 2007;293(1). Available from: http://ajpheart.physiology.org/content/293/1/H574.long
26. Durbeej M. Laminins. Cell Tissue Res. 2010;339(1):259–68. Available from: http://link.springer.com/10.1007/s00441-009-0838-2
27. Ichikawa-Tomikawa N, Ogawa J, Douet V, Xu Z, Kamikubo Y, Sakurai T, et al. Laminin α1 is essential for mouse cerebellar development. Matrix Biol. 2012;31(1):17–28. Available from: http://www.sciencedirect.com/science/article/pii/S0945053X11000904
28. Thyboll J, Kortesmaa J, Cao R, Soininen R, Wang L, Iivanainen A, et al. Deletion of the laminin alpha4 chain leads to impaired microvessel maturation. Mol Cell Biol. 2002;22(4):1194–202. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11809810
29. Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM. Expression and function of laminins in the embryonic and mature vasculature. Physiol Rev. 2005;85(3). Available from: http://physrev.physiology.org/content/85/3/979.long
30. Borg T, Rubin K, Lundgren E, Borg KÖB. Recognition of extracellular matrix components by neonatal and adult cardiac myocytes. Dev Biol. 1984;104(1):86–96. Available from: http://www.sciencedirect.com/science/article/pii/0012160684900381?via%3Dihub

31. Jin-long SUN, Xiao-yun Z, Xiao-dong CUI, Hong-ying LU, Qing-ling YIN, Xu J, et al. Effects of extracellular matrix on biological characteristics of late endothelial progenitor cells. Sheng Li Xue Bao. 2013;65(2011):409–16.
32. Hou L, Coller J, Natu V, Hastie TJ, Huang NF. Combinatorial extracellular matrix microenvironments promote survival and phenotype of human induced pluripotent stem cell-derived endothelial cells in hypoxia. Acta Biomater. 2016;44:188–99. Available from: http://www.sciencedirect.com/science/article/pii/S1742706116303907
33. Lee KM, Kim H, Nemeno JG, Yang W, Yoon J, Lee SLJ. Natural cardiac extracellular matrix sheet as a biomaterial for cardiomyocyte transplantation. Transplant Proc. 2015;47(3):751–6. Available from: http://www.sciencedirect.com/science/article/pii/S0041134515001086?via%3Dihub
34. Jung JP, Bache-Wiig MK, Provenzano PP, Ogle BM. Heterogeneous differentiation of human mesenchymal stem cells in 3D extracellular matrix composites. Biores Open Access. 2016;5(1):37–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26862471
35. Duval K, Grover H, Han L-H, Mou Y, Pegoraro AF, Fredberg J, et al. Modeling physiological events in 2D vs. 3D cell culture. Physiology (Bethesda). 2017;32(4):266–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28615311
36. Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FDP. 3D cell culture systems: advantages and applications. J Cell Physiol. 2015;230(1):16–26. Available from: http://doi.wiley.com/10.1002/jcp.24683
37. Jung JP, Hu D, Domian IJ, Ogle BM. An integrated statistical model for enhanced murine cardiomyocyte differentiation via optimized engagement of 3D extracellular matrices. Sci Rep. 2015;5:18705. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26687770
38. Santhakumar R, Vidyasekar P, Verma RS. Cardiogel: a nano-matrix scaffold with potential application in cardiac regeneration using mesenchymal stem cells. PLoS One. 2014;9(12):e114697. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25521816
39. Vanwinkle WB, Snuggs MB, Buja LM. Cardiogel: a biosynthetic extracellular matrix for cardiomyocyte culture. Vitr Cell Dev Biol Animal Soc Vitr Biol. 1996;32(9):478–85. Available from: https://link.springer.com/content/pdf/10.1007%2FBF02723051.pdf
40. Brown AC, Dysart MM, Clarke KC, Stabenfeldt SE, Barker TH. Integrin α3β1 binding to fibronectin is dependent on the ninth type III repeat. J Biol Chem. 2015;290(42):25534–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26318455
41. Hamaia SW, Pugh N, Raynal N, Némoz B, Stone R, Gullberg D, et al. Mapping of potent and specific binding motifs, GLOGEN and GVOGEA, for integrin α1β1 using collagen toolkits II and III. J Biol Chem. 2012;287(31):26019–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22654115
42. Schukur L, Zorlutuna P, Cha JM, Bae H, Khademhosseini A. Directed differentiation of size-controlled embryoid bodies towards endothelial and cardiac lineages in RGD-modified poly(ethylene glycol) hydrogels. Adv Healthc Mater. 2013;2(1):195–205. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23193099
43. Choi WS, Joung YK, Lee Y, Bae JW, Park HK, Park YH, et al. Enhanced patency and endothelialization of small-caliber vascular grafts fabricated by coimmobilization of heparin and cell-adhesive peptides. ACS Appl Mater Interfaces. 2016;8(7):4336–46. Available from: http://pubs.acs.org/doi/10.1021/acsami.5b12052
44. Ohta R, Niwa A, Taniguchi Y, Suzuki NM, Toga J, Yagi E, et al. Laminin-guided highly efficient endothelial commitment from human pluripotent stem cells. Sci Rep. 2016;6:35680. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27804979
45. Cai L, Dinh CB, Heilshorn SC. One-pot synthesis of elastin-like polypeptide hydrogels with grafted VEGF-mimetic peptides. Biomater Sci. 2014;2(5):757–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24729868
46. Mammadov R, Mammadov B, Guler MO, Tekinay AB. Growth factor binding on heparin mimetic peptide nanofibers. Biomacromolecules. 2012;13(10):3311–9. Available from: http://pubs.acs.org/doi/abs/10.1021/bm3010897

47. Rouet V, Hamma-Kourbali Y, Petit E, Panagopoulou P, Katsoris P, Barritault D, et al. A synthetic glycosaminoglycan mimetic binds vascular endothelial growth factor and modulates angiogenesis. J Biol Chem. 2005;280(38):32792–800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16014624
48. Rath R, Lee JB, Tran T-L, Lenihan SF, Galindo CL, Su YR, et al. Biomimetic microstructure morphology in electrospun fiber mats is critical for maintaining healthy cardiomyocyte phenotype. Cell Mol Bioeng. 2016;9(1):107–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28042345
49. LER O'l, Fallas JA, Bakota EL, Kang MK, Hartgerink JD. Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel. Nat Chem. 2011;3(10):821–8. Available from: https://ay17.moodle.umn.edu/pluginfile.php/987210/mod_resource/content/2/Collagen_Literature Learning.pdf
50. Que R, Mohraz A, Da Silva NA, Wang S-W. Expanding functionality of recombinant human collagen through engineered non-native cysteines. Biomacromolecules. 2014;15(10):3540–9. Available from: http://pubs.acs.org/doi/abs/10.1021/bm500735d.
51. Subbiah R, Hwang MP, Du P, Suhaeri M, Hwang J-H, Hong J-H, et al. Tunable crosslinked cell-derived extracellular matrix guides cell fate. Macromol Biosci. 2016;16(11):1723–34. Available from: http://doi.wiley.com/10.1002/mabi.201600280
52. Neuta PA, Rojas DM, Agredo W, Gutierrez JO. Evaluation of the repairing effect of collagen type I and MaxGel on the infarcted myocardium in an animal model. In: 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE; 2015. pp. 3529–32. Available from: http://ieeexplore.ieee.org/document/7319154/.
53. Ravi S, Caves JM, Martinez AW, Xiao J, Wen J, Haller CA, et al. Effect of bone marrow-derived extracellular matrix on cardiac function after ischemic injury. Biomaterials. 2012;33(31):7736–45. Available from: http://www.sciencedirect.com/science/article/pii/S0142961212007 79X?via%3Dihub
54. Seif-Naraghi SB, Salvatore MA, Schup-Magoffin PJ, Hu DP, Christman KL. Design and characterization of an injectable pericardial matrix gel: a potentially autologous scaffold for cardiac tissue engineering. Tissue Eng Part A. 2010;16(6):2017–27. Available from: http://www.liebertonline.com/doi/abs/10.1089/ten.tea.2009.0768
55. Dai W, Gerczuk P, Zhang Y, Smith L, Kopyov O, Kay GL, et al. Intramyocardial injection of heart tissue-derived extracellular matrix improves postinfarction cardiac function in rats. J Cardiovasc Pharmacol Ther. 2013;18(3):270–9. Available from: http://journals.sagepub.com/doi/10.1177/1074248412472257
56. Eitan Y, Sarig U, Dahan N, Machluf M. Acellular cardiac extracellular matrix as a scaffold for tissue engineering: *In Vitro* cell support, remodeling, and biocompatibility. Tissue Eng Part C Methods. 2010;16(4):671–83. Available from: http://www.liebertonline.com/doi/abs/10.1089/ten.tec.2009.0111
57. Singelyn JM, Christman KL. Modulation of material properties of a decellularized myocardial matrix scaffold. Macromol Biosci. 2011;11(6):731–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21322109
58. Williams C, Budina E, Stoppel WL, Sullivan KE, Emani S, Emani SM, et al. Cardiac extracellular matrix-fibrin hybrid scaffolds with tunable properties for cardiovascular tissue engineering. Acta Biomater. 2015;14:84–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25463503
59. Jacot JG, Martin JC, Hunt DL. Mechanobiology of cardiomyocyte development. J Biomech. 2010;43(1):93–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19819458
60. Pati F, Jang J, Ha D-H, Won Kim S, Rhie J-W, Shim J-H, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24887553
61. Jang J, Kim TG, Kim BS, Kim S-W, Kwon S-M, Cho D-W. Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Acta

Biomater. 2016;33:88–95. Available from: http://www.sciencedirect.com/science/article/pii/S1742706116300137?via%3Dihub
62. Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, Da Sie Y, et al. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. Circ Res. 2017;120(8):1318–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28069694
63. Syedain ZH, Meier LA, Lahti MT, Johnson SL, Tranquillo RT. Implantation of completely biological engineered grafts following decellularization into the sheep femoral artery. Tissue Eng Part A. 2014;20(11–12):1726–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24417686
64. Lawson JH, Glickman MH, Ilzecki M, Jakimowicz T, Jaroszynski A, Peden EK, et al. Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: two phase 2 single-arm trials. Lancet (London, England). 2016;387(10032):2026–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27203778
65. Ma B, Wang X, Wu C, Chang J. Crosslinking strategies for preparation of extracellular matrix-derived cardiovascular scaffolds. Regen Biomater. 2014;1(1):81–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26816627
66. Paulsen SJ, Miller JS. Tissue vascularization through 3D printing: will technology bring us flow? Dev Dyn. 2015;244(5):629–40. Available from: http://doi.wiley.com/10.1002/dvdy.24254
67. Visconti RP, Kasyanov V, Gentile C, Zhang J, Markwald RR, Mironov V. Towards organ printing: engineering an intra-organ branched vascular tree. Expert Opin Biol Ther. 2010;10(3):409–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20132061
68. Kinstlinger IS, Miller JS. 3D-printed fluidic networks as vasculature for engineered tissue. Lab Chip. 2016;16(11):2025–43. Available from: http://xlink.rsc.org/?DOI=C6LC00193A
69. Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue H-J, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv. 2015;1(9):e1500758. Available from: http://advances.sciencemag.org/cgi/doi/10.1126/sciadv.1500758
70. Costa-Silva B, da Costa MC, Melo FR, Neves CM, Alvarez-Silva M, Calloni GW, et al. Fibronectin promotes differentiation of neural crest progenitors endowed with smooth muscle cell potential. Exp Cell Res. 2009;315(6):955–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19331824
71. Wong JCY, Gao SY, Lees JG, Best MB, Wang R, Tuch BE. Definitive endoderm derived from human embryonic stem cells highly express the integrin receptors alphaV and beta5. Cell Adhes Migr. 2010;4(1):39–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20026907
72. Brafman DA, Phung C, Kumar N, Willert K. Regulation of endodermal differentiation of human embryonic stem cells through integrin-ECM interactions. Cell Death Differ. 2013;20(3):369–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23154389
73. Narayanan K, Lim VY, Shen J, Tan ZW, Rajendran D, Luo S-C, et al. Extracellular matrix-mediated differentiation of human embryonic stem cells: differentiation to insulin-secreting beta cells. Tissue Eng Part A. 2014;20(1–2):424–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24020641
74. Li Y, Gautam A, Yang J, Qiu L, Melkoumian Z, Weber J, et al. Differentiation of oligodendrocyte progenitor cells from human embryonic stem cells on vitronectin-derived synthetic peptide acrylate surface. Stem Cells Dev. 2013;22(10):1497–505. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23249362
75. Caiazzo M, Okawa Y, Ranga A, Piersigilli A, Tabata Y, Lutolf MP. Defined three-dimensional microenvironments boost induction of pluripotency. Nat Mater. 2016;15(3):344–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26752655
76. Derda R, Musah S, Orner BP, Klim JR, Li L, Kiessling LL. High-throughput discovery of synthetic surfaces that support proliferation of pluripotent cells. J Am Chem Soc. 2010;132(4):1289–95. Available from: http://pubs.acs.org/doi/abs/10.1021/ja906089g

77. Tan T-W, Huang Y-L, Chang J-T, Lin J-J, Fong Y-C, Kuo C-C, et al. CCN3 increases BMP-4 expression and bone mineralization in osteoblasts. J Cell Physiol. 2012;227(6):2531–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21898398
78. Saidak Z, Le Henaff C, Azzi S, Marty C, Da Nascimento S, Sonnet P, et al. Wnt/β-catenin signaling mediates osteoblast differentiation triggered by peptide-induced α5β1 integrin priming in mesenchymal skeletal cells. J Biol Chem. 2015;290(11):6903–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25631051
79. Dzobo K, Vogelsang M, Parker MI. Wnt/β-catenin and MEK-ERK signaling are required for fibroblast-derived extracellular matrix-mediated endoderm differentiation of embryonic stem cells. Stem Cell Rev Reports. 2015;11(5):761–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26022506
80. Cheng P, Andersen P, Hassel D, Kaynak BL, Limphong P, Juergensen L, et al. Fibronectin mediates mesendodermal cell fate decisions. Development. 2013;140(12):2587–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23715551
81. Alpy F, Jivkov I, Sorokin L, Klein A, Arnold C, Huss Y, et al. Generation of a conditionally null allele of the laminin α1 gene. Genesis. 2005;43(2):59–70. Available from: http://doi.wiley.com/10.1002/gene.20154
82. Li DY, Faury G, Taylor DG, Davis EC, Boyle WA, Mecham RP, et al. Novel arterial pathology in mice and humans hemizygous for elastin. J Clin Invest. 1998;102(10):1783–7. Available from: http://www.jci.org
83. Marjamaa J, Tulamo R, Abo-Ramadan U, Hakovirta H, Frösen J, Rahkonen O, et al. Mice with a deletion in the first intron of theCol1a1 gene develop dissection and rupture of aorta in the absence of aneurysms: high-resolution magnetic resonance imaging, at 4.7 T, of the aorta and cerebral arteries. Magn Reson Med. 2006;55(3):592–7. Available from: http://doi.wiley.com/10.1002/mrm.20798
84. SMART - Servier Medical ART. 2017. Available from: http://smart.servier.com/
85. Johnson TD, Braden RL, Christman KL. Injectable ECM scaffolds for cardiac repair. Methods Mol Biol. 2014;1181:109–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25070331

Chapter 8
Extracellular Matrix for Myocardial Repair

Jenna L. Dziki and Stephen F. Badylak

Abstract Multiple strategies have been investigated to restore functional myocardium following injury or disease including the local administration of cytokines or chemokines, stem/progenitor cell therapy, mechanical circulatory support, pharmacologic use, and the use of inductive biomaterials. The use of xenogeneic biologic scaffolds composed of extracellular matrix (ECM) has been shown to facilitate functional restoration of several tissues and organs including the esophagus, skeletal muscle, skin, and myocardium, among others. The present chapter describes the current understanding of specific components of biologic scaffolds composed of ECM, the mechanisms by which ECM bioscaffolds promote constructive cardiac remodeling after injury, determinants of remodeling outcome, and the versatility of ECM as a potential cardiac therapeutic.

Keywords Extracellular matrix · Decellularization · Macrophage · Heart valves · Cardiac patch · Whole-organ engineering · Hydrogels

8.1 ECM Bioscaffolds: Mother Nature's Template

The heart has minimal inherent regenerative capacity after injury, making its functional replacement after damage a challenging task. Though the field of transplant surgery and the development of pharmacologic and mechanical treatments for heart disease have made significant strides, a critical need remains for effective strategies

J. L. Dziki
McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

S. F. Badylak (✉)
McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA
e-mail: badylaks@upmc.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_8

to reconstruct or replace functional myocardium following injury. The fields of developmental biology, immunology, and tissue engineering/regenerative medicine have identified the extracellular matrix (ECM) as a powerful regulator of cell behavior, tissue homeostasis, and tissue and organ replacement in times of need [1–3]. The ECM consists of the structural and functional molecules secreted by resident cells, forming the ultrastructure and microenvironmental milieu of each tissue and organ. Logically therefore, biomaterials composed of the ECM represent a favorable inductive template to facilitate functional and constructive tissue remodeling following disease or injury by providing the appropriate microenvironmental milieu within which cells can maintain normal function. When appropriately decellularized and processed, ECM bioscaffolds retain the necessary structural and bioinductive cues to guide appropriately timed innate immune activation and phenotype transition [1–3], endogenous stem/progenitor cell chemotaxis [4–6], proliferation [7], differentiation [8, 9], innervation [4, 10, 11], and ultimately functional and constructive remodeling in multiple preclinical and clinical applications (Table 8.1, noninclusive list).

8.2 Mechanisms of Constructive Remodeling Mediated by ECM Bioscaffolds

8.2.1 Degradation and Release of Bioactive Constituents

The matrisome consists of a diverse and extensive inventory of structural and functional molecules. With current advances in genomic and proteomic technology and accumulated knowledge of the components of the ECM, a comprehensive molecular profile with associated functional attributes is within reach [12]. The ECM contains hundreds of constituents including collagens, glycoproteins, proteoglycans, ECM-modifying enzymes, ECM-binding growth factors, matrix-bound nanovesicles, and other ECM-associated proteins, most of which have a necessary or survival function [13]. The specific functions mediated by each ECM component are almost certainly dependent upon factors such as anatomic location and physiologic status (e.g., homeostasis vs. injury response) and are dependent upon release or exposure during matrix degradation. During normal physiologic processes, ECM is constantly degraded and replaced by the resident cells. Importantly, when the ECM is isolated and used as a biomaterial, it will rapidly degrade upon implantation and release these bioactive constituents. The degradation process is initiated immediately after in vivo implantation and is a necessary process to influence cell behavior and the downstream remodeling outcome [14, 15]. Degradation is mediated primarily by macrophages, one of the first responding innate immune cells to an implanted ECM biomaterial.

When properly prepared (i.e., when thoroughly decellularized and when chemical crosslinking agents are not utilized), the mechanical strength of the ECM bioscaffold diminishes as it degrades but is quickly followed by an increase in

Table 8.1 Preclinical and clinical applications of extracellular matrix bioscaffolds

Preclinical applications		Clinical applications	
Application	Report	Application	Report
Stroke	Ghuman et al. ECM hydrogel for the treatment of stroke: characterization of the host cell infiltrate. *Biomaterials.* 2016	Gingival recession	Gholami et al. Comparison of the clinical outcomes of connective tissue and acellular dermal matrix in combination with double papillary flap for root coverage: A 6-month trial. *Dent Res J.* 2013
Temporomandibular joint disk degeneration	Brown et al. Inductive, scaffold-based regenerative medicine approach to reconstruction of the temporomandibular joint disk. *J Oral Maxillofac Surg.* 2012	Facial reconstruction	Leventhal et al. Static facial suspension with Surgisis ES (enhanced strength) sling. *Laryngoscope.* 2008
Spinal cord injury	Tukmachev et al. Injectable extracellular matrix hydrogels as scaffolds for spinal cord injury repair. *Tissue Eng Part A.* 2016	Rotator cuff repair	Dopirak et al. Arthroscopic total rotator cuff replacement with an acellular human dermal allograft matrix. *Int J Shoulder Surg.* 2007
Ventricular wall cardiac patch	D'Amore et al. Bilayered polyurethane – extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model. *Biomaterials.* 2016	Esophageal neoplasia resection	Badylak et al. Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine approach with a biologic scaffold. *Tissue Eng Part A.* 2011
Small intestine replacement	Shaffiey S et al. Intestinal stem cell growth and differentiation on a tubular scaffold with evaluation in small and large animals. *Regen Med.* 2016	Breast reconstruction	Butterfield et al. 440 consecutive immediate, implant-based, single-surgeon breast reconstructions in 281 patients: a comparison of early outcomes and costs between SurgiMend fetal bovine and AlloDerm human cadaveric acellular dermal matrices. *Plast Reconstr Surg.* 2013

(continued)

Table 8.1 (continued)

Preclinical applications		Clinical applications	
Liver regeneration	Hammond et al. Scaffolds containing growth factors and extracellular matrix	Mitral valve replacement	Gerdisch MW et al. Clinical experience with CorMatrix extracellular matrix in the surgical treatment of mitral valve disease. *J Thorac Cardiovasc Surg.* 2014
Inflammatory bowel disease	Keane et al. Restoring mucosal barrier function and modifying macrophage phenotype with an extracellular matrix hydrogel: potential therapy for ulcerative colitis. *J Crohn's and Colitis.* 2017	Cardiac patch	Dharmapuram et al. Preliminary experience with the use of extracellular matrix to augment the native pulmonary valve during repair of tetralogy of Fallot. *World J Pediatr Congenit Heart Surg.* 2017
Digit tip injury	Agrawal et al. Epimorphic regeneration approach to tissue replacement in adult mammals. *Proc Natl Acad Sci U S A.* 2010	Volumetric muscle loss	Dziki et al. An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. *Nat Regen Med.* 2015
Cartilage	Benders et al. Extracellular matrix scaffolds for cartilage and bone regeneration. *Trends Biotechnol*. 2013	Ventral hernia repair	Kissane et al. A decade of ventral incisional hernia repairs with biologic acellular dermal matrix: What have we learned? *Plast Reconstr Surg.* 2012
Ligament-bone	Liu et al. Biomimetic tendon extracellular matrix composite gradient scaffold enhances ligament-to-bone junction reconstruction. *Acta Biomater.* 2017	Vascular patch	Ladowski et al. Ann Vasc Surg. Retrospective analysis of bovine pericardium (Vascu-Guard) for patch closure in carotid endarterectomies. *Ann Vasc Surg.* 2011
Compartment syndrome	Daly et al. A rabbit model of peripheral compartment syndrome with associated rhabdomyolysis and regenerative medicine approach for treatment. *Tissue Eng Part C Methods.* 2011	Diabetic ulcers	Lecheminant J. Porcine urinary bladder matrix: a retrospective study and establishment of protocol. *J Wound Care.* 2012

strength as a result of deposition of site-appropriate tissue [16]. Rapid in vivo degradation of these bioscaffolds also minimizes a foreign body reaction, a common host immune response to many nondegradable synthetic biomaterials and chemically crosslinked ECM biomaterials. Lack of ECM degradation may result in fibrous encapsulation and lack of integration with surrounding tissues [14, 17, 18]. Commonly used xenogeneic tissue heart valves, for example, are often crosslinked with glutaraldehyde, purportedly to mask antigenicity and obviate the need for decellularization. However, glutaraldehyde crosslinking is associated with structural alterations and calcification that are detrimental in the long term [19]. In contrast, ECM bioscaffolds that are not chemically crosslinked avoid the foreign body response and promote a favorable activation state of the immune system and constructive downstream remodeling [2, 14, 20–25]. As the ECM degrades, the bioactive constituents are gradually released and promote stem cell migration, proliferation, and differentiation [4, 5, 26, 27] and formation and/or release of antimicrobial peptides [28] and influence the host immune response toward a constructive and healing phenotype [2].

8.2.2 Activation and Phenotypic Transition of Macrophages

ECM scaffold degradation is mediated primarily by macrophages [14]. Historically, much effort has been focused upon evading or suppressing the immune system, particularly in the case of xenogeneic implants, for fear of immune-mediated rejection [29–31]. It is now apparent however that the benefits of immunosuppressive drugs barely outweigh the negative side effects, if at all [32].

Interaction of an ECM biomaterial with the host innate immune system is not only important but necessary for favorable tissue remodeling. The immune system is now recognized as a driver of normal cardiac repair after both transplant and ischemic events [33–36]. In fact, an active and robust immune system is necessary for normal tissue development, homeostasis, and wound healing [24, 37, 38]. Biologic scaffolds composed of ECM have the distinctive ability to modulate responding immune cells and activate them in an appropriate temporal sequence toward a pro-remodeling phenotype [6, 39]. For example, cardiac fibroblasts are profoundly influenced by the host macrophage signaling after myocardial infarction, a process regulated by the surrounding matrix, subsequently promoting or inhibiting cardiac remodeling [40]. Regulation of cardiac matrix synthesis and degradation by immune cell-secreted effector molecules such as TGF-ß and matrix metalloproteinases (MMPs) exemplify the dependence of efficient cardiac repair upon the immune response [41]. Pharmacologic inhibition or depletion of macrophages results in poor biomaterial-mediated tissue remodeling outcomes and altered normal regenerative processes [21, 42]. In fact, the early-responding immune cell phenotypic profile has been shown to be a predictor of downstream constructive remodeling outcome after implantation of many types of biomaterials, including ECM bioscaffolds [43]. In short summary, ECM bioscaffold degradation products

have been shown to both directly and indirectly (through stem cell-mediated signaling) activate macrophages and T cells toward a phenotype conducive to tissue repair and regeneration [44, 45]. The implications for cardiac remodeling are obvious.

8.3 Methods of ECM Bioscaffold Preparation and the Impact upon Remodeling Outcomes

The in vivo response to an implanted ECM bioscaffold is dependent upon the method by which it is prepared. Preferred preparation criteria include but are not limited to minimal cell remnants after decellularization, maintenance of the native tissue ECM composition and ultrastructure, avoidance of chemical crosslinking, and use of appropriate terminal sterilization methods.

8.3.1 Method of Decellularization

The differential ability of ECM derived from diverse source tissues to promote immune cell activation has been investigated [46] and is, in part, dependent upon the decellularization method utilized to prepare each ECM bioscaffold. Decellularization involves a balance of removing associated antigenic components that elicit an adverse immune response while retaining matrix ultrastructure and embedded bioactive molecules that support cell attachment, viability, proliferation, and differentiation. Decellularization protocols vary widely and have numerous adjustable parameters including use of freeze-thaw cycles, perfusion vs. immersion/agitation techniques, exposure time, and concentration of detergents and enzymes, among others. Optimization of decellularization protocols involves the fine-tuning of these and other parameters to achieve efficient decellularization while maintaining matrix integrity. Previous work has shown that decellularization of heart valves supplemented with granulocyte fractions influences the host response and rate of endogenous recellularization [47]. These findings directly implicate the efficiency of the decellularization process (i.e., presence of antigenic cellular components) as a critical step in the manufacturing of these tissue-derived scaffold materials. A recent study by Londono et al. further showed that cell remnants can negatively impact the early macrophage response [48]. A separate study by Keane also implicated decellularization efficacy as a strong determinant of downstream outcome [1, 48]. Standardization of criteria for determining the extent of decellularization has not been regulated by the FDA. At the present time, there is no consensus on the minimum acceptable criteria for residual cell products within ECM biomaterials that are commercially available; however, preclinical studies have suggested some guidelines [49]. The use of bovine and porcine source tissues

for preparing ECM bioscaffolds is common because of the compositional similarity of the matrix to human matrix and their relative availability. In summary, the problems associated with the lack of uniform decellularization standards have limited progress and accounted for variability in clinical outcomes.

An ambitious goal of the tissue engineering field is the decellularization of whole organs, including hearts, followed by recellularization of the resultant three-dimensional ECM bioscaffold to produce a substitute for donor organs in heart transplantation (i.e., whole-organ engineering). Typically, whole-organ decellularization is performed utilizing a perfusion method to maintain the resident vasculature and allow for ex vivo reperfusion with stem/progenitor cells. A recent study by Lee et al. showed that even orientation of the heart during perfusion can impact decellularization outcomes, indicating that flow dynamics is yet another variable to consider in optimizing decellularization of whole hearts [50]. Studies that attempt to use detergent-free, non-proteolytic, actin-disassembling methods for decellularization have shown preclinical success in achieving efficient decellularization [51]. As previously stated, the ultimate objective of decellularization is to avoid an adverse immune response due to antigens retained within the scaffold material.

8.3.2 Source Tissue

The impact of source tissue from which an acellular, ECM bioscaffold is derived (source age, species, and the use of heterologous vs. homologous tissue) upon a functional remodeling outcome is controversial. A study by Chen et al. showed that mammalian cardiac ECM may not be the optimal source tissue for cardiac applications. For example, zebra fish cardiac ECM harvested from healing hearts can promote functional cardiac recovery and regeneration of adult mouse heart tissue after acute myocardial infarction and induce proliferative and chemotactic effects upon human cardiac progenitor cells [52].

An additional factor to consider in ECM-based biomaterial design is the age of the source animal from which the ECM is derived. ECM derived from younger pigs vs. aged (>1 year) pigs, for example, is associated with less collagen deposition, more site-appropriate tissue formation, and an improved macrophage response [53]. A study by Li et al. suggests that cross talk between cardiac fibroblasts and the surrounding matrix is also age-dependent. In this example, the age of cardiac fibroblasts seeded within a 3D-engineered construct is a strong determinant of the structure, function, and bioactive milieu within the cardiac matrix which ultimately impacts cardiac function and maturation [54]. Brown et al. have recently provided a review of age-related effects upon cell-matrix interactions with respect to ECM biomaterials [55].

A study by Johnson et al. compared extracellular matrix hydrogels derived from both human and porcine myocardium. While extensive similarities were found between species' matrices, there were differences in sulfated glycosaminoglycan (sGAG) content, the ability to promote aortic smooth muscle and coronary artery

endothelial cell proliferation, and early induction of differentiation of human cardiomyocyte progenitor cells, with human-derived myocardial matrix performing slightly better. However, porcine matrix did not differ enough in performance compared to human matrix to warrant a switch to human tissue sourcing when pursuing clinical applications [56]. Sadtler et al. showed that cardiac ECM harbors advantageous immunomodulatory properties, inducing a higher expression of IL-4 after a volumetric muscle loss injury and promoting functional remodeling that is similar to that of the healthy control animals [45].

8.3.3 Terminal Sterilization

A frequently overlooked processing parameter when developing a biomaterial, and especially biologic scaffold-based materials, is the terminal sterilization method. Biologic scaffolds have been typically regulated as a medical device by the Food and Drug Administration (FDA), which requires validation of sterility prior to clinical use. However, many of the commonly used sterilization methods result in destruction of the matrix ultrastructure and bioactivity. Dearth et al. investigated the effects of ethylene oxide, gamma irradiation, and electron beam (e-beam) irradiation on structural, mechanical, biochemical properties, cytocompatibility, and the host response to ECM bioscaffold sheet materials and showed clear differential effects of sterilization methods on bioscaffold properties [57]. Alternative methods to sterilize acellular biologic scaffolds composed of cardiac ECM include ethanol and UV light sterilization [58] and simple peracetic acid (PAA) washes [59], though the efficacy of these methods to achieve clinically acceptable sterilization has yet to be determined.

Decellularized heart valves have proven to be a challenge for sterilization due to the delicacy and variability in thickness and structure across the cusps, root, and aortic wall. Hennessy et al. conducted a head-to-head comparison of the use of electrolyzed water, gamma radiation, ethanol and PAA, hydrogen peroxide, and supercritical CO_2 ($scCO_2$) to sterilize decellularized porcine aortic valves. Results showed only ethanol and PAA and supercritical CO_2 to achieve effective sterilization and still retain the tensile strength of the valve. Only the supercritical CO_2 method avoided extensive ECM crosslinking/denaturation of the ultrastructure according to calorimetric testing [60], making it a promising approach to pursue for terminal sterilization of ECM-based materials. Sterilization of other forms of ECM biomaterials such as powders (particulate forms), hydrogels, and soluble fractions is an important area of future investigation to expedite clinical translation.

8.3.4 Chemical Crosslinking

The use of chemical crosslinking for biologic scaffolds has relied on the rationale of (1) preventing scaffold degradation and thereby conferring preserved or increased mechanical strength and (2) preventing an adverse immune reaction.

Although cardiac tissue engineering and regenerative medicine research has focused on the replacement of cardiac tissue after myocardial infarction and ischemic injury, the use of xenogeneic, "fixed" (i.e., chemically crosslinked) porcine and bovine valves has a long history and is a commonly used approach to replace human valves. These porcine and bovine valves are often crosslinked with glutaraldehyde to increase the strength and durability of the valve while purportedly limiting the immunogenicity of the xenogeneic material. One reported source of immunogenic epitopes is the oligosaccharide galactose (galactosyl-α (1,3)-galactose (α-Gal) epitope), a xeno-antigen that is present in all mammals with the exception of humans and Old World monkeys. However, a study by Daly et al. showed that although remnants of the alpha-Gal epitope existed within decellularized ECM materials from porcine sources, implantation of these materials resulted in an increase in serum anti-Gal antibodies but had no adverse effect upon the remodeling response [61].

Glutaraldehyde crosslinking of heart valves, although common, causes a reduction in valve durability and increases the susceptibility to calcification [62, 63]. Other attempts to stabilize or strengthen valves, including quercetin crosslinking [64], have been successful, yet the rationale behind crosslinking remains only partly justified. In fact, studies have shown that chemical crosslinking promotes an adverse innate immune response to biologic scaffold materials, whereas non-crosslinked ECM materials are actually immune-friendly and stimulate endogenous tissue ingrowth.

8.3.5 Mechanical Properties of ECM Bioscaffolds Intended for Cardiac Applications

In addition to bioactive soluble and insoluble effector molecules within the ECM, mechanical stresses have a notable influence upon cell behavior. Cells sense deformation and mechanical forces from the surrounding matrix via actomyosin and cytosolic components. These signals are transmitted via focal adhesions, and in response these cells remodel the matrix by altering their secretome (i.e., an example of dynamic reciprocity) [65, 66]. One example of this reciprocal relationship is the rate and force of cardiomyocyte beating. Cardiomyocytes prefer a substrate with the same mechanical properties as native cardiac matrix [67]. Native ECM mechanics has also been shown to regulate migration and collective motion of cells and multicellular organization, and these forces can have direct effects upon gene expression [68]. Further, the dysregulation of mechanical properties of the matrix and/or mechanosensing of the cells affects disease progress including cancer progression [69], muscular dystrophy [70], inflammatory diseases [71], hypertension, atherosclerosis, and mitral valve disease, among others [72].

The design and successful implementation of ECM-based biomaterials for cardiac applications therefore is highly dependent upon mechanical properties of the

matrix itself and the applied physiologic loads imposed upon the matrix either ex vivo or in vivo. A study by Converse et al. evaluated the effect of decellularization and conditioning upon the mechanical properties of ovine leaflets showing the destruction and restoration of circumferential relaxation behavior with decellularization and reconditioning, respectively [73]. Cardiovascular cells and matrix are continually exposed to a variety of pulsatile, complex compressive and tensile strains and shear forces. Whether utilizing a whole-organ replacement ECM scaffold, a cardiac patch, a decellularized heart valve, or an ECM-based hydrogel, mechanical and rheologic properties of each scaffold material must provide the appropriate physical and biochemical environment to facilitate site-appropriate remodeling and withstand the mechanical forces exerted by the circulatory system. Both translational science and basic science would benefit from continued research to determine the precise effects of static and cyclic mechanical deformations upon cardiac ECM and remodeling.

8.4 Forms of ECM as a Cardiac Therapeutic

A trend toward utilizing biomaterial-based approaches for cardiac repair as opposed to cell-based, gene-based, or protein-based therapies has sparked the development of novel treatments for a wide range of cardiovascular diseases including valve degeneration, myocardial infarction, end-stage organ failure, and vascular insufficiency, among others. Regulatory hurdles, shelf life, delivery methods, and manufacturing challenges have prevented timely translation to widespread clinical use of many tissue engineering and regenerative medicine-based therapies; however, the pursuit of utilizing ECM-based biomaterials for cardiac therapy has significant translatable potential and will ultimately impact a large number of patients (Fig. 8.1).

8.4.1 Decellularized Heart Valves

Valvular heart disease is a major healthcare issue worldwide and is mainly addressed by replacement with mechanical or biologic prostheses. Mechanical valves have been in use since the mid-1950s including caged-ball, tilting-disk, and bi-leaflet valves (among other iterations). These mechanical valves are made from synthetic materials including titanium, pyrolytic carbon, polyester, Dacron, or polytetrafluorethylene (PTFE). While durable, these materials have limited hemocompatibility and require simultaneous and lifelong anticoagulation therapy to minimize the risk of thromboembolism [74]. Xenogeneic bioprosthetic heart valves are also widely used to treat valvular disease. As mentioned above, such valves are typically bovine or porcine sourced and are crosslinked with glutaraldehyde. While bioprosthetic heart valves have superior hemodynamic properties compared to mechanical valves, glutaraldehyde crosslinking increases susceptibility to calcification. These valves

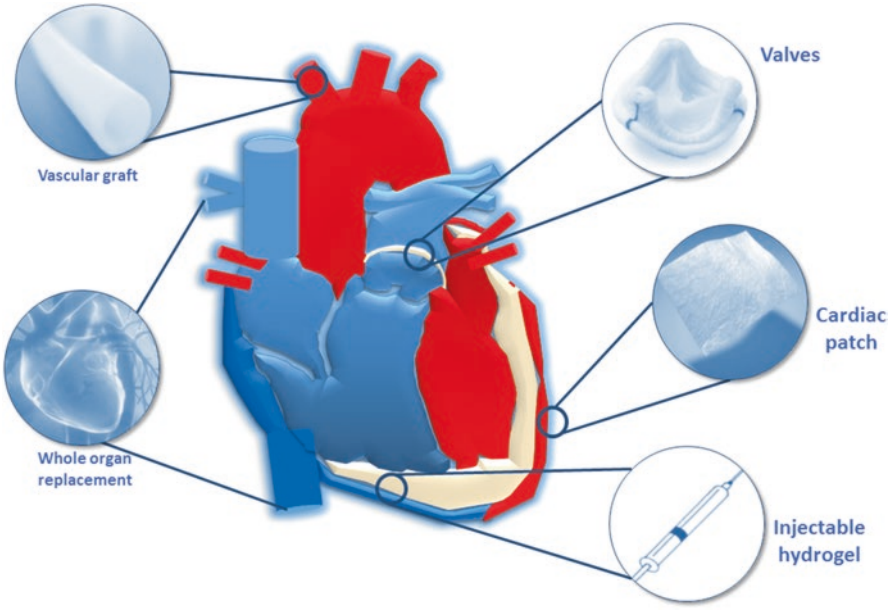

Fig. 8.1 Cardiovascular applications and formulations of ECM bioscaffolds and xenogeneic materials to treat disease

are also subject to premature degeneration and lack the ability to grow and remodel along with the patient for pediatric applications, contributing to the need for multiple reoperations. Since no suitable valve replacement exists as a long-term and/or pediatric solution, the field of tissue engineering and regenerative medicine has pursued the use of decellularized heart valves as an alternative treatment option that provides superior hemodynamics without the need for anticoagulation therapy, promotes active tissue remodeling, and maintains the ability to grow with the patient, thus minimizing the need for repeated operations.

Many methods for decellularization of heart valves have been proposed in preclinical studies including the use of anionic detergents and/or nonionic detergents [75], multi-detergents [76], enzymatic combinations [59], glycol radiation [77], osmotic shock [78], sequential antigen removal [79], and supercritical CO_2 treatment [80]. These methods have variable outcomes with respect to DNA removal (ranging from 30% to 98% DNA removal) and also upon mechanical properties of the ECM including an increased strain to stretch ratio [75], reduced glycosaminoglycan (GAG) and ECM protein content, misalignment of collagen fibers, and stiffening/dehydration of the ECM. All of these methods and their subsequent effects upon ECM properties will have implications in clinical and preclinical study performance. The use of decellularized heart valves has been associated with mixed clinical results ranging from catastrophic failure due to a severe immune response

in children [81] to long-term success and 100% freedom for reoperation [82]. While attempts have been made to recellularize and precondition tissue-engineered heart valves to create a non-thrombotic, durable, and living valve, cellular reseeding of valves is associated with its own issues including cell sourcing. However, recent studies have shown promising clinical results; specifically, Dohmen et al. showed 100% survival at 10 years and 3 months of biopsy point showing endothelialization and partial recellularization by fibroblasts with excellent hemodynamic performance [83].

Overall, the use of decellularized heart valves is an attractive alternative to the currently used technologies for treating valvular disease and remains a point of significant interest. Regulatory scrutiny for these valves is evolving, emphasizing valve functional safety. Development of performance markers in addition to traditional monitoring via MRI and echocardiography will be important in the successful development of treatment options that challenge existing paradigms.

8.4.2 Cardiac Patch

The use of ECM-based patch materials for cardiovascular applications has received widespread attention in preclinical animal studies including carotid artery grafts, aortic grafts, venous grafts, myocardial repair, pulmonary artery grafts, vascular patches, and ischemic heart failure. The use of such materials has shown promising results with respect to supporting endogenous cell infiltration and tissue growth and ultimately reorganized, functional tissue formation.

A study by Kochupura et al. in 2005 showed that an ECM-based myocardial patch can restore mechanical function in a canine full-thickness right ventricular defect, promoting an increase in systolic function and increased infiltration of cardiomyocytes [84]. ECM-based cardiac patch use in preclinical models has not been limited to myocardial repair but has also been investigated for the replacement of arteries and other vessels. A study by Fallon et al. in 2011 showed that an ECM-based cardiac patch can promote carotid artery repair in a sheep model and was associated with rapid endothelialization [85]. Cardiac patches have been particularly attractive for treating congenital defects. For example, Wainwright et al. showed that a cardiac ECM patch was superior to Dacron in promoting functional and histomorphologic remodeling in a rat model of right ventricular outflow tract reconstruction without causing rejection, stenosis, aneurysm, or calcification [86]. This response was evident not only with homologous ECM (i.e., cardiac ECM) but also was shown to occur when utilizing heterologous ECM like urinary bladder matrix (UBM) [87]. ECM-based materials have the added advantage of accommodating growth of a pediatric patient due to their degradation rate.

The results from preclinical studies investigating the use of ECM as a cardiac patch and vascular graft have been promising. While there are some reported thrombosis occurrences in preclinical models, in general ECM-induced remodeling has been shown to promote stronger arteries than that of native tissue [88], patent

grafts [89], low leakage rates [90], host tissue integration [91], and complete remodeling by 6 months [92].

ECM-based products are commercially available for cardiovascular surgery indications. For example, CorMatrix® (CorMatrix Cardiovascular Inc., Roswell, GA, USA) is FDA approved and has had the European CE mark for indications including pericardial repair and reconstruction, cardiac tissue repair, carotid artery repair, and enveloping implantable electronic devices to restrict their migration and impede infection [93]. The CorMatrix® graft has been used clinically since 2010. Clinical results have shown very few reports of complications when used as a venous repair device; however, higher-pressure intracardiac sites such as valve replacement are associated with increased risk of complications. Many of the complications have implicated an inadequate decellularization of the ECM material with the associated adverse immune response as the root cause [93]. However, the widespread use of ECM materials in preclinical and clinical cardiovascular applications still warrants further clinical studies to capitalize upon the favorable qualities including its ability to promote site-appropriate, functional remodeling, its usability in the operating room, its lack of calcification, the avoidance of thickening or retraction complications, and the potential use for pediatric applications.

8.4.3 Whole-Organ Replacement

Most commercially available ECM bioscaffolds that are utilized as cardiac therapeutics are prepared as thin sheets or hydrogels after decellularization of host tissues that are not necessarily cardiac in origin. The pursuit of a whole-organ replacement through whole-organ engineering requires different approaches of decellularization due to the density, mass, and complexity of an intact heart's 3D architecture. Preclinical studies involving perfusion decellularization through the vasculature have been successful when utilizing smaller rodent hearts [94]; however, scale-up to larger hearts that would be required in the clinical setting, such as porcine or bovine hearts, remains a challenge. In 2010, Wainwright et al. showed that while a reproducible and time-efficient (e.g., under 10 h) decellularization could be optimized for the porcine heart via retrograde aortic perfusion with hypertonic, hypotonic, enzymatic, acid, and detergent solutions, the cellular reseeding of large organs requires additional research [95]. Recently, Sánchez et al. showed that human hearts can be adequately decellularized while preserving its ultrastructure, 3D architecture, and vascularity and can be reseeded using human cardiac progenitor cells (hCPC), bone marrow-derived mesenchymal cells (hMSCs), human endothelial cells (HUVECs), and cardiomyocytes [96]. Guyette et al. also showed that the perfusion decellularization of rodent hearts could be scaled to humans using human hearts repopulated with cardiomyocytes derived from nontransgenic human iPS cells and the resulting heart, under biomimetic culture, developed functional, contractile tissue [97].

Repopulating the cardiac 3D ECM scaffold after decellularization has been the main pursuit in the whole-organ engineering community since the original description of perfusion decellularization. Ideally, reseeded cells would be autologous to minimize an adverse immune response and the requirement for immunosuppression. Should the eventual recipient have healthy cells remaining in their native tissue, biopsy samples can be taken, and cells can be expanded in culture to reseed the scaffold. Alternatively, other possibilities include the use of multipotent or induced pluripotent stem (iPS) cells [98], though the cost of culture and expansion techniques required to generate adequate numbers of cells provides an added challenge. The structural and cytologic variability of the whole heart also introduces challenges of reseeding techniques that can adequately cover the entire structure. Considerations of unidirectional or bidirectional vascular reseeding, the use of static or dynamic stretch, electrical stimulation, and different media components in the bioreactor, age matching of donor hearts if of human source, and a better understanding of the immune response to these materials are required prior to efficient translation of a functional heart [97].

Not only do technical challenges represent a significant hurdle to the translation of whole-organ engineered products, but regulatory and ethical concerns also slow progress. The competition between the desire to expedite the use of these products clinically due to demand, pursuit of prestige, and monetary gains must be balanced by the cautious execution of clinical trials initiated only after sufficient preclinical evidence is generated, cell source and surgical technique are explained with transparency, appropriate patient consent and explanation (including the financial burden) are obtained, and the ability to solve potential failures in clinical trials has been established. The promise of engineered whole organs attracts many to the field of tissue engineering and regenerative medicine; however, these barriers, among others, must be addressed head-on to achieve the desired end point.

8.4.4 ECM Hydrogels for Minimally Invasive Therapies

Scaffold biomaterials and whole-organ approaches to replacing damaged/diseased cardiac tissue have stimulated significant research efforts into identifying a minimally invasive approach. Patches require a thoracotomy, and most other injectable approaches for repairing cardiac tissues are not compatible with catheterization [99]. Because of this, an injectable ECM hydrogel has recently gained traction as a potential therapy for treating myocardial infarction (MI) patients. The hydrogel form of ECM can be injected directly into the infarct site and has been shown in preclinical models to promote left ventricular remodeling and preserve ejection fraction [100]. The cardiac ECM hydrogel is biocompatible (i.e., safe and effective) in a porcine MI model. This study showed reduced fibrosis following MI and promoted cardiac muscle infiltration while maintaining hemocompatibility. In addition, there was no embolization, ischemia, or adverse effects upon peripheral tissues or cardiac rhythm [101].

ECM hydrogels have desirable material properties for injection. For example, ECM hydrogels are shear thinning and possess gelation kinetics that allow them to remain liquid within a syringe or catheter but forming a solid gel to support cell infiltration and growth upon injection into myocardial tissue [102]. While synthetic hydrogels have also been investigated for use as a cardiac therapeutic due to the tunability of their properties, the advantage of utilizing an ECM hydrogel includes the favorable immune response, increased endogenous progenitor cell chemotaxis, proliferation, and differentiation, inclusion of inherent growth factors, and appropriate ultrastructural constituents that support cell survival, among others [103].

8.4.5 Conclusion

The promise of biomaterials derived from the extracellular matrix for cardiac repair including valve replacement, repair of functional myocardium, whole-organ replacement, or vascular repair is exciting. The hope that the extracellular matrix can provide a relevant strategy in multiple cardiac implications is not unfounded and has already achieved clinical success in some cases. The widespread application of ECM materials to treat patients with heart disease will hinge upon a multidisciplinary approach that supports not only continued progress in the laboratory but also progress with the regulatory and economic burdens associated with clinical trials that must be conducted to achieve the necessary data to fully evaluate this approach.

References

1. Keane TJ, Londono R, Turner NJ, Badylak SF. Consequences of ineffective decellularization of biologic scaffolds on the host response. Biomaterials. 2012;33(6):1771–81.
2. Sicari BM, Dziki JL, Siu BF, Medberry CJ, Dearth CL, Badylak SF. The promotion of a constructive macrophage phenotype by solubilized extracellular matrix. Biomaterials. 2014;35(30):8605–12.
3. Brown BN, Londono R, Tottey S, Zhang L, Kukla KA, Wolf MT, Daly KA, Reing JE, Badylak SF. Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials. Acta Biomater. 2012;8(3):978–87.
4. Agrawal V, Johnson SA, Reing J, Zhang L, Tottey S, Wang G, Hirschi KK, Braunhut S, Gudas LJ, Badylak SF. Epimorphic regeneration approach to tissue replacement in adult mammals. Proc Natl Acad Sci U S A. 2010;107(8):3351–5.
5. Agrawal V, Kelly J, Tottey S, Daly KA, Johnson SA, Siu BF, Reing J, Badylak SF. An isolated cryptic peptide influences osteogenesis and bone remodeling in an adult mammalian model of digit amputation. Tissue Eng Part A. 2011;17(23–24):3033–44.
6. Dziki JL, Sicari BM, Wolf MT, Cramer MC, Badylak SF. Immunomodulation and mobilization of progenitor cells by extracellular matrix bioscaffolds for volumetric muscle loss treatment. Tissue Eng Part A. 2016;22(19–20):1129–39.

7. Reing JE, Zhang L, Myers-Irvin J, Cordero KE, Freytes DO, Heber-Katz E, Bedelbaeva K, McIntosh D, Dewilde A, Braunhut SJ, Badylak SF. Degradation products of extracellular matrix affect cell migration and proliferation. Tissue Eng Part A. 2009;15(3):605–14.
8. Vorotnikova E, McIntosh D, Dewilde A, Zhang J, Reing JE, Zhang L, Cordero K, Bedelbaeva K, Gourevitch D, Heber-Katz E, Badylak SF, Braunhut SJ. Extracellular matrix-derived products modulate endothelial and progenitor cell migration and proliferation in vitro and stimulate regenerative healing in vivo. Matrix Biol. 2010;29(8):690–700.
9. Shaffiey SA, Jia H, Keane T, Costello C, Wasserman D, Quidgley M, Dziki J, Badylak S, Sodhi CP, March JC, Hackam DJ. Intestinal stem cell growth and differentiation on a tubular scaffold with evaluation in small and large animals. Regen Med. 2016;11(1):45–61.
10. Han N, Yabroudi MA, Stearns-Reider K, Helkowski W, Sicari BM, Rubin JP, Badylak SF, Boninger ML, Ambrosio F. Electrodiagnostic evaluation of individuals implanted with extracellular matrix for the treatment of volumetric muscle injury: case series. Phys Ther. 2016;96(4):540–9.
11. Turner NJ, Yates AJ Jr, Weber DJ, Qureshi IR, Stolz DB, Gilbert TW, Badylak SF. Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction. Tissue Eng Part A. 2010;16(11):3309–17.
12. Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. The extracellular matrix: tools and insights for the "omics" era. Matrix Biol. 2016;49:10–24.
13. Hynes RO, Naba A. Overview of the matrisome--an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol. 2012;4(1):a004903.
14. Valentin JE, Stewart-Akers AM, Gilbert TW, Badylak SF. Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds. Tissue Eng Part A. 2009;15(7):1687–94.
15. Carey LE, Dearth CL, Johnson SA, Londono R, Medberry CJ, Daly KA, Badylak SF. In vivo degradation of 14C-labeled porcine dermis biologic scaffold. Biomaterials. 2014;35(29):8297–304.
16. Costa A, Naranjo JD, Turner NJ, Swinehart IT, Kolich BD, Shaffiey SA, Londono R, Keane TJ, Reing JE, Johnson SA, Badylak SF. Mechanical strength vs. degradation of a biologically-derived surgical mesh over time in a rodent full thickness abdominal wall defect. Biomaterials. 2016;108:81–90.
17. Brown BN, Badylak SF. Expanded applications, shifting paradigms and an improved understanding of host-biomaterial interactions. Acta Biomater. 2013;9(2):4948–55.
18. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. Biomaterials. 2009;30(8):1482–91.
19. Ma B, Wang X, Wu C, Chang J. Crosslinking strategies for preparation of extracellular matrix-derived cardiovascular scaffolds. Regen Biomater. 2014;1(1):81–9.
20. Allman AJ, McPherson TB, Badylak SF, Merrill LC, Kallakury B, Sheehan C, Raeder RH, Metzger DW. Xenogeneic extracellular matrix grafts elicit a TH2-restricted immune response. Transplantation. 2001;71(11):1631–40.
21. Dearth CL, Slivka PF, Stewart SA, Keane TJ, Tay JK, Londono R, Goh Q, Pizza FX, Badylak SF. Inhibition of COX1/2 alters the host response and reduces ECM scaffold mediated constructive tissue remodeling in a rodent model of skeletal muscle injury. Acta Biomater. 2016;31:50–60.
22. Faulk DM, Londono R, Wolf MT, Ranallo CA, Carruthers CA, Wildemann JD, Dearth CL, Badylak SF. ECM hydrogel coating mitigates the chronic inflammatory response to polypropylene mesh. Biomaterials. 2014;35(30):8585–95.
23. Hachim D, LoPresti ST, Yates CC, Brown BN. Shifts in macrophage phenotype at the biomaterial interface via IL-4 eluting coatings are associated with improved implant integration. Biomaterials. 2016;112:95–107.
24. Lech M, Anders HJ. Macrophages and fibrosis: how resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair. Biochim Biophys Acta. 2013;1832(7):989–97.

25. Sorokin L. The impact of the extracellular matrix on inflammation. Nat Rev Immunol. 2010;10(10):712–23.
26. Brennan EP, Tang XH, Stewart-Akers AM, Gudas LJ, Badylak SF. Chemoattractant activity of degradation products of fetal and adult skin extracellular matrix for keratinocyte progenitor cells. J Tissue Eng Regen Med. 2008;2(8):491–8.
27. Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. Tissue Eng Part A. 2011;17(19–20):2435–43.
28. Brennan EP, Reing J, Chew D, Myers-Irvin JM, Young EJ, Badylak SF. Antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix. Tissue Eng. 2006;12(10):2949–55.
29. Ratner BD. New ideas in biomaterials science--a path to engineered biomaterials. J Biomed Mater Res. 1993;27(7):837–50.
30. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008;29(20):2941–53.
31. Kahan BD. Individuality: the barrier to optimal immunosuppression. Nat Rev Immunol. 2003;3(10):831–8.
32. Williams DF. There is no such thing as a biocompatible material. Biomaterials. 2014;35(38):10009–14.
33. Liu Q, Turnquist HR. Implications for Interleukin-33 in solid organ transplantation. Cytokine. 2013;62(2):183–94.
34. van den Bosch TP, Caliskan K, Kraaij MD, Constantinescu AA, Manintveld OC, Leenen PJ, von der Thusen JH, Clahsen-van Groningen MC, Baan CC, Rowshani AT. CD16+ monocytes and skewed macrophage polarization toward M2 type Hallmark heart transplant acute cellular rejection. Front Immunol. 2017;8:346.
35. Fakoya AO. New delivery Systems of Stem Cells for vascular regeneration in ischemia. Front Cardiovasc Med. 2017;4:7.
36. Kaplan A, Altara R, Eid A, Booz GW, Zouein FA. Update on the protective role of regulatory T cells in myocardial infarction: a promising therapy to repair the heart. J Cardiovasc Pharmacol. 2016;68(6):401–13.
37. Tidball JG, Dorshkind K, Wehling-Henricks M. Shared signaling systems in myeloid cell-mediated muscle regeneration. Development. 2014;141(6):1184–96.
38. Rogier EW, Frantz AL, Bruno ME, Wedlund L, Cohen DA, Stromberg AJ, Kaetzel CS. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. Proc Natl Acad Sci U S A. 2014;111(8):3074–9.
39. Liu W, Zhang X, Zhao M, Zhang X, Chi J, Liu Y, Lin F, Fu Y, Ma D, Yin X. Activation in M1 but not M2 macrophages contributes to cardiac remodeling after myocardial infarction in rats: a critical role of the calcium sensing receptor/NRLP3 Inflammasome. Cell Physiol Biochem. 2015;35(6):2483–500.
40. Howard CM, Baudino TA. Dynamic cell-cell and cell-ECM interactions in the heart. J Mol Cell Cardiol. 2014;70:19–26.
41. Jin YF, Han HC, Berger J, Dai Q, Lindsey ML. Combining experimental and mathematical modeling to reveal mechanisms of macrophage-dependent left ventricular remodeling. BMC Syst Biol. 2011;5:60.
42. Godwin JW, Pinto AR, Rosenthal NA. Macrophages are required for adult salamander limb regeneration. Proc Natl Acad Sci U S A. 2013;110(23):9415–20.
43. Badylak SF, Valentin JE, Ravindra AK, McCabe GP, Stewart-Akers AM. Macrophage phenotype as a determinant of biologic scaffold remodeling. Tissue Eng Part A. 2008;14(11):1835–42.
44. Dziki JL, Giglio RM, Sicari BM, Wang DS, Gandhi RM, Londono R, Dearth CL, Badylak SF. The effect of mechanical loading upon extracellular matrix bioscaffold-mediated skeletal muscle remodeling. Tissue Eng Part A. 2017;24:34.
45. Sadtler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, Patel CH, Luber BS, Wang H, Wagner KR, Powell JD, Housseau F, Pardoll DM, Elisseeff JH. Developing a pro-

regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science. 2016;352(6283):366–70.
46. Dziki JL, Wang DS, Pineda C, Sicari BM, Rausch T, Badylak SF. Solubilized extracellular matrix bioscaffolds derived from diverse source tissues differentially influence macrophage phenotype. J Biomed Mater Res A. 2017;105(1):138–47.
47. Sanders B, Driessen-Mol A, Bouten CVC, Baaijens FPT. The effects of scaffold remnants in Decellularized tissue-engineered cardiovascular constructs on the recruitment of blood cells. Tissue Eng Part A. 2017;23:1142.
48. Londono R, Dziki JL, Haljasmaa E, Turner NJ, Leifer CA, Badylak SF. The effect of cell debris within biologic scaffolds upon the macrophage response. J Biomed Mater Res A. 2017;105(8):2109–18.
49. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32(12):3233–43.
50. Lee PF, Chau E, Cabello R, Yeh AT, Sampaio LC, Gobin AS, Taylor DA. Inverted orientation improves decellularization of whole porcine hearts. Acta Biomater. 2017;49:181–91.
51. Assmann A, Struss M, Schiffer F, Heidelberg F, Munakata H, Timchenko EV, Timchenko PE, Kaufmann T, Huynh K, Sugimura Y, Leidl Q, Pinto A, Stoldt VR, Lichtenberg A, Akhyari P. Improvement of the in vivo cellular repopulation of decellularized cardiovascular tissues by a detergent-free, non-proteolytic, actin-disassembling regimen. J Tissue Eng Regen Med. 2017;11:3530.
52. Chen WC, Wang Z, Missinato MA, Park DW, Long DW, Liu HJ, Zeng X, Yates NA, Kim K, Wang Y. Decellularized zebrafish cardiac extracellular matrix induces mammalian heart regeneration. Sci Adv. 2016;2(11):e1600844.
53. Sicari BM, Johnson SA, Siu BF, Crapo PM, Daly KA, Jiang H, Medberry CJ, Tottey S, Turner NJ, Badylak SF. The effect of source animal age upon the in vivo remodeling characteristics of an extracellular matrix scaffold. Biomaterials. 2012;33(22):5524–33.
54. Li Y, Asfour H, Bursac N. Age-dependent functional crosstalk between cardiac fibroblasts and cardiomyocytes in a 3D engineered cardiac tissue. Acta Biomater. 2017;55:120–30.
55. Brown BN, Haschak MJ, LoPresti ST, Stahl EC. Effects of age-related shifts in cellular function and local microenvironment upon the innate immune response to implants. Semin Immunol. 2017;29:24–32.
56. Johnson TD, Dequach JA, Gaetani R, Ungerleider J, Elhag D, Nigam V, Behfar A, Christman KL. Human versus porcine tissue sourcing for an injectable myocardial matrix hydrogel. Biomater Sci. 2014;2014:60283D.
57. Dearth CL, Keane TJ, Carruthers CA, Reing JE, Huleihel L, Ranallo CA, Kollar EW, Badylak SF. The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold. Acta Biomater. 2016;33:78–87.
58. Wang B, Williams LN, de Jongh Curry AL, Liao J. Preparation of acellular myocardial scaffolds with well-preserved cardiomyocyte lacunae, and method for applying mechanical and electrical simulation to tissue construct. Methods Mol Biol. 2014;1181:189–202.
59. Luo J, Korossis SA, Wilshaw SP, Jennings LM, Fisher J, Ingham E. Development and characterization of acellular porcine pulmonary valve scaffolds for tissue engineering. Tissue Eng Part A. 2014;20(21–22):2963–74.
60. Hennessy RS, Jana S, Tefft BJ, Helder MR, Young MD, Hennessy RR, Stoyles NJ, Lerman A. Supercritical carbon dioxide-based sterilization of decellularized heart valves. JACC Basic Transl Sci. 2017;2(1):71–84.
61. Daly KA, Stewart-Akers AM, Hara H, Ezzelarab M, Long C, Cordero K, Johnson SA, Ayares D, Cooper DK, Badylak SF. Effect of the alphaGal epitope on the response to small intestinal submucosa extracellular matrix in a nonhuman primate model. Tissue Eng Part A. 2009;15(12):3877–88.
62. Schmidt D, Stock UA, Hoerstrup SP. Tissue engineering of heart valves using decellularized xenogeneic or polymeric starter matrices. Philos Trans R Soc Lond Ser B Biol Sci. 2007;362(1484):1505–12.

63. Tam H, Zhang W, Feaver KR, Parchment N, Sacks MS, Vyavahare N. A novel crosslinking method for improved tear resistance and biocompatibility of tissue based biomaterials. Biomaterials. 2015;66:83–91.
64. Zhai W, Lu X, Chang J, Zhou Y, Zhang H. Quercetin-crosslinked porcine heart valve matrix: mechanical properties, stability, anticalcification and cytocompatibility. Acta Biomater. 2010;6(2):389–95.
65. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126(4):677–89.
66. Chowdhury F, Na S, Li D, Poh YC, Tanaka TS, Wang F, Wang N. Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells. Nat Mater. 2010;9(1):82–8.
67. Engler AJ, Carag-Krieger C, Johnson CP, Raab M, Tang HY, Speicher DW, Sanger JW, Sanger JM, Discher DE. Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating. J Cell Sci. 2008;121(Pt 22):3794–802.
68. Sapir L, Tzlil S. Talking over the extracellular matrix: how do cells communicate mechanically? Semin Cell Dev Biol. 2017;71:99.
69. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, Weaver VM. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005;8(3):241–54.
70. Holland A, Murphy S, Dowling P, Ohlendieck K. Pathoproteomic profiling of the skeletal muscle matrisome in dystrophinopathy associated myofibrosis. Proteomics. 2016;16(2):345–66.
71. Zorzi F, Calabrese E, Monteleone G. Pathogenic aspects and therapeutic avenues of intestinal fibrosis in Crohn's disease. Clin Sci (Lond). 2015;129(12):1107–13.
72. Gupta V, Grande-Allen KJ. Effects of static and cyclic loading in regulating extracellular matrix synthesis by cardiovascular cells. Cardiovasc Res. 2006;72(3):375–83.
73. Converse GL, Armstrong M, Quinn RW, Buse EE, Cromwell ML, Moriarty SJ, Lofland GK, Hilbert SL, Hopkins RA. Effects of cryopreservation, decellularization and novel extracellular matrix conditioning on the quasi-static and time-dependent properties of the pulmonary valve leaflet. Acta Biomater. 2012;8(7):2722–9.
74. Maganti K, Rigolin VH, Sarano ME, Bonow RO. Valvular heart disease: diagnosis and management. Mayo Clin Proc. 2010;85(5):483–500.
75. Liao J, Joyce EM, Sacks MS. Effects of decellularization on the mechanical and structural properties of the porcine aortic valve leaflet. Biomaterials. 2008;29(8):1065–74.
76. Naso F, Gandaglia A, Formato M, Cigliano A, Lepedda AJ, Gerosa G, Spina M. Differential distribution of structural components and hydration in aortic and pulmonary heart valve conduits: impact of detergent-based cell removal. Acta Biomater. 2010;6(12):4675–88.
77. Ota T, Taketani S, Iwai S, Miyagawa S, Furuta M, Hara M, Uchimura E, Okita Y, Sawa Y. Novel method of decellularization of porcine valves using polyethylene glycol and gamma irradiation. Ann Thorac Surg. 2007;83(4):1501–7.
78. Somers P, De Somer F, Cornelissen M, Thierens H, Van Nooten G. Decellularization of heart valve matrices: search for the ideal balance. Artif Cells Blood Substit Immobil Biotechnol. 2012;40(1–2):151–62.
79. Marsh ME. Immunocytochemical localization of a calcium-binding phosphoprotein in hemocytes of heterodont bivalves. J Exp Zool. 1990;253(3):280–6.
80. Sawada K, Terada D, Yamaoka T, Kitamura S, Fujisato T. Cell removal with supercritical carbon dioxide for acellular artificial tissue. J Chem Technol Biotechnol. 2008;83(6):943–9.
81. Simon P, Kasimir MT, Seebacher G, Weigel G, Ullrich R, Salzer-Muhar U, Rieder E, Wolner E. Early failure of the tissue engineered porcine heart valve SYNERGRAFT in pediatric patients. Eur J Cardiothorac Surg. 2003;23(6):1002–6. discussion 1006
82. Cebotari S, Tudorache I, Ciubotaru A, Boethig D, Sarikouch S, Goerler A, Lichtenberg A, Cheptanaru E, Barnaciuc S, Cazacu A, Maliga O, Repin O, Maniuc L, Breymann T, Haverich A. Use of fresh decellularized allografts for pulmonary valve replacement may reduce

the reoperation rate in children and young adults: early report. Circulation. 2011;124(11 Suppl):S115–23.
83. Dohmen PM, Lembcke A, Holinski S, Pruss A, Konertz W. Ten years of clinical results with a tissue-engineered pulmonary valve. Ann Thorac Surg. 2011;92(4):1308–14.
84. Kochupura PV, Azeloglu EU, Kelly DJ, Doronin SV, Badylak SF, Krukenkamp IB, Cohen IS, Gaudette GR. Tissue-engineered myocardial patch derived from extracellular matrix provides regional mechanical function. Circulation. 2005;112(9 Suppl):I144–9.
85. Fallon A, Goodchild T, Wang R, Matheny RG. Remodeling of extracellular matrix patch used for carotid artery repair. J Surg Res. 2012;175(1):e25–34.
86. Wainwright JM, Hashizume R, Fujimoto KL, Remlinger NT, Pesyna C, Wagner WR, Tobita K, Gilbert TW, Badylak SF. Right ventricular outflow tract repair with a cardiac biologic scaffold. Cells Tissues Organs. 2012;195(1–2):159–70.
87. Remlinger NT, Gilbert TW, Yoshida M, Guest BN, Hashizume R, Weaver ML, Wagner WR, Brown BN, Tobita K, Wearden PD. Urinary bladder matrix promotes site appropriate tissue formation following right ventricle outflow tract repair. Organogenesis. 2013;9(3):149–60.
88. Sandusky GE Jr, Badylak SF, Morff RJ, Johnson WD, Lantz G. Histologic findings after in vivo placement of small intestine submucosal vascular grafts and saphenous vein grafts in the carotid artery in dogs. Am J Pathol. 1992;140(2):317–24.
89. Hiles MC, Badylak SF, Lantz GC, Kokini K, Geddes LA, Morff RJ. Mechanical properties of xenogeneic small-intestinal submucosa when used as an aortic graft in the dog. J Biomed Mater Res. 1995;29(7):883–91.
90. Pavcnik D, Uchida BT, Timmermans HA, Corless CL, O'Hara M, Toyota N, Moneta GL, Keller FS, Rosch J. Percutaneous bioprosthetic venous valve: a long-term study in sheep. J Vasc Surg. 2002;35(3):598–602.
91. Mewhort HE, Turnbull JD, Meijndert HC, Ngu JM, Fedak PW. Epicardial infarct repair with basic fibroblast growth factor-enhanced CorMatrix-ECM biomaterial attenuates postischemic cardiac remodeling. J Thorac Cardiovasc Surg. 2014;147(5):1650–9.
92. Padalino MA, Castellani C, Dedja A, Fedrigo M, Vida VL, Thiene G, Stellin G, Angelini A. Extracellular matrix graft for vascular reconstructive surgery: evidence of autologous regeneration of the neoaorta in a murine model. Eur J Cardiothorac Surg. 2012;42(5):e128–35.
93. Mosala Nezhad Z, Poncelet A, de Kerchove L, Gianello P, Fervaille C, El Khoury G. Small intestinal submucosa extracellular matrix (CorMatrix(R)) in cardiovascular surgery: a systematic review. Interact Cardiovasc Thorac Surg. 2016;22(6):839–50.
94. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 2008;14(2):213–21.
95. Wainwright JM, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, Badylak SF. Preparation of cardiac extracellular matrix from an intact porcine heart. Tissue Eng Part C Methods. 2010;16(3):525–32.
96. Sanchez PL, Fernandez-Santos ME, Costanza S, Climent AM, Moscoso I, Gonzalez-Nicolas MA, Sanz-Ruiz R, Rodriguez H, Kren SM, Garrido G, Escalante JL, Bermejo J, Elizaga J, Menarguez J, Yotti R, Perez del Villar C, Espinosa MA, Guillem MS, Willerson JT, Bernad A, Matesanz R, Taylor DA, Fernandez-Aviles F. Acellular human heart matrix: a critical step toward whole heart grafts. Biomaterials. 2015;61:279–89.
97. Guyette JP, Charest JM, Mills RW, Jank BJ, Moser PT, Gilpin SE, Gershlak JR, Okamoto T, Gonzalez G, Milan DJ, Gaudette GR, Ott HC. Bioengineering human myocardium on native extracellular matrix. Circ Res. 2016;118(1):56–72.
98. Badylak SF, Weiss DJ, Caplan A, Macchiarini P. Engineered whole organs and complex tissues. Lancet. 2012;379(9819):943–52.
99. Christman KL. Treating the leading killer. Sci Transl Med. 2012;4(146):146fs26.
100. Sonnenberg SB, Rane AA, Liu CJ, Rao N, Agmon G, Suarez S, Wang R, Munoz A, Bajaj V, Zhang S, Braden R, Schup-Magoffin PJ, Kwan OL, DeMaria AN, Cochran JR, Christman KL. Delivery of an engineered HGF fragment in an extracellular matrix-derived hydrogel prevents negative LV remodeling post-myocardial infarction. Biomaterials. 2015;45:56–63.

101. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, Kwan OL, Strachan GM, Wong J, Schup-Magoffin PJ, Braden RL, Bartels K, DeQuach JA, Preul M, Kinsey AM, DeMaria AN, Dib N, Christman KL. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med. 2013;5(173):173ra25.
102. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. Acta Biomater. 2017;49:1–15.
103. Singelyn JM, Christman KL. Injectable materials for the treatment of myocardial infarction and heart failure: the promise of decellularized matrices. J Cardiovasc Transl Res. 2010;3(5):478–86.

Chapter 9
Role of Extracellular Matrix in Cardiac Cellular Therapies

Peiman Hematti

Abstract The extracellular matrix (ECM) is an essential regulator of homeostasis at the cellular, tissue, and organ level. It is now very well known that ECM dynamic remodeling is indispensable not only for normal growth and development but also recovery from tissue injuries. Indeed, abnormal remodeling of the ECM plays a major role in many pathophysiological processes and contributes to many different pathologies including cardiovascular disorders. Recently, cellular therapies have emerged as a potential therapeutic strategy for restoration of lost cardiomyocytes or their rejuvenation after cardiac damage and injuries. Harnessing the biological properties of ECM could be a viable strategy to enhance the therapeutic effects of cellular therapies by improving the engraftment, integration, survival, and functional adaptation of newly transplanted cells in many different platforms. Conversely, transplanted cells could restore the functionality and original composition of damaged ECM by secreting and depositing new ECM or stimulating normal ECM production by cardiac tissue native cells. Although the ultimate role of cell therapy in treatment of cardiac disorders is still a matter of great debate, the potential utility of ECM in improving the therapeutic effect of transplanted cells and vice versa the potential role of cell therapy as a means to restore the structure and functionality of damaged ECM should be carefully considered in implementation of future clinical cardiovascular cell therapy trials.

Keywords Extracellular matrix · ECM · Stem cells · Cell therapy · Heart failure · Cardiac repair · Mesenchymal stem cells · Regeneration · Cell transplantation · Bioscaffolds · Cellularization

P. Hematti (✉)
Department of Medicine, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA
e-mail: pxh@medicine.wisc.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_9

9.1 Introduction

The extracellular matrix (ECM) is a very complex and highly dynamic structure that is present in all tissues and organs [1]. This three-dimensional acellular structure is composed of a diverse array of proteins and other molecules that defines the three-dimensional characteristics of tissues and organs by acting as a scaffold surrounding cells [2]. Although ECM has historically been considered as a biomechanical entity that its main function is to provide structural support, the ability of the ECM to undergo controlled remodeling to provide the contextual information responsible for controlling both individual and collective cellular behavior is now being increasingly recognized [3]. Indeed, homeostasis of ECM is crucial for keeping balance of many essential cellular processes, such as spatiotemporal development of tissues/organs in both prenatal and postnatal life, and restoring their balance and normalcy following tissue damages and injuries [4]. In pathological conditions, aberrant ECM reorganization following initial inflammatory cascade results in anomalous tissue organization mainly in the form of fibrotic processes, which is considered a main obstacle to normal tissue regeneration [5]. For cardiovascular disorders, fibrosis of cardiac tissue due to excessive and abnormal deposition of ECM by cardiac fibroblasts is the end result of many disease processes such as ischemic heart attack and long-standing hypertension [6]. Such pathological myocardial processes lead to stiffness of heart tissue and diastolic and systolic dysfunction, and these effects ultimately culminate into the development of heart failure [7]. Thus, restoration of ECM homeostasis, its biochemical composition, biomechanical characteristics, and its normal interactions with surrounding cells could all provide vibrant therapeutic strategies for a wide range of disorders [8].

Cardiovascular disorders are a major cause of morbidity and mortality in all societies, and its impact is expected to rise in the coming years and decades. Indeed, heart failure is now universally a major global health challenge, and its social and economic consequences are just becoming more formidable [9]. Current therapeutic options for heart diseases include pharmaceutical (small molecules and biologicals), revascularization procedures, medical devices, and surgical options including heart transplantation. However, there is no doubt that more effective alternatives for ischemic heart disease and congestive heart failure are urgently needed. Especially, critical advances are needed to replace the only available therapeutic option for advanced heart failure, i.e., heart transplantation, a drastic procedure only available to a minority of patients in need [10]. Over the last two decades, many regenerative biological and cellular therapies have emerged as a potential solution for these daunting medical issues. Regenerative medicine approaches focus on the healing of damaged heart through delivery of new (autologous or allogeneic) stem and progenitor cells, replacement of lost or damaged cells with differentiated cells, promotion of functional recovery of damaged heart via stimulation of endogenous tissue-resident stem and progenitor cells to generate new cells, delivery of angiogenic or immunomodulatory cells to stimulate repair processes, and finally replacement of part or all of the heart with bioengineered or artificial parts or even the

whole organ. Among regenerative medicine options, cellular therapies are the most advanced in clinical medicine as they have been tested in numerous clinical trials for cardiovascular disorders in the last decade and a half [11]. Other regenerative therapeutic options, such as cardiac patches, biomaterial scaffolds, biodegradable constructs, noncellular partial grafts, and whole-organ decellularized/recellularized scaffold grafts, despite some theoretical advantages, have remained mainly in the realm of basic and preclinical research [12]. However, such noncellular ECM-based therapies, if proven to be effective, could have advantages such as lack of immunogenicity and donor variability, potential for off-the-shelf accessibility, expanded shelf life, and potential practicality of administration alone or in combination with cells or other biologicals. Although the use of biomaterial technologies in the form of bioengineered ECM platforms for treatment of heart disorders is still in preliminary stages, it is expected that ultimately they will play a much bigger role in clinical cardiovascular medicine. On the other hand, although cellular therapies using a wide variety of cells from different sources, including bone marrow, have reached late-stage clinical trials and have been tested in thousands of patients, none has been approved yet for any cardiovascular indication [13]. In this chapter we will review the potential opportunities and challenges in cellular therapy for cardiac disorders, the potential contribution of cell therapy to ECM remodeling, and how ECM bioengineering could contribute to the advancement of cellular therapy approaches.

9.2 Stem Cells and ECM

Stem cell biology is one of the fastest growing fields in biomedical research as stem cells play fundamental roles not only in maintaining the homeostasis of tissues and organs during prenatal and postnatal growth and development but also in restoring normalcy after tissue damage or injury [14]. Stem cells are generally defined by their abilities to both give rise to different types of differentiated progenies and self-renew to make more daughter stem cells [15]. Adult stem cells usually reside in a quiescent state but undergo proliferation or differentiation based on specific demands in physiological and pathological conditions [16]. Even tissues that were for a long time considered to be terminally differentiated, such as the heart, are now considered to contain some populations of stem and progenitor cells that continue to proliferate in adult life. Such tissue-resident stem and progenitor cells in the heart could potentially participate in regeneration of heart tissue after injury, although to a much lesser degree compared to tissues such as bone marrow [17].

Physically, stem cells are localized in specialized niches, surrounded by specific cells that provide a dynamic milieu of extracellular cues and signals to support stem cell survival, their quiescence state, and when needed their proliferation and differentiation. This concept of stem cell niche was originally proposed, as a theoretical concept for hematopoietic system [18]. Now it is well accepted that stem cell niches provide a dynamic supportive microenvironment for cell-to-cell interaction via soluble factors, gap junctions, and cell surface receptor/ligand interactions for all types

of stem cells [19, 20]. Such tight and precise regulation of stem cell biological activity is not only essential for long-term maintenance of tissue homeostasis and regeneration [21] but also for keeping in check proliferation and differentiation of stem cells in response to tissue injuries, mainly to avoid uncontrolled proliferation that could lead to cancerous state [22].

Recently, the concept of the stem cell niche has expanded in its composition and complexity to include ECM, as ECM provides direct or indirect control and provision of the mechanical, biochemical, physicochemical, and structural support system for stem cells [23–25]. Also, due to heterogeneity of stem cells in different tissues, specialized composition and structure of ECM and its regulatory and signaling molecules are needed to provide specific niches for different types of stem cells accordingly [26]. Thus, tissue-specific ECM complex molecules, their physicochemical assembly, and their organization in time and space provide a multifaceted microenvironment that allows integration of reciprocal signaling between cellular and acellular components of the individual niches. This ultimately provides an environment capable of maintaining stem cell homeostasis [27, 28]. As will be discussed in more detail later, our current understanding of the role of ECM in stem cell biology has become the basis of development of bioengineering platforms to generate specialized ECM-mimetic biomaterials, for example, by depositing tissue-specific stem cell supportive factors into tissue-specific matrices, as a strategy to enhance the efficacy of transplanted stem cells [29].

It should be noted that interaction between stem cells and their niches is a reciprocal system, and tissue stem cells and their supportive cellular niches play an essential role in controlling and modulating the biochemical and functional integrity of ECM [30]. Thus, it could be postulated that cellular therapy could be a strategy for restoring the composition and function of damaged ECM as a component of supportive stem cell niche. However, it should be noted that the sheer size of human heart and its complex organization remain a major challenge for correction of ECM pathologies by localized cell therapeutic approaches. Correction of large ECM pathologies, such as advanced heart failure, necessitates multiple cell injections in the appropriate anatomical distribution to promote migration and integration of implanted cells throughout the damaged area. At the same time, the delivery method should avoid further damage to the ECM through manipulations such as injection of cells, before such cells could find the chance to deposit new and healthy ECM. And most importantly, for this strategy to be effective, the transplanted cells should be capable of depositing the right type and amount of cardiac-specific ECM [31].

9.3 Stem Cell Therapies for Cardiovascular Disorders

Regeneration of the heart by replacing lost cells or rejuvenating damaged cells is a very provocative therapeutic strategy. Indeed, use of stem cells for regeneration of the heart is one of the most active areas in the field of cellular therapies as cardiovascular disorders because current therapies for reversing already established heart

damage and heart failure including conventional drugs and newer biological therapies remain mainly ineffective [32, 33]. However, achieving optimal and effective heart repair strategies using regenerative cell therapy approaches remains challenging due to many factors such as complexity of the heart as an organ, its propensity for irreversible remodeling after tissue damage, and the ineffectiveness of its intrinsic repair mechanisms. As mentioned above for a long time, it was believed that mammalian heart muscle cells, including in humans, were incapable of proliferation. However, some recent discoveries challenge this dogma by showing that adult mammalian myocardium contains a population of resident cardiac stem cells (CSCs) that has the capability to self-renew and also differentiate into mature cardiomyocytes [34, 35]. Interestingly, despite extreme original enthusiasm in regard to this discovery, it is now believed that in the absence of injury, the rate of turnover of these cells is extremely low, and even in the case of massive cell loss, such as after myocardial infarction, tissue-resident cardiac stem and progenitor cells are not, at least on their own, capable of adequate proliferation and regeneration to restore adequate function of the heart to its pre-injury levels [36–39].

In general strategies for heart regeneration could be categorized into two main approaches, (a) administration of stem/progenitor cells or differentiated cells, either autologous or allogeneic, and via different routes, for regeneration and/or replacement of damaged or lost cardiac cells and (b) stimulation of resident stem and progenitor cells, via pharmacological, biological, ECM-based and even cell transplantation approaches, to promote autologous recovery [40]. Even for the former approaches, the potential mechanism for regeneration of the heart is still a matter of great debate and is not as straightforward as just replacing damaged cells [41]. Thus, current conceptual mechanism(s) of how a cell therapy approach might work depend on the type of cells administered, and proposed mechanisms range from potential replacement of lost cells, differentiation or transdifferentiation of transplanted stem cells into various types of heart tissue cells, improvement of angiogenesis, and immunomodulation of the inflammatory milieu to provide a more conducive environment for tissue repair, to stimulation of self-repair processes for regeneration of the heart via paracrine effects of transplanted cells [42–44].

For cardiac cell therapy, the types of cells used also range very widely and include minimally manipulated aspirated bone marrow cells, processed or purified bone marrow or mobilized peripheral blood progenitor and stem cells, ex vivo culture-expanded cells (from bone marrow or other sources), endothelial progenitor cells, skeletal myoblasts, and most recently pluripotent stem cells, including embryonic stem cells [45–51]. Also several different routes of cell delivery have been utilized including direct injection of cells to the myocardium, epicardium, and coronary arteries or simply intravenous infusion and have been all investigated in numerous clinical settings [52, 53]. In addition to the type of cells and the route administration, there are numerous other factors that could impact the ultimate effect of cell therapy. These include but not limited to dose of cells administered, frequency of cell administration, time of administration in relation to injury (acute myocardial infarction versus chronic heart failure), metabolic state of transplanted cells (fresh cells versus previously frozen and thawed cells), and use of other

concomitantly administered medications or simultaneously performed revascularization or surgical procedures [54–56]. The heterogeneity in this complex decision-making process makes it extremely difficult to compare results of these clinical studies head to head [57, 58]. Furthermore, results of most of the cellular clinical trials for heart disease evaluate only medical endpoints, such as clinical improvements or measured mechanical improvements such as ejection fraction by echo or other imaging modalities, and do not report on the ultimate benefit of such therapies, i.e., improvement in survival of patients [59, 60].

As mentioned, cellular therapies have been postulated to restore heart function through numerous mechanisms, depending on the type of cells used, their state of differentiation, and their route of delivery. One of the most striking challenges in the field of cardiovascular cell therapy is the heterogeneity of cell types that have been used in different clinical trials, which is reflective of the deficiency of our mechanistic insight at many levels [54, 61]. Many of the early clinical trials using bone marrow-derived cells, mainly hematopoietic or culture-expanded mesenchymal stem cells, were based on the assumption that these bone marrow-derived cells could differentiate into cardiomyocytes and thus repopulate the damaged/lost cells [62]. Later, investigators speculated that these cells might convert to endothelial cells and repair damaged heat by angiogenic promoting processes [63]. Next wave of hypothesis focused on the immunomodulatory and anti-inflammatory actions of transplanted cells [64]. The most recent iteration of the potential mechanism of action of bone marrow-based cell therapies is thought to be through their secretome. This secretome is believed to exert a wide range of paracrine effects, such as stimulation of heart tissue-resident stem and progenitor cells to proliferate and replace the lost cells via generation of new differentiated cells [65]. What that has been less paid attention to is the fact that cellular therapy, in addition to many already considered putative mechanisms, could also alter the biomechanical properties of the damaged zone by promoting changes in the ECM via depositing new ECM or making changes into the composition of ECM by secreting and injecting new molecules into the damaged native ECM. Also, similar to the current debate about the potential mechanism of action of transplanted cells, it could be argued that role of cell therapy in modulating/repairing the damaged ECM could be either as a direct deposition of ECM by transplanted cells, such as MSCs, or via indirect effect of transplanted cells to stimulate cardiac intrinsic cells to deposit new heart tissue-specific ECM. In other words, transplanted cells could induce structural changes inducing recovery and even proliferation of heart resident cells and that in turn lead to generation of ECM with appropriate biochemical and biomechanical properties to replace aberrant ECM typical of post-myocardial infarction or heart failure. Not surprisingly, there are only very limited and highly experimental data about the effect of human cell therapy on heart ECM [66], which seriously limits our understanding of effect of cell therapies on ECM components and their remodeling. Furthermore, ECM in every organ has a very complex, unique, and dynamic biochemical composition and biomechanical properties suited for the function of that organ [67]. Since most of cellular therapies so far tested are based on BM-derived cells, it is challenging to conceptualize how and to what extent these non-heart-derived cells could contribute to restoration of heart-specific ECM proteins and molecules.

9.4 Mesenchymal Stem Cells and ECM Repair

Mesenchymal stem cells, commonly also referred to as mesenchymal stromal cells (MSCs), have been one of the most commonly used types of cells in cardiovascular clinical trials [68–70]. Nevertheless, there is still no randomized, controlled clinical trial that shows unequivocal efficacy of MSCs to provide a meaningful clinical benefit. MSCs are generally characterized by their adherence to plastic, their multilineage differentiation potential, and their characteristic cell surface marker expression pattern [71]. Although bone marrow was the earliest source of MSCs, it is now well known that organ-specific MSCs have been found in almost all fetal and adult tissues and organs including the heart [72]. Interestingly, several lines of evidence suggest that MSCs have a very close and mostly undiscernible lineage relationship with fibroblasts/myofibroblasts [73, 74]. For example, in an in vivo infarct heart mouse model, it was shown that a population of heart-resident primitive mesenchymal cells gave rise to myofibroblasts [75]. This mesenchymal precursor population expressed murine MSC and fibroblast markers such as CD44, CD34, and telomerase reverse transcriptase (TERT). Since scar formation in the form of fibrosis is the result of a vicious cycle of myofibroblast proliferation leading to collagen deposition and disturbing normal ECM architecture, paying attention to the intricacies of ontological relationship between MSCs and fibroblasts is of paramount importance when designing clinical trials [76]. For example, it could be argued that transplanted MSCs could replace faulty fibroblasts and lead to resolution of ECM; on the other hand, too much of or the use of inappropriate source of MSCs could, at least theoretically, lead to exacerbation of fibrosis [77].

In an attempt to improve the results of BM-MSC trials for cardiac repair, use of cardiogenic promoting growth factors in cultures has been proposed to generate more effective MSCs. These so-called cardioprotective stem cells have been investigated in a multicenter randomized phase II C-CURE trial in which cardiopoietic BM-MSCs were delivered by endomyocardial injection. Although this trial showed the potential clinical feasibility and safety of this approach [78], potential efficacy of this approach needs to be investigated in larger trials. The use of cardiac tissue-derived stem cells has also reached clinical stage. Marban group has pioneered this approach in which an endomyocardial biopsy from the patients was used to generate autologous cardiosphere-derived cells. These cells, which have some characteristics of MSCs, such as high expression of CD105 and low expression of CD45 marker, were shown to be safe when delivered via intracoronary route after myocardial infarction [79]. It is noteworthy these cardiac-derived cells, when cultured as cardiospheres, recapitulated a stem cell niche microenvironment with enhanced expression of many ECM and adhesion molecules [80]. While advantage of these cells over other types of stem cells still needs to be verified in larger randomized clinical trials, biologically it is very plausible that cells derived from the heart could be superior source of cells for repair of the heart compared to cells derived from bone marrow or adipose tissue.

9.5 Role of Tissue-Engineered ECM and Biomaterials in Cellular Therapy

Results of many cellular therapies in preclinical animal models have shown that engraftment of transplanted cells is not a robust phenomenon. Thus, development of clinically applicable technologies to enhance cell engraftment, retention, and their ultimate functional integration is considered to be of vital importance to improve the results of cell therapy strategies [81, 82]. Such ex vivo and in vivo approaches to promote engraftment of cells upon their administration could be based on technologies that improve cell viability, cell migration, cell survival, and ultimately cell functionality upon reaching their target organs after administration of cells [83, 84]. Not surprisingly, utilization of bioengineered ECM technologies for such optimization of cell therapy platforms is under active investigation, based on the assumption that three-dimensional combination of appropriate cells with ECM or biodegradable scaffolds and tissue growth factors could lead to better tissue reconstruction [85, 86]. An example of such an approach could be implantation of cells, such as cardiomyocytes, cardiac progenitors, skeletal muscle cells, and MSCs, cultured as a sheet format construct on a variety of bioscaffolds [87], with the intention to enhance cell engraftment, survival, and integration. It is plausible that use of cells such as cardiomyocytes cultured in such a format with the aid of ECM and bioscaffolds could have further advantages as this strategy may also lead to development of functional cellular connections and synchronicity in their contractibility. However, matching the physical and biomechanical needs of a constantly contracting heart is not an easy task [88], as a cardiac construct must not only be capable of withstanding the biomechanical forces of the cardiac contractile cycle but should also be biocompatible with the host tissue from immunological and biological points [89]. Direct injection of cellular-derived biomaterials could be also a feasible strategy for treatment of heart disorders by modulating ventricle remodeling [90]. Furthermore, injectable biomaterials could also provide a strategy for enhancing the effects of cell transplantation/implantation when administered with cells [91]. Even more encouraging is use of cardiac-specific ECM in combination with cellular products that could prove beneficial for enhancing cell retention and improving the outcomes of cellular therapies [92].

Another example of bioengineered ECM strategy that has generated intense interest is use of decellularized whole-heart matrix scaffolds. An established approach for treatment of end-stage heart failure is heart transplantation; however, organ shortage from deceased donors remains the rate-limiting step in this process. A potential solution to shortage of cadaveric hearts, aside from use of biomechanical heart devices, is generation of new hearts using biological or synthetic scaffolds that had been repopulated with cells to generate a new functional heart. In this scenario use of biological scaffolds provides some theoretical advantages due to biologically natural ECM components and presence of heart-specific growth factors and signaling molecules in the bioscaffold that has been generated from a heart. Theoretically, such bioscaffolds could promote the migration, engraftment, maturation and

survival of the cells, and ultimately their functional adaptation. Thus, development of bioengineered artificial hearts, through decellularization/recellularization of whole-heart scaffolds has become an area of active research, as whole-organ decellularized matrices are presumed to provide near physiological, biophysical, and biochemical cues and thus support generation of an organ ex vivo [93]. Indeed, utilizing decellularized tissues has verified the essential role of ECM in the regulation and promotion of seeding, engraftment, expansion, differentiation and fate of transplanted cells, and their ultimate biomechanical and physiological functionality [94]. Experiments using ECM scaffolds derived from natural decellularized tissues could instruct differentiation of stem cells into the specific cell types that naturally reside in the tissues of origin for those corresponding ECM [95], which have further expedited the development of tissue engineering and cellular therapy approaches based on use of ECM in the form of decellularized organs [96].

The source of these decellularized heart matrices could be allogeneic, such as human cadaveric hearts that are deemed not suitable for transplant. However, xenogeneic hearts could also be used because it is assumed that during the decellularization process, cellular antigens are eliminated, and thus the bioscaffold becomes hypo-immunogenic. As mentioned, these decellularized ECM biological scaffolds generated from human or pig heart have potential advantages over synthetic bioscaffolds, such as biosynthetic scaffolds generated using three-dimensional bioprinting technologies, as they better provide a tissue-specific recapitulation of heart ECM biophysical and biochemical structure, intrinsic heart micro- and macro-anatomy, and physiological composition and contractile strength [97]. Successful recellularization of acellular scaffolds with appropriate cell types should ultimately generate organ-specific cell composition in appropriate proportion and cell density.

For generating whole-heart scaffolds, either human cadaveric or xenogeneic hearts, different decellularization technologies have been developed to remove the cells from the organ to generate a cell-free whole-heart scaffold with all its ECM components. The lysis of cells could be done using different techniques such as chemical or enzymatic procedures, followed by physical perfusion to remove lysed cell fragments. The next step in the process will be recellularizing the scaffold, either ex vivo in bioreactors or in vivo or combined. At this stage, the cell type and concentrations and appropriate recellularization strategies become important parameters. One major challenge to overcome is to recellularize the scaffold with the right type and right ratio of cells in correct spatial organization, using the appropriate method of cell delivery such as direct injection, perfusion, or both. Currently, investigators are using more and more sophisticated technologies to further mimic physiological conditions to promote proper organ function. For example, rat heart scaffolds were not only repopulated with rat neonatal cardiomyocytes but also primary rat aortic endothelial cells and then were cultured with pulsatile antegrade left heart perfusion to simulate functional preload, ventricular filling, and afterload, while pacing hearts with electrical stimulation [98]. With current technologies ex vivo cellularization of a whole heart may take weeks of culture; however, partially repopulated bioartificial organs such as heart could be implanted in recipients, to allow further in vivo maturation of the organ via the regenerative potential of the

body. Under such circumstances, in vivo maturation of the organ depends on the trafficking of the stem and progenitor cells to the organ, their engraftment, maturation and integration, and ultimately functional tissue formation. However, such strategies are only applicable to tissues and organs that do not need to be fully functional at time of implantation or when a therapeutic bridge is available, such as total artificial heart pump. It is noteworthy that clinical use of decellularized/recellularized human pulmonary heart valves implanted with autologous endothelial progenitor cells, isolated from peripheral mononuclear cells, has been already reported. In this study monolayers of cells were cultured and differentiated on the luminal surface of the scaffolds in a bioreactor for up to 3 weeks and then implanted into two pediatric patients (ages 13 and 11) with congenital pulmonary valve failure. Postoperatively, a mild pulmonary regurgitation was documented in both children, but 3.5 years of follow-up did not show any safety issues or signs of valve degeneration and indeed showed that the implanted valves had the potential to remodel and grow accordingly to the growth of the children [99]. Despite these preliminary achievements, successful recellularization of decellularized whole-heart bioscaffolds to recapitulate a native heart and generate a functionally integrated unit remains a very challenging task. This is not only due to the many remaining technological challenges to generate the optimal whole-heart bioscaffold but also because much more progress is needed to generate fully functional types of cells and sophisticated biomedical engineering techniques for their exact spatiotemporal delivery and implantation into the scaffolds. Thus, there is no doubt that achieving the goal of delivering a complex organ such as whole heart starting with a bioscaffold from bench to beside will remain a daunting task for the foreseeable future.

9.6 Conclusion

Importance of ECM in restoring tissue organization and functionality to the heart after myocardial injury is now very well understood. However, further research is needed to develop practical and effective technologies to restore normal biochemical and biomechanical properties of ECM after tissue damage. Recently use of cellular therapies, using a wide range of natural or ex vivo generated/modified cells, has received great attention for repair and regeneration of heart tissue. Although the exact mechanism by which cellular therapies could benefit heart recovery and function, and also the magnitude of this effect, is still a matter of great debate, it could be hypothesized that one potential mechanism of action of transplanted cells to restore heart function is through depositing new and healthy ECM to restore the lost/damaged ECM. Conversely, use of different bioengineered ECM-based platforms, such as decellularized scaffolds or use of injectable ECM biomaterials, as adjunct to cell therapy approaches could be considered as a strategy to improve the effect of administered cells. Understanding how transplantation of cells could restore ECM function and vice versa how use of ECM could enhance the therapeutic effect of cell therapy provides new and exciting venues for regenerating the heart.

References

1. Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326(5957):1216–9. PubMed PMID: 19965464. Pubmed Central PMCID: 3536535.
2. Hynes RO, Naba A. Overview of the matrisome--an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol. 2012;4(1):a004903. PubMed PMID: 21937732. Pubmed Central PMCID: 3249625.
3. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15(12):786–801. PubMed PMID: 25415508. Pubmed Central PMCID: 4316204.
4. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol. 2010;341(1):126–40. PubMed PMID: 19854168. Pubmed Central PMCID: 2854274.
5. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165–78. PubMed PMID: 21324931. Pubmed Central PMCID: 3046088.
6. Suthahar N, Meijers WC, Sillje HHW, de Boer RA. From inflammation to fibrosis-molecular and cellular mechanisms of myocardial tissue remodelling and perspectives on differential treatment opportunities. Curr Heart Fail Rep. 2017;14(4):235–50. PubMed PMID: 28707261. Pubmed Central PMCID: 5527069.
7. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: the fibroblast awakens. Circ Res. 2016;118(6):1021–40. PubMed PMID: 26987915. Pubmed Central PMCID: 4800485.
8. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol. 2011;3(12). PubMed PMID: 21917992. Pubmed Central PMCID: 3225943.
9. Cahill TJ, Choudhury RP, Riley PR. Heart regeneration and repair after myocardial infarction: translational opportunities for novel therapeutics. Nat Rev Drug Discov. 2017;16(10):699–717. PubMed PMID: 28729726.
10. Kittleson MM, Kobashigawa JA. Cardiac transplantation: current outcomes and contemporary controversies. JACC Heart Fail. 2017;5(12):857–68. PubMed PMID: 29191293.
11. Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair--lessons from clinical trials. Nat Rev Cardiol. 2014;11(4):232–46. PubMed PMID: 24594893.
12. Taylor DA, Parikh RB, Sampaio LC. Bioengineering hearts: simple yet complex. Curr Stem Cell Rep. 2017;3(1):35–44. PubMed PMID: 28261549. Pubmed Central PMCID: 5315720.
13. A futile cycle in cell therapy. Nat Biotechnol. 2017;35(4):291. PubMed PMID: 28398319.
14. Biteau B, Hochmuth CE, Jasper H. Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. Cell Stem Cell. 2011;9(5):402–11. PubMed PMID: 22056138. Pubmed Central PMCID: 3212030.
15. Wagers AJ, Christensen JL, Weissman IL. Cell fate determination from stem cells. Gene Ther. 2002;9(10):606–12. PubMed PMID: 12032706.
16. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. Science. 2010;327(5965):542–5. PubMed PMID: 20110496. Pubmed Central PMCID: 4105182.
17. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell. 2000;100(1):157–68. PubMed PMID: 10647940.
18. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4(1-2):7–25. PubMed PMID: 747780.
19. Li L, Xie T. Stem cell niche: structure and function. Annu Rev Cell Dev Biol. 2005;21:605–31. PubMed PMID: 16212509.
20. Moore KA, Lemischka IR. Stem cells and their niches. Science. 2006;311(5769):1880–5. PubMed PMID: 16574858.

21. Bardelli S, Moccetti M. Remodeling the human adult stem cell niche for regenerative medicine applications. Stem Cells Int. 2017;2017:6406025. PubMed PMID: 29090011. Pubmed Central PMCID: 5635271.
22. Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. Cancer Res. 2006;66(9):4553–7. PubMed PMID: 16651403.
23. Voog J, Jones DL. Stem cells and the niche: a dynamic duo. Cell Stem Cell. 2010;6(2):103–15. PubMed PMID: 20144784. Pubmed Central PMCID: 3012646.
24. Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. Biochim Biophys Acta. 2014;1840(8):2506–19. PubMed PMID: 24418517. Pubmed Central PMCID: 4081568.
25. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nat Rev Mol Cell Biol. 2013;14(8):467–73. PubMed PMID: 23839578.
26. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci. 2008;121(Pt 3):255–64. PubMed PMID: 18216330.
27. Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Curr Opin Cell Biol. 2012;24(5):645–51. PubMed PMID: 22898530.
28. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science. 2009;324(5935):1673–7. PubMed PMID: 19556500. Pubmed Central PMCID: 2847855.
29. Peerani R, Zandstra PW. Enabling stem cell therapies through synthetic stem cell-niche engineering. J Clin Invest. 2010;120(1):60–70. PubMed PMID: 20051637. Pubmed Central PMCID: 2798701.
30. Votteler M, Kluger PJ, Walles H, Schenke-Layland K. Stem cell microenvironments--unveiling the secret of how stem cell fate is defined. Macromol Biosci. 2010;10(11):1302–15. PubMed PMID: 20715131.
31. Rienks M, Papageorgiou AP, Frangogiannis NG, Heymans S. Myocardial extracellular matrix: an ever-changing and diverse entity. Circ Res. 2014;114(5):872–88. PubMed PMID: 24577967.
32. Metra M, Teerlink JR. Heart failure. Lancet. 2017;390(10106):1981–95. PubMed PMID: 28460827.
33. McDonald MA, Ashley EA, Fedak PWM, Hawkins N, Januzzi JL, McMurray JJV, et al. Mind the gap: current challenges and future state of heart failure care. Can J Cardiol. 2017;33(11):1434–49. PubMed PMID: 29111107.
34. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003;114(6):763–76. PubMed PMID: 14505575.
35. Hsieh PC, Segers VF, Davis ME, MacGillivray C, Gannon J, Molkentin JD, et al. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. Nat Med. 2007;13(8):970–4. PubMed PMID: 17660827. Pubmed Central PMCID: 2754571.
36. Rubart M, Field LJ. Cardiac regeneration: repopulating the heart. Annu Rev Physiol. 2006;68:29–49. PubMed PMID: 16460265.
37. Laflamme MA, Murry CE. Heart regeneration. Nature. 2011;473(7347):326–35. PubMed PMID: 21593865. Pubmed Central PMCID: 4091722.
38. Cai CL, Molkentin JD. The elusive progenitor cell in cardiac regeneration: slip slidin' away. Circ Res. 2017;120(2):400–6. PubMed PMID: 28104772. Pubmed Central PMCID: 5260844.
39. Zebrowski DC, Becker R, Engel FB. Towards regenerating the mammalian heart: challenges in evaluating experimentally induced adult mammalian cardiomyocyte proliferation. Am J Physiol Heart Circ Physiol. 2016;310(9):H1045–54. PubMed PMID: 26921436.
40. Silvestre JS, Menasche P. The evolution of the stem cell theory for heart failure. EBioMedicine. 2015;2(12):1871–9. PubMed PMID: 26844266. Pubmed Central PMCID: 4703721.

41. Dai W, Kloner RA. Bone marrow-derived cell transplantation therapy for myocardial infarction: lessons learned and future questions. Am J Transplant. 2011;11(11):2297–301. PubMed PMID: 21929617.
42. Bolli R, Ghafghazi S. Stem cells: cell therapy for cardiac repair: what is needed to move forward? Nat Rev Cardiol. 2017;14(5):257–8. PubMed PMID: 28361979.
43. Cambria E, Pasqualini FS, Wolint P, Gunter J, Steiger J, Bopp A, et al. Translational cardiac stem cell therapy: advancing from first-generation to next-generation cell types. NPJ Regen Med. 2017;2:17. PubMed PMID: 29302353. Pubmed Central PMCID: 5677990.
44. Chamuleau SAJ, van der Naald M, Climent AM, Kraaijeveld AO, Wever KE, Duncker DJ, et al. Translational research in cardiovascular repair: a call for a paradigm shift. Circ Res. 2018;122(2):310–8. PubMed PMID: 29348252.
45. Menasche P, Vanneaux V, Hagege A, Bel A, Cholley B, Cacciapuoti I, et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. Eur Heart J. 2015;36(30):2011–7. PubMed PMID: 25990469.
46. Ott HC, Davis BH, Taylor DA. Cell therapy for heart failure--muscle, bone marrow, blood, and cardiac-derived stem cells. Semin Thorac Cardiovasc Surg. 2005;17(4):348–60. PubMed PMID: 16428043.
47. Nasseri BA, Ebell W, Dandel M, Kukucka M, Gebker R, Doltra A, et al. Autologous CD133+ bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: the Cardio133 trial. Eur Heart J. 2014;35(19):1263–74. PubMed PMID: 24497345.
48. Rodrigo SF, van Ramshorst J, Beeres SL, Al Younis I, Dibbets-Schneider P, de Roos A, et al. Intramyocardial injection of bone marrow mononuclear cells in chronic myocardial ischemia patients after previous placebo injection improves myocardial perfusion and anginal symptoms: an intra-patient comparison. Am Heart J. 2012;164(5):771–8. PubMed PMID: 23137509.
49. Quyyumi AA, Vasquez A, Kereiakes DJ, Klapholz M, Schaer GL, Abdel-Latif A, et al. PreSERVE-AMI: a randomized, double-blind, placebo-controlled clinical trial of intracoronary administration of autologous CD34+ cells in patients with left ventricular dysfunction post STEMI. Circ Res. 2017;120(2):324–31. PubMed PMID: 27821724.
50. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol. 2003;41(7):1078–83. PubMed PMID: 12679204.
51. Patel AN, Henry TD, Quyyumi AA, Schaer GL, Anderson RD, Toma C, et al. Ixmyelocel-T for patients with ischaemic heart failure: a prospective randomised double-blind trial. Lancet. 2016;387(10036):2412–21. PubMed PMID: 27059887.
52. Raval AN. Therapeutic potential of adult progenitor cells in the management of chronic myocardial ischemia. Am J Cardiovasc Drugs. 2008;8(5):315–26. PubMed PMID: 18828643.
53. Ang KL, Chin D, Leyva F, Foley P, Kubal C, Chalil S, et al. Randomized, controlled trial of intramuscular or intracoronary injection of autologous bone marrow cells into scarred myocardium during CABG versus CABG alone. Nat Clin Pract Cardiovasc Med. 2008;5(10):663–70. PubMed PMID: 18711405.
54. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. Nature. 2008;451(7181):937–42. PubMed PMID: 18288183.
55. Schmuck EG, Koch JM, Hacker TA, Hatt CR, Tomkowiak MT, Vigen KK, et al. Intravenous followed by X-ray fused with MRI-guided transendocardial mesenchymal stem cell injection improves contractility reserve in a swine model of myocardial infarction. J Cardiovasc Transl Res. 2015;8(7):438–48. PubMed PMID: 26374144. Pubmed Central PMCID: 5006679.
56. Tongers J, Losordo DW, Landmesser U. Stem and progenitor cell-based therapy in ischaemic heart disease: promise, uncertainties, and challenges. Eur Heart J. 2011;32(10):1197–206. PubMed PMID: 21362705. Pubmed Central PMCID: 3094549.
57. Choi SH, Jung SY, Kwon SM, Baek SH. Perspectives on stem cell therapy for cardiac regeneration. Advances and challenges. Circ J. 2012;76(6):1307–12. PubMed PMID: 22739079.

58. Dinsmore JH, Dib N. Stem cells and cardiac repair: a critical analysis. J Cardiovasc Transl Res. 2008;1(1):41–54. PubMed PMID: 20559957.
59. Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Cochrane Database Syst Rev (Online). 2016;12:CD007888. PubMed PMID: 28012165.
60. Bruyneel AA, Sehgal A, Malandraki-Miller S, Carr C. Stem cell therapy for the heart: blind alley or magic bullet? J Cardiovasc Transl Res. 2016;9(5-6):405–18. PubMed PMID: 27542008. Pubmed Central PMCID: 5153828.
61. Dixit P, Katare R. Challenges in identifying the best source of stem cells for cardiac regeneration therapy. Stem Cell Res Ther. 2015;6:26. PubMed PMID: 25886612. Pubmed Central PMCID: 4357059.
62. Singh A, Singh A, Sen D. Mesenchymal stem cells in cardiac regeneration: a detailed progress report of the last 6 years (2010-2015). Stem Cell Res Ther. 2016;7(1):82. PubMed PMID: 27259550. Pubmed Central PMCID: 4893234.
63. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells. 2004;22(3):377–84. PubMed PMID: 15153614.
64. van den Akker F, de Jager SC, Sluijter JP. Mesenchymal stem cell therapy for cardiac inflammation: immunomodulatory properties and the influence of toll-like receptors. Mediators Inflamm. 2013;2013:181020. PubMed PMID: 24391353. Pubmed Central PMCID: 3872440.
65. Fontaine MJ, Shih H, Schafer R, Pittenger MF. Unraveling the mesenchymal stromal cells' paracrine immunomodulatory effects. Transfus Med Rev. 2016;30(1):37–43. PubMed PMID: 26689863.
66. Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014;510(7504):273–7. PubMed PMID: 24776797. Pubmed Central PMCID: 4154594.
67. Roskelley CD, Srebrow A, Bissell MJ. A hierarchy of ECM-mediated signalling regulates tissue-specific gene expression. Curr Opin Cell Biol. 1995;7(5):736–47. PubMed PMID: 8573350. Pubmed Central PMCID: 2933201.
68. Karantalis V, DiFede DL, Gerstenblith G, Pham S, Symes J, Zambrano JP, et al. Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: The prospective randomized study of mesenchymal stem cell therapy in patients undergoing cardiac surgery (PROMETHEUS) trial. Circ Res. 2014;114(8):1302–10. PubMed PMID: 24565698. Pubmed Central PMCID: 4104798.
69. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA. 2012;308(22):2369–79. PubMed PMID: 23117550. Pubmed Central PMCID: 4762261.
70. Guijarro D, Lebrin M, Lairez O, Bourin P, Piriou N, Pozzo J, et al. Intramyocardial transplantation of mesenchymal stromal cells for chronic myocardial ischemia and impaired left ventricular function: Results of the MESAMI 1 pilot trial. Int J Cardiol 2016;209:258-265. PubMed PMID: 26901787.
71. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–7. PubMed PMID: 16923606. eng.
72. da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006;119(Pt 11):2204–13. PubMed PMID: 16684817. eng.
73. Denu RA, Nemcek S, Bloom DD, Goodrich AD, Kim J, Mosher DF, et al. Fibroblasts and mesenchymal stromal/stem cells are phenotypically indistinguishable. Acta Haematol. 2016;136(2):85–97. PubMed PMID: 27188909. Pubmed Central PMCID: 4988914.

74. Hematti P. Mesenchymal stromal cells and fibroblasts: a case of mistaken identity? Cytotherapy. 2012;14(5):516–21. PubMed PMID: 22458957. Epub 2012/03/31. eng.
75. Carlson S, Trial J, Soeller C, Entman ML. Cardiac mesenchymal stem cells contribute to scar formation after myocardial infarction. Cardiovasc Res. 2011;91(1):99–107. PubMed PMID: 21357194. Pubmed Central PMCID: 3112022.
76. Ramkisoensing AA, de Vries AA, Atsma DE, Schalij MJ, Pijnappels DA. Interaction between myofibroblasts and stem cells in the fibrotic heart: balancing between deterioration and regeneration. Cardiovasc Res. 2014;102(2):224–31. PubMed PMID: 24576955.
77. Trial J, Entman ML, Cieslik KA. Mesenchymal stem cell-derived inflammatory fibroblasts mediate interstitial fibrosis in the aging heart. J Mol Cell Cardiol. 2016;91:28–34. PubMed PMID: 26718722. Pubmed Central PMCID: 4764495.
78. Bartunek J, Behfar A, Dolatabadi D, Vanderheyden M, Ostojic M, Dens J, et al. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. J Am Coll Cardiol. 2013;61(23):2329–38. PubMed PMID: 23583246.
79. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012;379(9819):895–904. PubMed PMID: 22336189. Pubmed Central PMCID: 4326004.
80. Li TS, Cheng K, Lee ST, Matsushita S, Davis D, Malliaras K, et al. Cardiospheres recapitulate a niche-like microenvironment rich in stemness and cell-matrix interactions, rationalizing their enhanced functional potency for myocardial repair. Stem Cells. 2010;28(11):2088–98. PubMed PMID: 20882531. Pubmed Central PMCID: 3405979.
81. Bartczak A, McGilvray I, Keating A. Mesenchymal stromal cell therapy to promote cardiac tissue regeneration and repair. Curr Opin Organ Transplant. 2017;22(1):86–96. PubMed PMID: 28005573.
82. Terzic A, Behfar A. Stem cell therapy for heart failure: Ensuring regenerative proficiency. Trends Cardiovasc Med. 2016;26(5):395–404. PubMed PMID: 27020904. Pubmed Central PMCID: 4912443.
83. Ezquer FE, Ezquer ME, Vicencio JM, Calligaris SD. Two complementary strategies to improve cell engraftment in mesenchymal stem cell-based therapy: Increasing transplanted cell resistance and increasing tissue receptivity. Cell Adh Migr. 2017;11(1):110–9. PubMed PMID: 27294313. Pubmed Central PMCID: 5308221.
84. Karpov AA, Udalova DV, Pliss MG, Galagudza MM. Can the outcomes of mesenchymal stem cell-based therapy for myocardial infarction be improved? Providing weapons and armour to cells. Cell Prolif. 2017 ;50(2). PubMed PMID: 27878916.
85. Fuchs JR, Nasseri BA, Vacanti JP. Tissue engineering: a 21st century solution to surgical reconstruction. Ann Thorac Surg. 2001;72(2):577–91. PubMed PMID: 11515900.
86. Langer R, Vacanti JP. Tissue engineering. Science. 1993;260(5110):920–6. PubMed PMID: 8493529.
87. Ishii O, Shin M, Sueda T, Vacanti JP. In vitro tissue engineering of a cardiac graft using a degradable scaffold with an extracellular matrix-like topography. J Thorac Cardiovasc Surg. 2005;130(5):1358–63. PubMed PMID: 16256789.
88. Bouten CV, Dankers PY, Driessen-Mol A, Pedron S, Brizard AM, Baaijens FP. Substrates for cardiovascular tissue engineering. Adv Drug Deliv Rev. 2011;63(4-5):221–41. PubMed PMID: 21277921.
89. Reis LA, Chiu LL, Feric N, Fu L, Radisic M. Biomaterials in myocardial tissue engineering. J Tissue Eng Regen Med. 2016;10(1):11–28. PubMed PMID: 25066525. Pubmed Central PMCID: 4933503.
90. Wassenaar JW, Gaetani R, Garcia JJ, Braden RL, Luo CG, Huang D, et al. Evidence for mechanisms underlying the functional benefits of a myocardial matrix hydrogel for post-MI treatment. J Am Coll Cardiol. 2016;67(9):1074–86. PubMed PMID: 26940929. Pubmed Central PMCID: 4779189.

91. Wang RM, Christman KL. Decellularized myocardial matrix hydrogels: In basic research and preclinical studies. Adv Drug Deliv Rev. 2016;96:77–82. PubMed PMID: 26056717. Pubmed Central PMCID: 4670814.
92. Singelyn JM, Sundaramurthy P, Johnson TD, Schup-Magoffin PJ, Hu DP, Faulk DM, et al. Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction. J Am Coll Cardiol. 2012;59(8):751–63. PubMed PMID: 22340268. Pubmed Central PMCID: 3285410.
93. Moser PT, Ott HC. Recellularization of organs: what is the future for solid organ transplantation? Curr Opin Organ Transplant. 2014;19(6):603–9. PubMed PMID: 25304814.
94. Lane SW, Williams DA, Watt FM. Modulating the stem cell niche for tissue regeneration. Nat Biotechnol. 2014;32(8):795–803. PubMed PMID: 25093887. Pubmed Central PMCID: 4422171.
95. Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. Tissue Eng Part A. 2010;16(7):2207–16. PubMed PMID: 20156112. Pubmed Central PMCID: 2947947.
96. Faulk DM, Johnson SA, Zhang L, Badylak SF. Role of the extracellular matrix in whole organ engineering. J Cell Physiol. 2014;229(8):984–9. PubMed PMID: 24347365.
97. Wainwright JM, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, et al. Preparation of cardiac extracellular matrix from an intact porcine heart. Tissue Eng Part C Methods. 2010;16(3):525–32. PubMed PMID 19702513. Pubmed Central PMCID: 2945869.
98. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 2008;14(2):213–21. PubMed PMID: 18193059.
99. Cebotari S, Lichtenberg A, Tudorache I, Hilfiker A, Mertsching H, Leyh R, et al. Clinical application of tissue engineered human heart valves using autologous progenitor cells. Circulation. 2006;114(1 Suppl):I132–7. PubMed PMID: 16820562.

Chapter 10
Regulation of Regenerative Medicine Products

Adrian P. Gee

Abstract Cellular therapies have moved to the forefront based upon promising results from clinical trials using both chimeric antigen receptor T lymphocytes to treat leukemia and other cell types to restore structure and function to tissues that have been damaged by disease or physical injury. The pace at which these treatments have evolved has posed a regulatory challenge to agencies, such as the Food and Drug Administration (FDA). This chapter describes how a specific regulatory strategy was developed and how it has evolved in response to the demand for these new therapies.

Keywords Extracellular matrix · Regenerative medicine · Cellular therapies · Regulatory authority · Food and Drug Administration · FDA guidance · GDraft guidance · Good manufacturing practices · GMP manufacturing · Investigational new drug application · Minimally manipulated · More than minimally manipulated

10.1 Introduction

For many years, it has been proposed that cellular therapies could provide cures for otherwise untreatable diseases. These claims were based on two concepts. The first was that a specific immune response could be engineered to destroy diseased target cells. The second was based on the concept that all cells evolved from stem and progenitor populations, which could be expanded and differentiated ex vivo or in vivo to produce a population that could be used to elicit a regenerative or corrective effect in the recipient. Clinical proof of principle was provided by bone marrow transplantation, in which functioning immune and blood-forming systems could be restored in cancer patients treated with myeloablative chemo/radiotherapy. This therapy has achieved the status of practice of medicine and is not regulated by the

A. P. Gee (✉)
cGMP Facilities, Center for Cell & Gene Therapy, Baylor College of Medicine, Houston, TX, USA
e-mail: agee@bcm.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_10

FDA. Subsequently, different cell sources, new in vitro cell manipulations, and novel indications have proliferated. This has posed a problem to the international regulatory agencies as to what laws should govern this area. In the following sections, the evolution of specific regulations is reviewed together with how they have been recently streamlined to increase patient access.

10.2 Regulatory Authority

In the United States, the responsibility for regulation of drugs, foodstuffs, cosmetics, animal feeds, etc. falls to the FDA. These regulations are published in Title 21 of the Code of Federal Regulations (CFR), which is available online. On an ongoing basis, the FDA monitors developments in their area of responsibility and determines whether they require regulation. If so, the initial approach is to decide whether there are pre-existing regulations that could be applied to the new issue. Cellular therapy products are regulated as pharmaceuticals, and as such, there were some existing laws that were deemed to be relevant. There were, however, obvious differences between exiting regulated small molecule drugs and biological therapeutics that required specific attention. The problem was identifying these differences and developing a consistent regulatory strategy that would address them.

The method by which this is achieved is often through a survey of what new products are being developed. This may be followed by publication of draft guidances, which suggest a regulatory strategy that may be adopted and how compliance could be achieved. Stakeholders in the field have the opportunity to submit comments to the FDA. These will be officially reviewed and may result in changes to the proposed strategy. Eventually, the FDA will produce a final guidance document, and subsequently the contents may appear in the regulations published in the CFR.

This process was adopted when the FDA reviewed developments in cellular therapies. The data gathering stage consisted of a variety of meetings held between the FDA and stakeholders. At these, the stakeholders could propose regulatory strategies, outline needs and problems, and indicate how they believed the field would evolve. In parallel, the FDA introduced Annual Establishment Registration. This requires facilities involved in preparation of cellular therapy products to register with the FDA and indicate the activities they perform, e.g., collection, processing, distribution, etc., and the types of cellular products involved, etc. This provides ongoing data on how the field is developing and whether new cellular products and practices are emerging.

Based upon an extensive review of the field, the FDA identified existing regulations that could be applied. These included the requirement to evaluate the new product under an Investigational New Drug (IND) approval [1]. The IND mechanism requires submission of data on the rationale for the treatment, preclinical studies to support this rationale, how the product will be manufactured and tested, and design of the clinical trial, to include patient numbers with inclusion and exclusion criteria, doses to be evaluated, stopping rules, statistical analysis methods, etc.

An IND study requires that the test product is manufactured under current good manufacturing practices (GMP). This is a system that ensures that the cells are prepared using a controlled, auditable, reproducible procedure that results in a safe and potentially effective product. GMP regulations have been in place for other types of therapeutics for many years and can be found in Parts 200 and 600 of Title 21 of the CFR (the electronic version of the current CFR is available online at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm.

Having made the decision, the FDA then had to address how the IND and GMP mechanisms would be applied to living therapeutics.

10.3 Risk-Based Regulations

There are a number of issues that arise quickly when trying to implement existing regulations to cellular products. These are living entities with inherent variability that are then subjected to ex vivo manipulation, possible long-term storage, shipment to treatment centers, and additional manipulation before patient administration. It would be very difficult to develop a single regulatory strategy that could be applied to a potentially very wide range of product types. The FDA elected to use a risk-based approach. This evaluated the potential degree of risk to the donor, the risks posed by ex vivo manipulation, and the risks to the intended recipient. Using this system, it was possible to implement two regulatory strategies; however, to do so, the risks needed to be specified. The major risks are outlined in Table 10.1 for somatic cell therapy products. Products defined as posing high risk would follow the existing GMP/IND mechanism and are referred to as Type 351 products. Lower-risk products (Table 10.1) would be subject to new regulations. These were named good tissue practices (GTP) and are described in 21 CFR Part 1271 and described in an FDA guidance [2] and cover Type 361 products. They regulate minimally manipulated products, which immediately raises the question of how this term is defined.

Table 10.1 Major factors used to classify high- and low-risk cell therapy products

Low-risk (Type 361 products) Good tissue practice regulations	High-risk (Type 351 products) Good manufacturing practice regulations
Simple collection procedure, e.g., peripheral blood draw	Complex collection procedure, e.g., surgical procedure required
Minimal manipulation of cells ex vivo, e.g., • Plasma reduction • Red cell removal • Selective removal of cells	More-than-minimal manipulation of cells ex vivo, e.g., • Ex vivo cell culture • Genetic modification • Activation of cells
No change to relevant or biological characteristics of the cells	Changes to relevant and/or biological characteristics of the cells
No combination of cells with another article	Combination with another article or device
Homologous use	Nonhomologous use

For structural tissue, it is defined as processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement, and, for cells or nonstructural tissues, it is processing that does not alter the relevant biological characteristics of tissues. This would appear to put many regenerative products under the GTP regulations; however, this is not always true. The FDA was requested by stakeholders to provide a clearer definition of manipulation, and in 2006, it published the "Guidance for Industry and FDA Staff: Minimal Manipulation of Structural Tissue Jurisdictional Update" [3]; this described a request for designation process, by which the investigator could ask for an official manipulation designation on their particular processing. There is an associated Guidance for Industry and FDA: "How to Write a Request for Designation (RFD)" [4]. This document did not specifically define what in vitro procedures would constitute more-than-minimal manipulation, and so this issue continued to be debated. In parallel, there was controversy on the term "homologous use," which is a requirement for regulation under GTP. Many investigators felt that GTP regulations covered use of bone marrow or adipose-derived cells implanted in different tissues to achieve different functions. The FDA argued that homologous use only included applications where the cells were expected to perform the same function at the sites of collection and administration. This interpretation was still questioned, and to try to resolve both of these areas of contention, the FDA in December 2017 [5] published the "Guidance for Industry and Food and Drug Administration Staff—Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use." This guidance aims to provide clear definitions for both minimal manipulation and homologous use.

In general terms, more-than-minimal manipulation, for the purposes of this article, covers ex vivo culture, genetic manipulation, and cell activation, some or all of which are used when preparing cells for regenerative applications. In turn, depending on the origin of the cells and their final application, the cells or tissues may be for homologous or nonhomologous use. As a result, most early-phase regenerative medicine protocols are regulated under the IND mechanism and employ product manufacturing under GMP, rather than GTP regulations (Table 10.1).

10.4 GMP Manufacturing

Many academic institutions contemplating opening Phase 1 clinical trials of a regenerative medicine product may be unfamiliar with GMP regulations. A full description of the requirements is outside the scope of this article, but reviews are available [6, 7]. Not all components of full GMP are required for Phase 1 studies. The FDA Guidance "CGMP for Phase 1 Investigational Drugs" provides a summary of the expectations [8]. These include policies and procedures that cover staff, quality control, facility and equipment, control of components, manufacturing and records, laboratory controls, packaging, labeling, distributing, and recordkeeping. Recognizing that cell therapy products differ from traditional small drug

pharmaceuticals, in 2013 the FDA issued a Guidance [9] specifically relating to cellular and gene therapy products: "Draft Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products", which provided important supplementary information.

The IND application contains the Chemistry, Manufacturing and Control (CMC) section, which describes in detail the origin, manufacturing, testing, labeling and distribution of the product, and the facility in which it is to be manufactured. Luckily, there is an excellent FDA Guidance [10] on how the CMC should be written and formatted, "Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)". There is a parallel Guidance [11], which provides the same information for gene therapy products, "Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)". These documents streamline writing of the CMC section, and should be followed closely when preparing information for IND submission.

10.5 Extracellular Matrix (ECM) Regulation

This volume discusses the types and properties of ECMs and how they exert their beneficial effects. This chapter addresses the regulation of ECM and how it is evolving. Therapeutic products that consist of cells plus a matrix [12, 13], or scaffold, are regulated as combination products, and assignment falls under the FDA Office of Combination Products. The designation as a combination product can be disputed using the request for designation mechanism described earlier. Additional information can be found in a recent final guidance "Classification of Products as Drugs and Devices & Additional Product Classification Issues" [14]. Combination products are specifically intended to be used together, and both components are required to mediate the therapeutic effect. Jurisdiction as to which part of the FDA (the Center for Biologics Evaluation and Research (CBER) or the Center for Devices and Radiological Health (CDRH)) the combination product is assigned and is determined by its primary mode of action. In some cases, two applications may be required. Table 10.2 shows the information that will be requested on the cell and scaffold components. The variety and properties of ECM are continually evolving, making it difficult to describe a single common regulatory strategy for all. The most recent development has been signing into law of the twenty-first Century Cures Act in 2016, which is designed to accelerate the development and review of novel medical products [15]. It also established new expedited product development programs: (i) the Regenerative Medicine Advanced Therapy (RMAT) [16] and (ii) the Breakthrough Devices program. RMAT designation is intended for regenerative medicine therapies that are required to treat, modify, reverse, or cure serious or life-threatening diseases or conditions where there is preliminary clinical evidence that the therapy has the potential to address unmet medical needs. RMAT designation is

Table 10.2 Information required by FDA on combination products

Cells	Scaffold (Device)
Source (auto, allo) Donor eligibility, master cell bank testing	Starting materials Material selection, design, and fabrication Biocompatibility
Cell processing GMP compliance, in-process testing	Design and properties Mechanical and physical
Release testing Safety, identity, purity, potency Cell number	Manufacturing and testing Resorption profile, design control, performance Sterility assurance, quality system regulations, device GMP
Cells + Device	
Dose response, cell growth, cell function, cell-scaffold interactions	
Final product	
Safety, potency, durability, cell fate, structural and biomaterial decomposition	

This table is based on information presented by MH Lee, Office of Cellular, Tissue, and Gene Therapies, CBER, FDA, at the 2nd Annual Symposium on Stem Cell Strategies, Best Practices and Regulatory Considerations, San Francisco, CA. September 2010

obtained by filing a request with CBER or by including the request in the IND application. Since many regenerative therapies involve the use of a device as part of a combination product, the FDA subsequently issued a draft guidance "Evaluation of Devices used with Regenerative Medicine Advanced Therapies" [17]. It is important to note, however, that this guidance specifically states that the "FDA does not consider scaffolds combined with a cellular product to be within the scope of this Guidance." The rationale is that the scaffold would not generally be considered solely "a device used in the delivery of RMAT," because it provides more than a delivery function, and that "both the scaffold and the cellular product are typically necessary for the RMAT to achieve its intended purpose."

In spite of an evolving regulatory landscape, the FDA has approved a number of scaffolds and combination products. These include autologous cellularized scaffolds, CorMatrix ECM, and hydrogels, such as polyethylene glycol (PEG) or polyethylene oxide (PEO), which is a biocompatible and hydrophilic polymer approved for several biomedical applications (12).

10.6 Interacting with the FDA

The best approach when seeking regulatory approval for an ECM/cell product is to obtain advice from the FDA early in the process. In 2017, the FDA published a procedural draft guidance "Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products" [18]. This summarizes the types of meetings that can be held with the agency. The most important are the types A, B, and C meetings. Initial contact should be made through a Type C meeting. This is described as

"any meeting other than a Type A, Type B, or Type B (end of production) meeting regarding the development and review of a product." It offers the IND sponsor the opportunity to obtain regulatory feedback on an IND study under early development. The normal procedure is to contact the FDA to request a Type C meeting (often referred to as a pre-pre-IND meeting) and provide them with a list of issues that require clarification. This is usually done by outlining the proposed action to be taken, e.g., we proposed to assess product functionality using the following assay, will that be acceptable? The meeting takes place by conference call between the investigators and selected FDA staff. Careful notes should be taken of the proceedings, and it is advisable to follow up with an e-mail or letter to the FDA outlining your understanding of the points that were raised. This advice is invaluable in clarifying how the IND will be written, the product manufactured and tested, and the studies designed.

Type B meetings occur subsequently. These are used as the "official" pre-IND meetings and are held shortly before the IND application is submitted. They provide an opportunity to briefly present points where clarification from the agency is required. The format is the same as described above for the Type C meeting, and an outline of what is to be discussed should be submitted to the agency in advance. Type B meetings can also be used to discuss other issues, e.g., risk evaluation. Type A meetings are used for dispute resolution, follow-up after regulatory action, etc.

Before holding a meeting with the FDA, it is advisable to become familiar with the various draft and final guidances that have been published. These allow an investigator to get an overview of current thinking on regulatory strategies. Some of the most valuable guidances are listed in Table 10.3. All are accessible from the FDA website at http://www.fda.gov/. It is also possible to subscribe to the CBER website to automatically receive notification of new information.

10.7 Summary

Recent successes achieved by cellular therapies for leukemia, and in regenerative medicine applications, have caught the public interest. As a result, there has been increasing pressure on the FDA to develop new regulatory approaches to accelerate the evaluation and approval of these treatments. For the investigator wishing to implement a clinical trial using a new cellular product, this poses a challenge, since the existing regulatory approach has taken some time to evolve and continues to do so. The concepts of "manipulation," "homologous use," and "combination products," all of which affect the regulatory pathway, have been long debated, and their final definitions were only recently resolved. We now face additional mechanism to improve the evaluation and approval process. This chapter aims to provide a "snapshot view" of where the field is currently and how it may evolve in the future. Given rapid developments in ECM biology, cardiac cell therapy science and indications, and governmental regulations, it is impossible to hit such a rapidly moving target

Table 10.3 Selected FDA guidances

Guidance	Subject	Date
Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products: Draft Guidance for Industry	Description of types of meeting that can be held with the FDA	December 2017
Chemistry, Manufacturing, and Controls Changes to an Approved Application: Certain Biological Products—Draft Guidance for Industry	Making changes to the chemistry, manufacturing, and control section of an approved IND	December 2017
Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue- Based Products: Minimal Manipulation and Homologous Use—Guidance for Industry and Food and Drug Administration Staff	Clarification of definitions of homologous use and manipulation	December 2017
Evaluation of Devices Used with Regenerative Medicine Advanced Therapies: Draft Guidance for Industry	Evaluation of combination products in regenerative medicine	November 2017
Expedited Programs for Regenerative Medicine Therapies for Serious Conditions	Mechanisms for accelerated approval of regenerative medicine products	November 2017
Current Good Manufacturing Practice Requirements for Combination Products: Final Guidance for Industry and FDA Staff	GMP regulations for manufacturing combination products	January 2017
Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products	Difference between designs for cell and gene therapy clinical trials and those for small molecule drugs	June 2015
Preclinical Assessment of Investigational Cellular and Gene Therapy Products: Final Guidance	Types of preclinical data required for cell and gene therapy IND applications	November 2013
Process Validation: General Principles and Practices: Guidance for Industry	Design of validation studies	January 2011
Potency Tests for Cellular and Gene Therapy Products: Guidance for Industry	Types of potency tests that can be used for cell and gene therapy products	January 2011
Cellular Therapy for Cardiac Disease: Guidance	Overview of considerations for product manufacturing, testing, delivery, and clinical trial design	October 2010
Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs): Guidance for Industry and FDA Reviewers	Template for writing somatic cell therapy product CMC section	April 2008
Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)	Template for writing gene therapy product CMC section	April 2008

(continued)

Table 10.3 (continued)

Guidance	Subject	Date
CGMP for Phase 1 Investigational Drugs: Guidance for Industry	Minimal GMP requirements for manufacturing products for use in a Phase 1 clinical trial	July 2008
Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice: Guidance for Industry	Sterile manufacturing for products that cannot be terminally sterilized	September 2004

accurately. The reader is strongly advised to keep abreast of scientific and clinical developments and to use FDA resources to determine the best way to translate these into early-phase clinical trials.

Acknowledgments This work was supported in part by the Cancer Prevention and Research Institute of Texas (CPRIT) Core Grant Number 3300017409. The author wishes to thank colleagues at the Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas.

References

1. Investigational New Drug Applications Prepared and Submitted by Sponsor-Investigators: Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER); 2015.
2. Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps):Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research; 2011.
3. Minimal Manipulation of Structural Tissue Jurisdictional Update: Guidance for Industry and FDA Staff. U.S. Department of Health and Human Services Food and Drug Administration, Office of the Commissioner, Office of Combination Products and Center for Biologics Evaluation and Research; 2006.
4. How to Write a Request for Designation (RFD): Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Office of the Commissioner, Office of Combination Products; 2011
5. Guidance for Industry and Food and Drug Administration Staff: Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research Center for Devices and Radiological Health Office of Combination Products; 2017.
6. Aghayan HR, Arjmand B, Burger SR. GMP Facilities for Clinical Cell Therapy Product Manufacturing: A Brief Review of Requirements and Design Considerations. In: Arjmand B, editor. Perinatal Tissue-Derived Stem Cells Stem Cell Biology and Regenerative Medicine. Cham: Humana Press; 2016.
7. Giancola R, Bonfini T, Iacone A. Cell therapy: cGMP facilities and manufacturing. Muscles, Ligaments and Tendons Journal. 2012;2(3):243–7.
8. CGMP for Phase 1 Investigational Drugs: Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research

(CDER) Center for Biologics Evaluation and Research (CBER) Office of Regulatory Affairs (ORA); 2008.
9. Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products: Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research;2015
10. Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)". Guidance for Industry and FDA Reviewers. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research; 2008
11. Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research; 2008.
12. Saldin LT, Cramer MC, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: Structure and function. Acta Biomater. 2017;49:1–15.
13. Nielsen SH, Mouton AJ, DeLeon-Pennell KY, Genovese F, Karsdal M, Lindsey ML. Understanding cardiac extracellular matrix remodeling to develop biomarkers of myocardial infarction outcomes. Matrix Biol. 2017. pii: S0945-053X(17)30268–30268. doi: https://doi.org/10.1016/j.matbio.2017.12.001. [Epub ahead of print]
14. Classification of Products as Drugs and Devices & Additional Product Classification Issues: Guidance for Industry and FDA Staff. U.S. Department of Health and Human Services, Food and Drug Administration, Office of Combination Products, Office of Special Medical Programs, Office of the Commissioner; 2017
15. FDA announces comprehensive regenerative medicine policy framework 16 Nov 2017. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm585345.htm
16. Regenerative Medicine Advanced Therapy Designation. https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ucm537670.htm
17. Evaluation of Devices Used with Regenerative Medicine Advanced Therapies: Draft Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research Center for Devices and Radiological Health Office of Combination Products November 2017
18. Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products: Draft Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER).December 2017.

Chapter 11
Clinical Trial Design for Investigational Cardio-Regenerative Therapy

Amish N. Raval

Abstract Human trials of cardio-regenerative biologic therapies are being performed worldwide to address a growing, unmet need for durable treatments of cardiovascular disease. A well-constructed clinical trial design for these novel therapies requires careful attention to defining a clear hypothesis, a patient population, and anticipated outcomes. The scope of screening, method of randomization, blinding approach, data monitoring, and statistical analysis plan are the foundational elements that must be addressed in any clinical trial. Although the experience of human trials involving extracellular matrix constructs for cardiovascular disease treatment is limited, numerous lessons have been learned in the field of cell therapy that are translatable across all biologic treatment options. Future progress in this field may include testing combinations of cells, gene-transfer agents, and matrix and identifying treatment responders versus nonresponders.

Keywords Heart failure · Clinical trial · Stem cell · Treatment · Regenerative medicine · Myocardial infarction · Cardiomyopathy

11.1 Introduction

Cardiovascular disease is the most common cause of death and reason for hospitalization in the United States [1]. For example, an estimated six million Americans suffer from heart failure, and this number is expected to increase to more than eight million by 2030 [2]. Mortality is high in these patients and similar to that of advanced cancer. Standard therapy involves beta-adrenergic and angiotensin II-inhibiting medications that block maladaptive neurohormonal pathways, but these drugs are only partially effective and are not universally tolerated [3]. Left ventricular assist devices and heart transplant may be offered, but device failure, stroke, infection, and organ shortages limit these approaches [4]. A dizzying array of stem/progenitor

A. N. Raval (✉)
School of Medicine & Public Health, University of Wisconsin-Madison, Madison, WI, USA
e-mail: anr@medicine.wisc.edu

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_11

cells or cell-*derivative* therapies are being tested in human trials to replenish heart and vascular cells worldwide. Candidate cardio-regenerative therapies have targeted patients who suffer from large acute myocardial infarction, chronic refractory heart failure, and chronic refractory angina. However, despite nearly three decades of investigation, there are still no Food and Drug Administration (FDA)-approved cardio-regenerative therapies. On the other hand, the accumulating experience has resulted in several "lessons learned" for designing and executing cardio-regenerative clinical trials. In this chapter, we will review the fundamentals of clinical trial design, therapeutic delivery, endpoints, potency, and novel adaptive trial design approaches in the context of cardio-regenerative medicine.

11.2 Clinical Trial Design for Biologics Therapy for Cardiac Disease

Investigators who design clinical trials must have a clear understanding of the target patient population, a clearly stated goal or hypothesis, and clinical endpoints. The Food and Drug Administration (FDA) provides regulatory oversight of pharmaceuticals, biologics, and medical devices in the United States. The Center for Biologics Evaluation and Research (CBER) is a branch of the FDA that provides specific oversight of human trials pertaining to vaccines, blood products, and biologics including cell-based and extracellular matrix therapies [5]. Additional regulatory oversight of human trials is provided by local institutional review boards (IRB). Clinical trials for regenerative medicine applications must follow the same rigorous principles and ethical standards of those of traditional pharmaceutical trials.

In the United States, human trials that test medical/device treatments are conducted in "phases" (see Table 11.1). In Phase I, the objective is to demonstrate safety and feasibility of the approach; to define the product's kinetics, biodistribution, and traceability; and to test the feasibility of any exploratory endpoints that may be useful in future phases. Phase I studies may be randomized or open label without a control group. "Phase Ia" studies imply a single dose that is being tested

Table 11.1 Phases for cardio-regenerative therapeutic clinical trials

Trial phase	Objective	Design	Typical sample size
I	Feasibility Safety	Open-label or randomized placebo controlled	20–40
II	Safety Efficacy using surrogate endpoints	Randomized placebo controlled	40–200
III	Efficacy using clinically meaningful endpoints	Randomized placebo controlled	>200
IV	Post-marketing surveillance To monitor for long-term effects	Open-label	>1000

to confirm safety. “Phase Ib” studies imply multiple, ascending doses to define dose response. With pharmaceutical trials, it is typical for Phase I studies to be performed in healthy volunteers, whereas in cardio-regenerative trials, patients with advanced disease who are refractory to conventional therapy are enrolled. In Phase II, the objective is to confirm safety in a larger randomized placebo-controlled trial. It is common to test for exploratory surrogate endpoints in Phase II for foundational evidence to justify and support a larger Phase III trial. “Phase IIa” studies imply an exploratory safety study with pharmacodynamics and biologic activity as the primary endpoint. “Phase IIb” studies imply defining an optimum dose range related to both safety and efficacy. In Phase III, the goal is to confirm that the investigational agent is effective in a randomized, placebo-controlled trial. The primary endpoint in a Phase III trial must be clinically relevant such as survival, reduction in symptoms, improvement in quality of life, freedom from hospitalization, improvement in walking distance, or combinations thereof. Phase III “pivotal” trials are designed with an intent to submit the findings to the FDA to establish a “labeled” indication, so that the therapy can be marketed and applied in clinical practice. Following FDA approval, post-marketing surveillance (sometimes called “Phase IV”) may be required. At this point, the treatment is commercially available, but patient information related to their demographics and follow-up observations are entered into a registry. The objective for Phase IV is to monitor for long-term side effects of the treatment.

A clear, succinct, and prospectively declared trial hypothesis is the hallmark of any reputable clinical trial. Once the hypothesis is defined, the trial will require a logistical framework that includes (i) sample size, (ii) screening, (iii) randomization, (iv) blinding, (v) treatment, (vi) endpoints, and (vii) data monitoring and statistical analysis plan. These are discussed below.

11.2.1 Sample Size

The trial sample size must be sufficiently robust to test the trial hypothesis for the primary endpoint. The greater the number of events anticipated in the target patient population, the smaller the sample size, and vice versa; the fewer the number of anticipated events, the larger the sample size. For the latter, this can translate to an unacceptable amount of time and expense to accrue subjects and delay approval of potentially effective therapies for vulnerable patient populations.

Recently, clinical trialists have suggested prospectively defined “adaptive” trial design models to use accumulating data obtained while the trial is ongoing to modify its course [6, 7]. One adaptation that has been proposed is to adjust the sample size at a pre-specified point in the trial when the primary endpoint is reached (such as improvement in 6-min walk distance), but there is a strong trend to improvement in a different but clearly important endpoint such as survival. By adapting to the larger sample size, the trial boosts statistical power to show that the investigational therapy significantly improves both ambulatory function and survival. Other adaptations

include stopping early due to futility in reaching the primary endpoint, adjusting therapeutic dose to efficiently assess dose response, adding or dropping randomization arms or dosing strata, and narrowing or widening the screening criteria. Although the FDA has embraced adaptive trial designs and, as such, has issued a guidance document [8], this approach has not been universally accepted by other stakeholders such as healthcare providers, community representatives, administrators, and payers [9].

11.2.2 Screening

Cardio-regenerative trials have traditionally focused on advanced heart disease, such as large myocardial infarction, chronic refractory angina, and heart failure despite optimized conventional medical, revascularization, and device therapy. Rigorous inclusion and exclusion screening criteria are typical in Phase I/II trials to narrow the patient population under study and avoid confounding variables that could alter prognosis independent of the investigational therapy. For example, patients with serious comorbid conditions such as dialysis-dependent renal failure are typically excluded. Most cardio-regenerative therapies tout angiogenic and immunomodulatory paracrine properties, and therefore, conditions such as proliferative diabetic retinopathy, active cancer, and active infectious diseases such as human immunodeficiency virus and viral hepatitis are exclusionary. When the primary endpoint is a test of ambulatory function such as walking distance, non-ambulatory patients are excluded. Also, most cardio-regenerative clinical trials exclude patients who have received an investigational biologic therapy in the past, to avoid confounding toxicity with the new investigational therapy.

There has been recent interest to narrow the scope of the trial to include only patients who are likely to be treatment "responders" based on biomarker parameters. For example, the Phase III CardiAMP Heart Failure trial [10] (NCT02438306) screens ischemic heart failure patients by performing a small volume bone marrow aspirate and testing the aspirate for cell potency markers. Individuals who have potent cells are then randomized to receive transendocardial injection of autologous bone marrow mononuclear cells obtained through a second, larger volume aspirate. This specific design was based on numerous post hoc analyses of cardio-regenerative studies that have suggested a variety of demographic features and other clinical biomarkers that isolate responders from non-responders to biologic therapies [11–15].

11.2.3 Randomization

Randomization in clinical trials improves the scientific validity of the results and should be performed as close in time to the investigational treatment as possible. This is straightforward for off-the-shelf pharmaceutical products, synthesized

biomaterials, or allogenic cell products, but not for autologous therapies that require a tissue/cell harvesting step. For example, autologous bone marrow cell therapy trials, circulating progenitor cell therapy trials, and cardiac progenitor cell therapy trials require bone marrow aspirate, leukapheresis, and heart biopsy, respectively, in order to derive the investigational product. If the goal is to test the hypothesis that the overall therapeutic strategy of harvest/autologous treatment is safe and effective against traditional care, then the randomization step would have to occur prior to the harvest. In the Stem Cell Revascularization in Patients with Critical Limb Ischemia (SCRIPT-CLI) clinical trial, the circulating progenitor cells were harvested and isolated the day prior to treatment [14]. An alternative approach is to harvest cells from all patients and then randomize them to treatment or control. In this case, patients randomized to the control group would be exposed to the risk of a harvesting procedure but would not receive the investigational product.

Randomization may be equal (1:1, treated to control patients) or unequal. Unequal randomization schemes are usually designed for more treated patients than controls (i.e., 2:1 or 3:1) to incentivize patient recruitment. Randomization should occur at an independent, centralized facility. For multicenter placebo-controlled trials, the randomization is typically conducted using permuted blocks of random sizes, to ensure each center represents the population with a similar ratio of treated to control patients. Phase III multicenter trials often compare pooled results from geographic regions such as comparing outcomes in US centers versus European centers, for example.

11.2.4 Treatment

The treatment phase of the study follows the screening phase and randomization. The patient receives either the investigational treatment or placebo (or sham). With a few exceptions, the vast majority of cardio-regenerative trials to date have applied the investigational treatment at only one time point and at one dose. Delivery methods to date have included intravenous delivery, intracoronary artery infusion, direct epicardial injection, transvascular interstitial injection, coronary sinus infusion, transendocardial injection, and surgical application of cell-loaded and cell-free patches [16, 17].

11.2.5 Blinding

Ideally, the investigational team and patient should not have knowledge about their treatment/placebo assignment to avoid future decision bias. A common example of this issue is when a patient drops out of a study if they knew they received placebo or sham treatment. When the therapy involves an invasive procedure to deliver the study agent such as surgical exposure or catheter-based delivery, maintaining blinding of the investigational team and the patient (i.e., “double-blind”) can be challenging.

Sham or "mocked up" procedures can be performed to sufficiently deceive the patient while at the same time investigators are obliged to minimize exposing patients to potential harm. A questionnaire at the end of the treatment phase may be posed to the patient to determine if they could determine which treatment assignment they received, in order to confirm that the sham procedure was successful.

11.2.6 Endpoints

Defining the primary endpoint of a clinical trial is one of the most crucial elements of trial design [18]. The primary endpoint of the trial should reflect the disease at issue and be relevant to patients, healthcare providers, and payers with minimal bias. Where possible, endpoints should be evaluated by independent and blinded core laboratories and investigators and adjudicated by independent event committees. The primary endpoint is the key determinate of the sample size of the trial and FDA regulatory approval in Phase III studies. Safety endpoints, surrogate endpoints, clinically meaningful endpoints, clinician-interpreted endpoints, and patient-reported endpoints are further discussed below.

Safety Endpoints In cardio-regenerative clinical trials, adverse events (AEs) are documented and tallied. Significant events are also referred to as serious adverse events (SAEs). Posttreatment SAEs including death, myocardial infarction, stroke, and hospitalization are the focus of Phase I and II studies. Consensus documents have emerged that propose monitoring of tissue injury and arrhythmogenesis in cardio-regenerative trials [19, 20]. In addition, efforts should be made to establish the interaction of dose and safety readouts in Phase I/II trials. A goal of Phase I and II trials is to show no excess mortality or other major adverse events with the investigational therapy. In contrast, Phase III trials are designed to show these events occur less often, or at least not more, with the investigational therapy compared to placebo.

The timing of when safety events should be recorded and analyzed in the follow-up period remains controversial and depends on the clinical condition and endpoints being studied. In Phase I/II studies, it is not unusual to reach a safety endpoint conclusion based on 3–6 months follow-up. In Phase III, a longer follow-up window (i.e., >2 years) is often desirable to ensure no late adverse effects from the investigational therapy. This is particularly the case for cardio-regenerative studies when live cells or gene transfer agents are administered.

Surrogate Efficacy Endpoints Surrogate endpoints are typically continuous parameters that closely predict a robust clinical endpoint such as survival and provide plausible interpretation of how the investigation agent may be efficacious. These endpoints may provide a pathophysiologic explanation for how the agent will affect prognosis. Predicting the extent of improvement in surrogate endpoints is challenging in Phase II trials, as there is minimal prior experience with the study agent. In fact, observing a close relationship between the predicted and actual treatment effect is a good measure of success for the trial. In heart failure trials, for example, common surrogate endpoints include pro-N-terminal pro-brain natriuretic peptide

and other biomarkers [21], left ventricular chamber size and ejection fraction, viability on positron emission tomography (PET) or magnetic resonance imaging (MRI), arrhythmia burden on electrocardiogram (ECG) monitoring, and ischemia territory on stress imaging. Surrogate parameters alone or in aggregate are insufficient to achieve FDA approval for the investigational treatment. Instead, demonstrating success using robust clinically meaningful endpoints is required to achieve an FDA-labeled indication.

Clinically Meaningful Efficacy Endpoints By Phase III, clinically meaningful benefit must be demonstrated in a randomized, double-blind, placebo-controlled trial with a sufficiently large sample size. Often, Phase III cardiac drug and device trials emphasize all-cause mortality as the primary endpoint. For example, this is the primary endpoint for the ongoing bone marrow cell therapy in acute myocardial infarction (BAMI) Phase III trial which will randomize 3000 acute myocardial infarction patients to receive bone marrow-derived cells or standard of care [22]. The choice of all-cause mortality over cardiovascular mortality as the primary endpoint for these trials is controversial [20]. Additional clinically meaningful endpoints in heart failure trials include walking distance, freedom from hospitalization, freedom from life-threatening arrhythmias, transplant, left ventricular assist device placement, stroke, and myocardial infarction.

Clinician-Interpreted Endpoints Versus Patient-Reported Endpoints An example of clinician-interpreted outcomes is the New York Heart Association (NYHA) heart failure functional class. NYHA classification is considered a soft and unreliable endpoint as it is highly subjective and dependent on the absence of other intervening comorbidities such as degenerative joint disease or lung disease. An example of a patient-reported outcome is the Minnesota Living with Heart Failure Questionnaire (MLWHFQ) score [23]. The Minnesota Quality of Life Questionnaire is a common, simple to use tool to assess and monitor over time the quality of life of a heart failure patient. In contrast to NYHA classification, patient-reported quality of life is viewed favorably by the FDA as one variable that could sway regulatory approval of a therapy.

11.2.7 Data Monitoring and Statistical Analysis Plan

In most US clinical trials, independent data monitoring occurs by blinded clinical research associates (CRAs) who represent an independent clinical research organization (CRO). This involves careful documentation review of each enrolling site. This data is then entered into a web-based portal, to be reviewed by an independent data monitoring committee (DMC). The DMC typically consists of unblinded individuals with varied clinical backgrounds, including a statistician. The DMC follows a DMC "Charter" that may stipulate frequency of DMC meetings, trial stopping rules, and the management of blinded and unblinded data. The outcomes of these DMC meetings are summarized in a letter, which is made available to the investigators, the sponsor, the local institutional review board, and the FDA.

All clinical trials must develop, a priori, a data analysis plan to evaluate the safety and efficacy readouts. In Phase I trials, it is common to test escalating study agent doses to identify dose-related toxicity. The design may include patient cohorts that are stratified in terms of escalating doses, where investigators proceed to next dose strata as long as the current dose strata show a freedom from serious toxicity. In Phase II trials, the most successful one or two doses are tested, and serious adverse events are tallied and adjudicated for their *relatedness* to the investigational treatment. Whether the SAE was related to the study treatment is first assessed by the site principal investigator and then verified by the DMC. In larger trials, there may be a separate event adjudication committee tasked to categorize SAEs and their relatedness to the investigational agent. Phase III clinical trials are so-called "pivotal" trials when they are designed and executed to obtain FDA approval for a disease-specific labeled indication. The primary objective is to show the treatment is effective. Phase III studies must have a pre-defined statistical analysis plan for evaluating the primary and secondary efficacy endpoints of the trial.

Many clinical trials list a composite endpoint of clinically meaningful variables (such as death, MI, and stroke) with the goal of showing therapeutic benefit with a reasonable population sample size. There are several challenges with using composite outcomes. First, double or triple counting of events is a problem that can confuse the result. For example, a patient has an MI in the follow-up period and dies from it. This issue is dealt with by stipulating the composite endpoint as *time to first event.* Secondly, clinicians, patients, hospital systems, payers, and society can view the importance of each individual component of the composite differently. This issue was illustrated by Kipp et al. where patients were compared to physicians in terms of their perception of the relative importance of death or stroke following either coronary artery bypass grafting or percutaneous coronary revascularization [24]. In this study, patients feared stroke far more than death, in contrast to physicians who had the opposite view of these events.

In general, composite endpoint scores should have objective clinically meaningful event categories that are interrelated and directionally concordant. The greater the number of clinically meaningful event categories, the higher the likelihood of one event category moving in a directionally discordant way from chance alone. For example, the primary endpoint of the Phase II Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial had seven event categories and required all seven to move concordantly in a favorable direction, with no significant worsening in any event category [25].

Recently, cardio-regenerative trials have emerged with more sophisticated statistical analysis plans. For example, the CardiAMP trial has 6-minute walk distance as the primary endpoint but allocates death, hospitalization, and quality of life scores in a stepwise hierarchical fashion. In this approach, the most significant clinical outcome supersedes less clinically important outcomes. A priori, each outcome is assigned a weight (e.g., 1 = death, 0.5 = MI, 0.2 = hospitalization).

Another approach to reduce the need for unmanageable sample size is to compare treatment/control patients head to head on clinically meaningful outcomes within the composite. One example of this is described by Finkelstein and Schoenfeld [26]. Each patient in the treatment arm is assigned a score of 1, 0, and − 1 depending on

whether they were better, the same, or worse than a comparison patient in the control arm for clinically meaningful events that are ordered in a hierarchy of clinical importance. At the pre-specified follow-up time to evaluate the primary outcome, net scores are compared between treatment and control groups using a test statistic.

11.3 Examples of Cardio-Regenerative Clinical Trials Testing Extracellular Matrix

Human trial experience using acellular and cellularized extracellular matrix or patches for cardio-regenerative repair is limited. The PRESERVATION I study (n = 303) by Rao et al. tested a bioabsorbable alginate polymer Algisyl (LoneStar Heart Inc., Dallas, Texas) in patients with ST elevation myocardial infarction who were successfully reperfused after percutaneous coronary intervention [27, 28]. The alginate was infused into the infarct-related artery up to 5 days following reperfusion. The primary objective of the trial was to demonstrate safety. The primary efficacy surrogate endpoint was chance in left ventricular diastolic volume index measured by three-dimensional echocardiography from baseline to 6 months. There were no safety concerns with the alginate polymer. Further, there was no significant difference in left ventricular geometry or function after 6 months, compared to the placebo group.

Human embryonic stem cell-derived cardiac progenitors were embedded within a fibrin scaffold and then surgically delivered onto the epicardial surface of a patient with chronic heart failure [29]. This experience led to the ongoing open-label Phase 1 ESCORT study (NCT02057900) which is testing human embryonic stem cell-derived CD15+ Isl1+ progenitors delivered within a fibrin patch in severe heart failure patients. The primary endpoint of this study is safety. Exploratory efficacy endpoints will include left ventricular function, viability of the grafted area, ambulatory function, and major adverse cardiovascular events.

The porcine small intestine submucosa (CorMatrix, Aziyo Biologics, Silver Spring, MD) has been used in cardiac surgery for pericardial closure and tissue repair during left ventricular assist device explant [30] and in children undergoing congenital heart disease repair [31]. Overall, the results have been mixed, and concerns have been raised due to undesirable inflammatory responses [32, 33]. Nummi et al. are performing an open-label pilot study (NCT02672163) that tests surgical placement of a tissue engineered sheet combined with autologous atrial appendage derived cells in patients with post myocardial infarction heart failure [34]. The cells are applied on an extracellular matrix sheet (CorMatrix, ECM Technology, CorMatrix Cardiovascular Inc., Atlanta, GA). Fibrin sealant (Tisseel, Baxter Healthcare, Westlake Village, CA) is added to the cells/CorMatrix to secure adherence of the suspense to the matrix. The micrograft is then sutured to the epicardial surface adjacent to the infarcted myocardium. Safety and feasibility of the procedure are the primary objective, but cardiac MRI will measure left ventricular function, geometry, and viability at 6 months.

Another open-label Phase I study (NCT02305602) is administering a semisolid, porous, porcine-derived hydrogel (VentriGel, Ventrix, San Diego, CA) via catheter-based transendocardial injection into patients between 60 days and 3 years following myocardial infarction with left ventricular ejection fractions 25–45%. The Myostar/NOGA (Biosense Webster) electro-anatomic mapping and catheter-injection system is being used for this procedure. The primary endpoint is freedom from serious adverse events in 6 months. Secondary endpoints will look at left ventricular function, geometry, and viability.

11.4 Conclusion

Large myocardial infarction, chronic refractory heart failure, and chronic refractory angina afflict a growing number of patients who desperately require durable treatment. A wide array of cells and cell-derivative cardio-regenerative technologies, including extra cellular matrix, are being studied in human trials worldwide. Lessons learned from past experiences have led to improvements in the design and efficient execution of contemporary cardio-regenerative clinical trials. A clear hypothesis and succinct methods for screening, blinding, randomization, treatment delivery, endpoints, and data analysis are the backbone of a well-designed clinical trial.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, DK MG, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB. Executive summary: Heart disease and stroke statistics--2016 update: a report from the American heart association. Circulation. 2016;133:447–54.
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, DK MG, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB. Heart disease and stroke statistics-2016 update: a report from the American heart association. Circulation. 2016;133:e38–360.
3. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 accf/aha guideline for the management of Heart failure (HF): a report of the American college of cardiology foundation/American heart association task force on practice guidelines. Circulation. 2013;128:e240–327.

4. Raikhelkar J, Uriel N. Contemporary perspectives in durable mechanical circulatory support: what did we learn in the last 3 years? Curr Cardiol Rep. 2018;20:6.
5. Kusinitz M, Braunstein E, Wilson CA. Advancing public health using regulatory science to enhance development and regulation of medical products: food and drug administration research at the center for biologics evaluation and research. Front Med (Lausanne). 2017;4:71.
6. Collins SP, Lindsell CJ, Pang PS, Storrow AB, Peacock WF, Levy P, Rahbar MH, Del Junco D, Gheorghiade M, Berry DA. Bayesian adaptive trial design in acute heart failure syndromes: moving beyond the mega trial. Am Heart J. 2012;164:138–45.
7. Lai TL, Lavori PW, Tsang KW. Adaptive design of confirmatory trials: advances and challenges. Contemp Clin Trials. 2015;45:93–102.
8. Food And Drug Administration CfBEaR. Adaptive designs for medical device clinical studies. 2016. www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm446729.pdf.
9. Meurer WJ, Legocki L, Mawocha S, Frederiksen SM, Guetterman TC, Barsan W, Lewis R, Berry D, Fetters M. Attitudes and opinions regarding confirmatory adaptive clinical trials: a mixed methods analysis from the adaptive designs accelerating promising trials into treatments (adapt-it) project. Trials. 2016;17:373.
10. Raval AN, Cook TD, Duckers HJ, Johnston PV, Traverse JH, Abraham WT, Altman PA, Pepine CJ. The cardiamp heart failure trial: A randomized controlled pivotal trial of high dose autologous bone marrow mononuclear cells using the cardiamp cell therapy system in patients with post myocardial infarction Heart failure (HF): trial rationale and study design. Am Heart J. 2018;201:141–8.
11. Zemljic G, Poglajen G, Sever M, Cukjati M, Frljak S, Androcec V, Cernelc P, Haddad F, Vrtovec B. Electroanatomic properties of the myocardium predict response to cd34+ cell therapy in patients with ischemic and nonischemic heart failure. J Card Fail. 2017;23:153–60.
12. Jokerst JV, Cauwenberghs N, Kuznetsova T, Haddad F, Sweeney T, Hou J, Rosenberg-Hasson Y, Zhao E, Schutt R, Bolli R, Traverse JH, Pepine CJ, Henry TD, Schulman IH, Moye L, Taylor DA, Yang PC. Circulating biomarkers to identify responders in cardiac cell therapy. Sci Rep. 2017;7:4419.
13. Shahrivari M, Wise E, Resende M, Shuster JJ, Zhang J, Bolli R, Cooke JP, Hare JM, Henry TD, Khan A, Taylor DA, Traverse JH, Yang PC, Pepine CJ, Cogle CR. Peripheral blood cytokine levels after acute myocardial infarction: Il-1beta- and il-6-related impairment of bone marrow function. Circ Res. 2017;120:1947–57.
14. Raval AN, Schmuck EG, Tefera G, Leitzke C, Ark CV, Hei D, Centanni JM, de Silva R, Koch J, Chappell RG, Hematti P. Bilateral administration of autologous cd133+ cells in ambulatory patients with refractory critical limb ischemia: lessons learned from a pilot randomized, double-blind, placebo-controlled trial. Cytotherapy. 2014;16:1720–32.
15. Taylor DA, Perin EC, Willerson JT, Zierold C, Resende M, Carlson M, Nestor B, Wise E, Orozco A, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Traverse JH, Cooke JP, Schutt RC, Bhatnagar A, Grant MB, Lai D, Johnstone BH, Sayre SL, Moye L, Ebert RF, Bolli R, Simari RD, Cogle CR. Identification of bone marrow cell subpopulations associated with improved functional outcomes in patients with chronic left ventricular dysfunction: an embedded cohort evaluation of the focus-cctrn trial. Cell Transplant. 2016;25:1675–87.
16. Raval AN. Therapeutic potential of adult progenitor cells in the management of chronic myocardial ischemia. Am J Cardiovasc Drugs. 2008;8:315–26.
17. Raval AN, Kamp TJ, Hogle LF. Cellular therapies for heart disease: unveiling the ethical and public policy challenges. J Mol Cell Cardiol. 2008;45:593–601.
18. Banovic M, Loncar Z, Behfar A, Vanderheyden M, Beleslin B, Zeiher A, Metra M, Terzic A, Bartunek J. Endpoints in stem cell trials in ischemic heart failure. Stem Cell Res Ther. 2015;6:159.
19. Bartunek J, Dimmeler S, Drexler H, Fernandez-Aviles F, Galinanes M, Janssens S, Martin J, Mathur A, Menasche P, Priori S, Strauer B, Tendera M, Wijns W, Zeiher A. The consensus of the task force of the European society of cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J. 2006;27:1338–40.

20. Zannad F, Garcia AA, Anker SD, Armstrong PW, Calvo G, Cleland JG, Cohn JN, Dickstein K, Domanski MJ, Ekman I, Filippatos GS, Gheorghiade M, Hernandez AF, Jaarsma T, Koglin J, Konstam M, Kupfer S, Maggioni AP, Mebazaa A, Metra M, Nowack C, Pieske B, Pina IL, Pocock SJ, Ponikowski P, Rosano G, Ruilope LM, Ruschitzka F, Severin T, Solomon S, Stein K, Stockbridge NL, Stough WG, Swedberg K, Tavazzi L, Voors AA, Wasserman SM, Woehrle H, Zalewski A, JJ MM. Clinical outcome endpoints in heart failure trials: a European society of cardiology heart failure association consensus document. Eur J Heart Fail. 2013;15:1082–94.
21. Chow SL, Maisel AS, Anand I, Bozkurt B, de Boer RA, Felker GM, Fonarow GC, Greenberg B, Januzzi JL Jr, Kiernan MS, Liu PP, Wang TJ, Yancy CW, Zile MR. Role of biomarkers for the prevention, assessment, and management of Heart failure (HF): a scientific statement from the American heart association. Circulation. 2017;135:e1054–91.
22. Mathur A, Arnold R, Assmus B, Bartunek J, Belmans A, Bonig H, Crea F, Dimmeler S, Dowlut S, Fernandez-Aviles F, Galinanes M, Garcia-Dorado D, Hartikainen J, Hill J, Hogardt-Noll A, Homsy C, Janssens S, Kala P, Kastrup J, Martin J, Menasche P, Miklik R, Mozid A, San Roman JA, Sanz-Ruiz R, Tendera M, Wojakowski W, Yla-Herttuala S, Zeiher A. The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: rationale and design of the bami trial. Eur J Heart Fail. 2017;19:1545–50.
23. Kelkar AA, Spertus J, Pang P, Pierson RF, Cody RJ, Pina IL, Hernandez A, Butler J. Utility of patient reported outcome instruments in heart failure JACC heart fail. 2016;4(3):165–75.
24. Kipp R, Lehman J, Israel J, Edwards N, Becker T, Raval AN. Patient preferences for coronary artery bypass graft surgery or percutaneous intervention in multivessel coronary artery disease. Catheter Cardiovasc Interv. 2013;82:212–8.
25. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, Hajjar RJ. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (cupid): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum ca2+−atpase in patients with advanced heart failure. Circulation. 2011;124:304–13.
26. Finkelstein DM, Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. Stat Med. 1999;18:1341–54.
27. Rao SV, Zeymer U, Douglas PS, Al-Khalidi H, Liu J, Gibson CM, Harrison RW, Joseph DS, Heyrman R, Krucoff MW. A randomized, double-blind, placebo-controlled trial to evaluate the safety and effectiveness of intracoronary application of a novel bioabsorbable cardiac matrix for the prevention of ventricular remodeling after large st-segment elevation myocardial infarction: rationale and design of the preservation i trial. Am Heart J. 2015;170:929–37.
28. Rao SV, Zeymer U, Douglas PS, Al-Khalidi H, White JA, Liu J, Levy H, Guetta V, Gibson CM, Tanguay JF, Vermeersch P, Roncalli J, Kasprzak JD, Henry TD, Frey N, Kracoff O, Traverse JH, Chew DP, Lopez-Sendon J, Heyrman R, Krucoff MW. Bioabsorbable intracoronary matrix for prevention of ventricular remodeling after myocardial infarction. J Am Coll Cardiol. 2016;68:715–23.
29. Menasche P, Vanneaux V, Hagege A, Bel A, Cholley B, Cacciapuoti I, Parouchev A, Benhamouda N, Tachdjian G, Tosca L, Trouvin JH, Fabreguettes JR, Bellamy V, Guillemain R, Suberbielle Boissel C, Tartour E, Desnos M, Larghero J. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. Eur Heart J. 2015;36:2011–7.
30. Ferng A, Connell A, Nunez M, Johnson K, Braunhut B, Lick S, Desai A, Kazui T, Runyan R, Khalpey Z. Cardiac regeneration in the human left ventricle after cormatrix implantation. Ann Thorac Surg. 2017;104:e239–41.
31. Nelson JS, Heider A, Si MS, Ohye RG. Evaluation of explanted cormatrix intracardiac patches in children with congenital heart disease. Ann Thorac Surg. 2016;102:1329–35.
32. Mosala Nezhad Z, Poncelet A, de Kerchove L, Gianello P, Fervaille C, El Khoury G. Small intestinal submucosa extracellular matrix (cormatrix(r)) in cardiovascular surgery: a systematic review. Interact Cardiovasc Thorac Surg. 2016;22:839–50.

33. Woo JS, Fishbein MC, Reemtsen B. Histologic examination of decellularized porcine intestinal submucosa extracellular matrix (cormatrix) in pediatric congenital heart surgery. Cardiovasc Pathol. 2016;25:12–7.
34. Nummi A, Nieminen T, Patila T, Lampinen M, Lehtinen ML, Kivisto S, Holmstrom M, Wilkman E, Teittinen K, Laine M, Sinisalo J, Kupari M, Kankuri E, Juvonen T, Vento A, Suojaranta R, Harjula A. Epicardial delivery of autologous atrial appendage micrografts during coronary artery bypass surgery-safety and feasibility study. Pilot Feasibility Stud. 2017;3:74.

Chapter 12
Regenerative Medicine Venturing at the University-Industry Boundary: Implications for Institutions, Entrepreneurs, and Industry

Adam J. Bock and David Johnson

Abstract Regenerative medicine research at university laboratories has outpaced commercial activity. Legal, regulatory, funding, technological, and operational uncertainty have slowed market entry of regenerative medicine treatments. As a result, commercial development has often been led by entrepreneurial ventures rather than large biopharma firms. Translating regenerative medicine across the university-industry boundary links academic scientists, technology transfer organizations, funders, and entrepreneurs. Conflicting motivations among the participants may significantly hinder these efforts. Unproven downstream business models for regenerative medicine delivery further complicate the entrepreneurial process. This chapter explores the challenges associated with entrepreneurial activity commercializing regenerative medicine science developed at research institutions.

Keywords Commercialization · Entrepreneurship · Technology transfer · Venturing · Regenerative medicine

12.1 Introduction

Regenerative medicine is a cutting-edge science and potentially big business. Billions of dollars are being invested in research and development and infrastructure amidst shifting government policies, complex lawsuits, uncertain regulatory and pricing policies, and capital-intensive market entry requirements. Long-term estimates for the market value of regenerative medicine range widely from the tens of

A. J. Bock (✉)
University of Wisconsin-Madison, Madison, WI, USA
e-mail: bock2@wisc.edu

D. Johnson
Edinburgh Napier University Business School, Edinburgh, United Kingdom

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7_12

billions to hundreds of billions of dollars. The majority of current revenues were derived from tools and research products; few therapeutics have been approved for use, with limited commercial success.

The developing regenerative medicine industry has been driven by academic scientists and clinical entrepreneurs rather than large established life science companies [40, 63]. Academic entrepreneurs possess innovative technical experience but usually lack knowledge in venturing, scale-up, manufacturing, regulatory environments, and health-care reimbursement systems. These capabilities are crucial to translate concepts from the bench to the market. Regenerative medicine ventures are, therefore, considered very risky and prone to failure.

Based on 5 years of research, this chapter examines regenerative medicine commercialization at the university-industry (U-I) boundary from an entrepreneurial perspective. In Sect. 12.2, we discuss the complexities faced by university technology transfer offices (TTOs) in translating regenerative medicine. Using the technology transfer process as a backdrop, we explore how individual-, structural-, cultural-, and market-based factors impact regenerative medicine venturing. In Sect. 12.3, we discuss how the academic entrepreneur and regenerative medicine venture operate within a wider entrepreneurial ecosystem. In Sect. 12.4, we explore business models in regenerative medicine venturing. We note the complex resource requirements, disparate motivations, and market-facing validation challenges of commercializing regenerative medicine innovations. In Sect. 12.5, we discuss the implications for academic scientists, TTOs, industry, and policymakers.

12.2 Regenerative Medicine Technology Transfer

Regenerative medicine venturing immerses scientists and TTOs in complex legal, ethical, and commercial environments. The unique aspects of regenerative medicine technology create conflicts within and among these parties at the U-I boundary [51, 56]. In this section, we provide a brief overview of the university technology transfer process and identify the challenges faced by inventing entrepreneurs, TTOs, and spinout ventures associated with commercializing regenerative medicine inventions.

12.2.1 A Brief Introduction to Technology Transfer

Many TTOs operate as a department within the host university, but there are other models. The Wisconsin Alumni Research Foundation (WARF), for example, is an independent, not-for-profit foundation supporting research at the University of Wisconsin [66]. Imperial Innovations, the TTO for Imperial College London, was spun out of the university as a separate legal entity and is now listed on a public stock exchange.

The university technology transfer process model is relatively straightforward. Scientists disclose inventions to the TTO. The office evaluates each innovation for novelty and long-term value. The TTO may seek intellectual property (IP) protection for the invention, usually by filing a patent application. If no patents are filed, ownership rights may devolve to the inventor, a research funder, or even the public, depending on the institution's policies, the provenance of research funding, and other factors [58]. The TTO may try to monetize IP by licensing or selling patents or use rights.

Some technology transfer activities have generated significant profits for universities. Examples of significant IP monetization include the patents for Google's search engine, which generated $330 million in stock value for Stanford, and the royalty stream for the patented pharmaceutical Lyrica, which Northwestern University sold for $700 million. Major successes are, however, the exception. Relatively few TTO patents are actually licensed, and a minority of licensed patents yield successful products [21]. More recently, traditional licensing is increasingly being replaced by new technology venturing (NTV) activity and, in some instances, direct financial investments by universities into technology ventures through university venture funds. In the NTV model, the inventing scientists or related parties license the technology to a new venture in exchange for equity rather than upfront payment. Licensing patented innovations to NTVs generate tensions for the academic entrepreneurs and TTOs, especially in the field of regenerative medicine technology.

12.2.2 Technology Transfer Offices and Regenerative Medicine

TTOs face numerous challenges supporting translation of regenerative medicine innovations. Many TTOs have been charged with four separate and potentially conflicting missions: obtaining IP rights for innovations, maximizing financial returns from IP, enforcing and punishing IP rights infringement, and generating social benefits from university research [57]. For example, Oxford University's TTO, Oxford University Innovations, states that it "…manages the University's intellectual property portfolio, working with University researchers on *identifying*, *protecting* and *marketing* technologies…" in order to "maximise *social* and *economic* benefits in a *commercial* manner" ([32] – italics for emphasis).

Regenerative medicine venturing presents complex institutional and legal issues for TTOs. Regenerative medicine IP has been more contentious and restrictive than many other technologies, especially in the USA. A limited number of key foundational patents cover fundamental technologies; some TTOs have been relatively aggressive in prosecuting infringement [6, 43]. The narrow IP regime combined with disparate international patenting policies has dramatically increased the cost of global licensing and enforcement of patent infringement. Further, TTOs must

attempt to evaluate regenerative medicine discoveries 5, 10, or even 20 years before the market value of an innovation can be credibly tested. Simply, most lack the necessary skill and expertise to forecast market value and access.

Unclear metrics for regenerative medicine commercialization increase the coordination costs of licensee selection and material transfer agreement (MTA) execution. Assigning exclusive rights for cell lines or foundational stem cell technology to a start-up venture risks locking up the technology for years [23]. Apparently simple technology transfer activities such as MTAs are more complex for stem cells, because agreements must explicitly identify the chain of custody, use rights, and informed consent [45]. TTOs may bear the legal burden of ensuring that donor informed consent is not violated at any stage from lab work to downstream product use. MTA pricing has been affected by these challenges as well as a variety of other factors [39].

Regenerative medicine creates unique tensions for the TTO's social mission. Political and scientific interpretations of the "public good" associated with regenerative medicine have not always been aligned. WiCell, an independent scientific research institute, addresses this directly, noting that its role at founding was in part to serve as "a safe haven for the advancement of stem cell research in the politically charged environment of the time" [67].

Promoting social good through technology transfer is not, in fact, explicit in the dominant legal frameworks governing technology transfer. The Bayh-Dole Act only requires that university-based inventions be entrusted to the free enterprise system, protected from "unreasonable use," and commercialized via small businesses where possible [64]. For TTOs, the balance between potential social good and the obligation to engage with commercial markets may be subject to controversy, even when TTO and university policies are relatively well codified. This is unfortunate, because studies suggest that TTO participation in research commercialization also improves long-term scientific outcomes [57].

12.3 Regenerative Medicine Entrepreneurs, Ventures, and Ecosystems

Innovations do not commercialize themselves. Most TTOs would prefer to license patented technologies to established businesses in exchange for direct financial compensation. In some cases, however, there may be no obvious licensees, often because the technology will require significant additional investment prior to market entry. Relatively few business people actively seek unlicensed university technologies for commercialization. When licensing to an established organization is not evident, the innovators and related members of the academic community are the most likely agents for early-stage commercialization activities [24].

12.3.1 Academic Entrepreneurs in Regenerative Medicine

Successful academics often demonstrate entrepreneurial characteristics: tolerance for ambiguity, inventiveness and creativity, perseverance, and social networking [4]. Yet some academic scientists are discouraged by the myth that academics participating in commercial activity produce less high-quality research. In fact, participation in the patenting process is generally associated with higher publication productivity [17]. Further, academic scientists who participate in commercialization demonstrate an increase in high-quality research output, especially in the life sciences, where collaboration addresses resource scale requirements [65].

At the same time, scientist-entrepreneurs experience a variety of personal and professional conflicts when they engage in commercial activities. These "inventing entrepreneurs" struggle to reorient from a scientific or technological focus to a market-driven identity [24]. Academic scientists may perceive commercially oriented activity as secondary or tertiary to their research and career. These inventing entrepreneurs may receive positive or negative feedback from their institutions [20]. At some research institutions, a stigma may be attached to pursuing commercial outcomes rather than pure knowledge goals. Academic and entrepreneurial roles require significant time commitments; relatively few scientists can invest in both effectively, much less incorporate family and other life activities [33].

Inventing entrepreneurs may struggle to objectively assess their own affinity for a given technological innovation, the relative importance of that innovation, and the risks associated with commercializing different technology types. For example, life scientists may perceive work on blockbuster therapeutic applications to be more fulfilling—and perhaps rewarding—than inventing research tools or diagnostics:

> *You have to sell an awful lot of kits to equal one Prozac. It is much more interesting to develop a therapeutic, [even though] there are many more challenges.* (Ron Raines, University of Wisconsin-Madison)

Perceptions that career and financial incentives are greater for work in therapeutics are strengthened by funders, which tend to provide more resources for clinical applications than for developing tools, techniques, or methodologies.

In regenerative medicine, entrepreneurial scientists must also assess how venturing activity aligns with university and government policies on cell line access. Marketing, distributing, and supporting regenerative medicine-based products requires more administrative and legal oversight than most other university technologies, which may discourage entrepreneurial interest. Further, commercializing regenerative medicine technology brings inventing entrepreneurs into complex ethical and legal discussions. Rarely, if ever, has so much media attention been focused on the potential health benefits—and ethical controversies—of a novel technology. Most academic researchers are inexperienced in dealing with the media and various public groups. As entrepreneurs, their work may become even more visible than they would have in their academic labs.

Enthusiasm for technology transfer and de novo venturing has mediated the stigma of participating in commercial activity at some research institutions [13].

This has been aided by changes in social context, institutional norms, and measures of research funding impact [69]. Regardless, many scientists question whether market-based financial incentives are the appropriate motivation for regenerative medicine technology transfer.

Just as regenerative medicine innovations have multiple possible technology transfer paths, regenerative medicine scientists experience idiosyncratic entrepreneurial journeys. Some remain dedicated academic scientists, while some transition temporarily or permanently into the commercial realm [24]. The experience of Professor Jamie Thomson at the University of Wisconsin, Madison, highlights the rapidly changing nature of role-identity alignment for regenerative medicine scientists:

> *I went into science having no clue that you could actually make money doing it. When I was in high school, that was when recombinant DNA came out and nobody started biotech companies. Ultimately you go into academics because you love a certain area…. It is very good to keep track of the fact that what you do does have commercial interest, and it is important when you're about to publish that you actually think it through and say, "Does this have commercial value?*" (James Thomson, quoted in [24]: 269)

The regenerative medicine scientist engaged in entrepreneurial activity must commit to extensive knowledge acquisition and significant investments of time and energy. Partnering with experienced industry professionals and companies with the expertise in resource management, marketing, regulatory affairs, public relations, and other areas the scientist may lack will go a long way toward making venturing more successful.

Our research into this field has revealed that regenerative medicine venturing is driven by how academic entrepreneurs make sense of the uncertainties associated with commercialization activities. When information gathering and analysis cannot resolve uncertainty, academic entrepreneurs must make decisions under significant uncertainty. Entrepreneurs address this uncertainty with a problem-based coping mechanism or ignore it via an emotion-based coping mechanism [34]. The choice of coping response has important implications for the regenerative medicine ecosystem. Our investigations into regenerative medicine venturing across the UK, the USA, and Russia reveal that entrepreneurial coping mechanisms are directly linked to the entrepreneurial culture within the university [11].

While individual cognitive factors are important, successful commercialization of a regenerative medicine invention still requires a market-facing commercial entity: the regenerative medicine venture.

12.3.2 The Regenerative Medicine Venture

New technology venturing is a complex and resource-intensive process. Regenerative medicine spinouts face special hurdles associated with knowledge collaboration, operations, funding, and exit uncertainty.

Knowledge sharing and partnership, including U-I collaborations, are valuable in technologically complex industries [50, 70]. Regenerative medicine therapeutics could resolve the shrinking pipeline of novel pharmaceutical compounds without cannibalizing existing products and generate returns beyond the patent life horizon because treatments could be based on cell lines inaccessible to competitors [19]. While partnering activity has increased recently, the major pharmaceutical companies have not made significant investments in foundational and early-stage regenerative medicine technologies due to concerns about efficacy, regulations, liability, and unproven cost/benefit against extant treatments [40]. Additionally, market access and reimbursement uncertainties further complicate matters. Pharmaceutical companies are taking fewer risks in collaborative activities and tying payments to results rather than funding exploratory research [52].

> *The industry has struggled under burdensome regulations, unreasonable expectations, and increasingly conservative venture capitalists, while trying to push forward an untested form of therapy that everyone knows will be expensive to implement* [36].

Without partnering, new regenerative medicine ventures are less likely to develop deep capabilities needed to explore related technological and market opportunities [27]. In our research, we found that entrepreneurial propensities for knowledge exchanges and partnering are culturally driven. When the host university emphasizes a high degree of entrepreneurial culture, we recorded greater levels of partnering and development of knowledge capabilities. When entrepreneurial culture at the research institution is inhibited, academic entrepreneurs are less likely to seek out partnerships and knowledge-based collaborations. The latter situation is especially problematic for regenerative medicine ventures.

Regenerative medicine ventures must also overcome numerous operations challenges. To date, only a few companies have demonstrated successful scale-up of cell production under good manufacturing practice (GMP). The legal and regulatory environments for research and commercial use of stem cells in the USA remain uncertain [22]. Although Japan has made significant efforts to streamline the regulatory process for regenerative medicines, the global regulatory context is complex and inconsistent. As one example, the CEO of one UK regenerative medicine venture we interviewed noted that initiating clinical trials required consultation and document filings with five different government departments.

Regenerative medicine therapeutics ventures face especially significant funding hurdles. Therapeutic development and clinical testing generally exceed venture capital fund time horizons and investment limits. The time and costs for development and testing of regenerative medicine therapeutics are beyond the reach of all but the largest venture capital and pharmaceutical firms.

Tools and diagnostics companies may not share this problem but may be significantly hampered by uncertain product specification. Early-stage regenerative medicine tool companies may not be certain about downstream use, because manufacturing, distribution, and use characteristics cannot be confirmed until clinicians actually test products on site.

Regenerative medicine spinouts led by scientists may face skepticism from commercial funders, who may perceive that scientists are not prepared for venture management. Scientists often prefer to maintain multiple technological options, while venture capitalists prefer to invest in ventures that focus resources on one high-potential innovation. Further, the skills associated with managing large projects and teams at the university may not fully transfer to the commercial context [24]. While the efficacy of broad-based entrepreneurship training remains uncertain, targeting specific skill deficiencies to a self-selected and engaged audience of potential inventing entrepreneurs could prove valuable [55]. In regenerative medicine, experiential training that directly addresses coping with failure and knowledge capability development is most likely to be the most effective form of training over any other skill-based emphasis.

Meanwhile, the broader context for funding biotechnology ventures has been challenging. High-profile government funding schemes in some countries can complement, not replace private capital sources. Venture capital funding for biotechnology has fluctuated dramatically with broader economic and market conditions, as well as industry-specific events, such as the exit of Geron from the regenerative medicine field. Venture capitalists remain skeptical of regenerative medicine technologies and product distribution because of the inherent uncertainties and unknown unknowns [28].

Many of the challenges associated with regenerative medicine venture financing and development can be attributed to the exit problem. Venture capitalists prefer investments with a clear path to exit, usually through the sale of the organization, monetization of its assets, or more rarely, an initial public offering. Successful exit events tend to be large transactions requiring high levels of capital liquidity. Funding for exit activity in the life science sector generally has been limited since 2001. Regenerative medicine company funding is further hampered by the limited number of potential acquirers and concerns about long-term liabilities associated with regenerative medicine treatments [18, 61].

Starting, sustaining, and growing a regenerative medicine venture does not happen in a vacuum. Although most critical success factors for regenerative medicine venturing can be linked to the team and technology, the venture must operate within a larger industrial organizational context. Within the management literature, the focus of analysis has shifted from a *cluster* of competitors to an *ecosystem* of related and co-dependent organizations. Of particular interest are *entrepreneurial ecosystems* that nurture high growth and technology ventures.

12.3.3 Regenerative Medicine Entrepreneurial Ecosystems

Entrepreneurial ecosystems have become important considerations in university and government policymaking [38]. The development of regenerative medicine entrepreneurial ecosystems at the U-I boundary is a complex, context-specific phenomenon. Prior research into entrepreneurial ecosystem development has

predominantly emphasized the importance of environmental conditions and policy. In the context of venturing uncertainty, however, these factors alone are insufficient to explain ecosystem outcomes at the U-I boundary [11]. Institutional policies, practices, and resources are fundamental requirements to support entrepreneurial ecosystems. However, uncertainty during regenerative medicine commercialization highlights the agency of the academic entrepreneur.

In particular, high levels of unresolvable uncertainty in regenerative medicine requires entrepreneurs to makes sense of venturing uncertainties through the implementation of preferential coping mechanisms and collaboration strategies. Our prior research revealed that when information gathering and analysis cannot resolve uncertainty, the decision-making abilities of academic entrepreneurs is challenged. Entrepreneurs have the choice to either address this uncertainty directly through a problem-based coping response or choose to ignore it, acting through an emotion-based coping mechanism [34].

Our investigations into regenerative medicine venturing across the UK, the USA, and Russia reveal that entrepreneurial coping mechanisms to uncertainty are largely driven by the entrepreneurial culture within the university. When universities emphasized a strong culture for entrepreneurship, the result was a focus on problem-based coping strategies. In contrast, a low culture for entrepreneurship at the university was associated with emotion-based coping strategies [11].

Both coping and culture are associated with collaboration efforts at the U-I boundary. More specifically, we witnessed greater collaboration effort at the U-I boundary when the ecosystem emphasized problem-based coping along with higher levels of entrepreneurial culture. Reduced collaboration efforts were indicative of an ecosystem that emphasized emotion-based coping and reduced entrepreneurial culture. Since we have already discussed the importance of collaboration and partnering from a knowledge and capability perspective, the latter situation is problematic for regenerative medicine ventures.

Thus, the choice of coping response has important implications for the types of spinout ventures at the U-I boundary and the regenerative medicine ecosystem. Our prior research revealed a typology of regenerative medicine ecosystem types. Shown in Fig. 12.1, these ecosystem types derive from the degree of partnership development at the U-I boundary and the dominant coping response to uncertainty [10].

Healthy ecosystems are characterized by problem-based coping and collaborative knowledge development. We believe these ecosystems best adapt and respond to the uncertainties inherent to regenerative medicine technology transfer and venturing [27].

Insular domains are ecosystems where firms problem-solve but collaborate poorly. This generates a parochial network of firms that rely predominantly on internal knowledge and resources for venturing. In these ecosystems, entrepreneurs and ventures utilize their problem-solving capabilities to primarily focus on challenges within the organization and its immediate context, rather than exploring broader market opportunities. When the technology, team, and application are well-designed (and, perhaps, lucky) these ecosystems may appear healthy, despite lacking the ability to collaborate for knowledge creation and new opportunities. Since regenerative

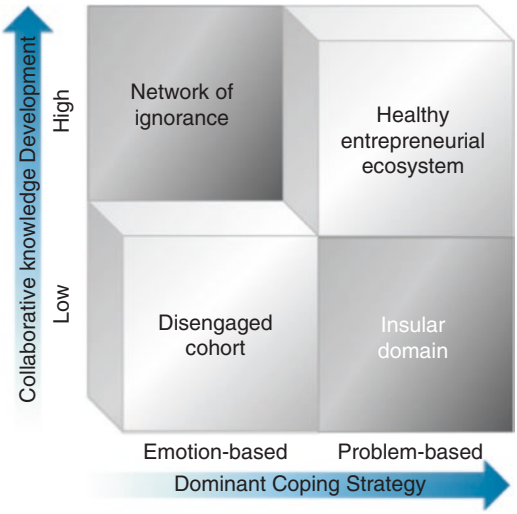

Fig. 12.1 Entrepreneurial ecosystem types. (Originally published in [10], European Business Review)

medicine venturing operate within a high-uncertainty environment, we believe that ventures that rely solely on their extant capabilities and knowledge are significantly at risk for missing key trends and market changes.

Networks of ignorance form when venturing activity utilizes collaboration in the context of an emotion-based coping strategy. Emotion-based coping ignores the impact of uncertainty and the need for adaptation. This type of collective behavior can be especially dangerous, because it presents the appearance of market-facing activity, via networking, while actually encouraging entrepreneurs and ventures to connect only with like-minded participants. Homophily effects can lead these firms to collaborate with similar firms who equally have similar misconceptions of specific technologies and/or commercialization models.

Disengaged cohorts result from poor knowledge collaboration and an emotion-based coping strategy. In these groups and ecosystems, entrepreneurs and ventures attempt to execute one commercialization model without feedback or the ability to adapt. We believe that such ecosystems are almost certain to fail.

12.4 Regenerative Medicine Business Models

A key inhibitor to regenerative medicine commercialization is the lack of proven business models. In this section, we describe the business model challenge for regenerative medicine ventures, summarize regenerative medicine business model research, and suggest an approach to characterize general regenerative medicine business models. We also discuss business model innovation in the regenerative medicine industry.

12.4.1 Business Model Design in the Life Sciences

A business model is the design of the organization to exploit a commercial opportunity [25]. In other words, a business model provides a map of key organizational elements that shows how the entity creates and captures value to succeed as a viable commercial venture.

Various studies have assessed dominant business models in the life sciences industry, focusing on one of three organizational characteristics. Some distinguish between innovation-driven versus market-driven activities [68]. Others distinguish product, service, and platform businesses [46]. Finally, some emphasize vertical integration from research and materials provision through end use [8, 44].

Broadly speaking, new life science ventures utilize a technology innovation or platform to target a specific application or market space. Inherent resource limitations require most technology ventures to focus on one product or indication at a time.

The "blockbuster" pharmaceutical business model is predicated on a treatment modality in which patients receive long-term treatments over many years. The advent of regenerative medicine *cures* for long-term, chronic ailments presents challenges for pricing and reimbursement. A value-based pricing policy for a cure, rather than lifetime treatment, may not present sufficient financial returns to justify the required investment in R&D and clinical trials.

In the near term, services and tools ventures have the clearest path to viable business models. No clinical trials are required to sell such products for R&D use by other organizations. These firms therefore require smaller investments and generate, on average, commensurately smaller returns. Similarly, diagnostics and related pretreatment mechanisms have a significantly reduced regulatory requirement compared to treatments.

12.4.2 Exploring Business Models in Regenerative Medicine

Regenerative medicine ventures will likely use a wide spectrum of models to serve a variety of business and patient markets. Some general business models in the life sciences industry will be relevant for regenerative medicine ventures. Some will be highly localized, adapted to rapid physical delivery of cultured cells from production site to clinical application. It is conceivable that this type of business model could be franchised, with disparate, small-scale production facilities supporting local clinical treatments. Others will be global and fully integrated, competing with or subsumed by the pharmaceutical companies. It is quite possible that the treatment potential for certain diseases will become matters of government interest. This could generate quasi-governmental organizations, or special purpose public-private partnerships, that oversee or even implement production and distribution of certain tools or treatments.

Some regenerative medicine ventures, however, will require entirely new business models, especially as the industry evolves [30]. Entrepreneurs and policymakers must remember that the major pharmaceutical companies have significant advantages in slack resources, scale-up capacity, market knowledge, and distribution relationships. They will be favored by innovations that utilize explicit and transferable capabilities, assets, and IP. Tacit and time-based capabilities are therefore critical to the success of new entrants. If innovative business models utilize tacit capabilities and technologies that are costly to transfer or acquire, then new, innovative regenerative medicine ventures could achieve significant success.

12.4.2.1 Toward a Typology of Business Models in Regenerative Medicine

To develop a more useful typology of regenerative medicine business models, we utilize a general framework from organization studies. Although many different frameworks have been proposed for understanding business models, the simplest and most clear relies on three general components or structures: resources, transaction, and value [25]. The resource structure of the firm is the configuration of assets, knowledge, and capabilities leveraged to meet market needs. These are, in effect, all the "things" that give an organization an advantage over other organizations. Transactive structure describes the type, content, and governance of interactions with suppliers, partners, and customers [3]. These are all the connections and exchanges that the organization engages in, with both individuals and entities, in order to convert resources into outcomes that are valuable. The value dimension represents the type and form of value actually created for customers and other stakeholders [2].[1]

A generalized business model typology for regenerative medicine companies must incorporate whether the firm targets therapeutics or enables therapeutic development by other firms, the nature of the cell technology, manufacturing requirements, and distribution characteristics. Limitations on cell survival as well as idiosyncratic and specialized delivery requirements may be key determinants in the structure of successful regenerative medicine businesses.

Based on the prior literature of life science venturing, regenerative medicine business models can be described with eight determining categories. The resource structure includes cell delivery model, level of vertical integration, and resource differentiator. The transactive structure includes customer type, manufacturing specificity, and distribution scale. The value structure includes value form and type. This classification for regenerative medicine business model categories and organizational elements is shown in Table 12.1.

Corporate strategy suggests that a viable business model uses one and only one element from each category [31]. Addressing more than one element within a

[1] It is, unfortunately, impossible to quickly recapitulate the organizational literature on business models and how they function. Interested readers are directed to the following for an introduction to business models in theory and practice [9, 25, 44, 71, 72].

Table 12.1 An integrated typology of regenerative medicine business models

Business model structure	Regenerative medicine business model categories	Regenerative medicine business model elements
Resources	Cell model	Autologous
		Allogenic
		Acellular
	Vertical integration	Niche product
		Platform technology
		Vertically integrated
	Resource differentiator	Research innovation
		Operational efficiency
Transaction	Customer type	End user
		Therapeutic developer
	Manufacturing specificity	Industrial scale
		Moderate scale
		Small or batch scale
		Customer scale
	Distribution scale	Local
		Regional/national
		Global
Value	Value form	Product
		Service
		Solution
	Value type	Profit
		Impact

category makes the organization vulnerable to focused competitors via inefficient operations or poor fit with market segment characteristics [37, 49]. It is important to note, of course, that new and established ventures often operate parallel business models or test multiple business models during exploratory periods. Business model theory suggests that such experimentation is valuable [26]; corporate strategy theory suggests that longer-term competitive success depends on moving from experimentation to implementation.

The eight dimensions and twenty elements in the typology suggest hundreds of business models are *possible*, but only a limited number will prove to be *viable*. To date, relatively few have been tested. The only proven models have been demonstrated primarily at firms providing niche products and services supporting therapeutic development. Equally important, not all possible business models are viable at scale.

To explore the business model space in the context of regenerative medicine, we consider three case examples. Cellular Dynamics International, acquired by FujiFilm, commercialized foundational stem cell and induced pluripotent stem cell (iPSC) technology developed by James Thomson. Cellular Logistics, a spinout of the Wisconsin Institute for Medical Research, is commercializing an extracellular matrix as a cardiac therapeutic and cell delivery platform. Censo Biotechnologies,

formerly Roslin Cells, is a hybrid entity originally spun out of the University of Edinburgh. These mini-cases present only the minimum information to explore possible business models. The examples provide only a thin slice of the variety of regenerative medicine ventures already in existence and business models yet to be tested.

12.4.2.2 Testing and Selecting Basic Business Models in Regenerative Medicine: Cellular Logistics

Cellular Logistics[2] (CLI) was formed in 2016 to commercialize an extracellular matrix developed at the Wisconsin Institute for Medical Research at The University of Wisconsin-Madison. Dr. Eric Schmuck discovered the biomaterial during doctoral research on cardiac fibroblasts. The innovation was patented by the WARF in 2014. Working with Dr. Amish Raval, an interventional cardiologist, Schmuck determined that the matrix demonstrated pro-regenerative characteristics in a myocardial infarction mouse model. Since that time, the technology has also been demonstrated to significantly improve cell retention when delivered as a combination product.

As of the writing of this chapter, CLI is still exploring possible business models. The use of the company's acellular ECM as a cardiac therapeutic represents a relatively standard life science therapeutic business model. As previously noted, however, the economics of a business model based on curative rather than long-term treatment have not been fully demonstrated.

The cell delivery embodiment presents at least two alternative business model options. For example, the company could in-license cell or regenerative medicine products and attempt to commercialize combination therapeutic products. Alternately it could out-license or sell the cell delivery product for other regenerative medicine therapeutics companies.

Three possible business models for CLI are shown in Table 12.2. Each requires different resources and presents a distinct risk-reward profile to founders, partners, and investors. Many, if not most, de novo ventures in the regenerative medicine field will face similar business model challenges and choices.

12.4.2.3 Hybrid Business Models in Regenerative Medicine Venturing: Censo Biotechnologies

Some universities have participated in novel, hybrid institutional activities to support regenerative medicine venturing [1]. These include one-off entities such as incubators and government funding schemes. It is unclear, however, whether such systems promote success by supporting the organization during the critical, fragile launch period or facilitate failure by shielding the organizations from the market

[2] Full disclosure: Adam J. Bock, Eric Schmuck, Amish Raval, and Peiman Hematti are the co-founders of Cellular Logistics. Data collection and analysis for this mini-case were implemented by David Johnson who has no relationship to the company.

Table 12.2 Possible business models for Cellular Logistics

Business model categories	ECM cardiac therapeutic	ECM cell delivery platform – in-license	ECM cell delivery platform – tool sales
Cell model	Acellular	Acellular	Acellular
Vertical integration	Niche	Vertically integrated	Platform
Resource differentiator	Research innovation	Research innovation	Operational efficiency
Customer type	End user	End user	Therapeutic developer
Manufacturing specificity	Moderate scale	Moderate scale	Industrial scale
Distribution scale	Regional/national	Regional/national	Global
Value form	Product	Product	Solution
Value type	Profit	Profit	Profit

ECM extracellular matrix

and industry forces that it must eventually address and overcome [53]. Even direct investments by universities into regenerative medicine centers of excellence may not be enough to promote commercialization activities, which may be ultimately driven at the level of the central research institution. Censo Biotechnologies Ltd. is a regenerative medicine technology company providing human cells and contract research services for drug discovery, toxicity testing, and cell banking. It began operations in 2006 as Roslin Cells, which was spun out of The University of Edinburgh's Roslin Institute. Roslin Cells was a not-for-profit organization, whose activities spanned the development of cell therapies, and GMP manufacturing services for cell therapy products and mammalian cell banks for clinical use. Roslin Cells also provided a range of pluripotent stem cells for clinical use and research. In 2008, Roslin Cellab was created as a sister company to Roslin Cells since certain commercialization activities were felt to be incompatible with the not-for-profit status of Roslin Cells. This for-profit organization was responsible for assisting organizations in developing products and services for the regenerative medicine space via access to both stem cells and regenerative medicine know-how. Both Roslin Cell and Roslin Cellab had to undergo some critical adjustments to their respective business models. The ethical controversies surrounding the use of embryonic stem (ES) cells saw both organizations alter their commercialization activities to one that centered around the use of iPSCs rather than ES cells.

In 2016, Roslin Cells announced the creation of two subsidiaries. The first subsidiary, Roslin Cell Therapies, would accelerate the process development and manufacture of cell-based therapies and advanced therapy medicinal products (ATMPs). The second subsidiary, Roslin Cell Sciences, would focus on the development of iPSCs for commercial and academic drug use. In May 2016, Roslin Cellab and Roslin Cells Sciences announced a merger, with the parent company being renamed Censo Biotechnologies. Roslin Cell Sciences would continue to trade as a wholly owned subsidiary.

This case clearly highlights the complexities of operating within the regenerative medicine commercialization space. Since founding in 2006 as a spinout from the

University of Edinburgh, Censo's business model changed from a not-for-profit organization to a hybridized for-profit and not-for profit organization. Censo also switched from human ES cells to iPSCs due to ethics-related concerns. Finally, the new organization spans both services and therapeutics (Table 12.3).

12.4.2.4 Business Model Innovation in Regenerative Medicine Venturing: Cellular Dynamics International

Although business models have received extensive attention in organizational literature, business model innovation is relatively unstudied. Business model innovation is a "known unknown" in regenerative medicine venturing. Successful business model innovation may not be predicted by prior innovation success [14]. In other words, today's failing spinout company may be tomorrow's regenerative medicine business model innovator. Even successful business model innovators, however, may not capture the value of their pioneering efforts. The winners of radical innovation stages are determined by whether inventions rely on extant capabilities or require entirely new technology and process capabilities [62]. Innovative business models can dramatically disrupt industries, bringing new competitors to the field, function alongside incumbent communities, or even reinforce the advantages of the incumbents.

It is, therefore, useful to consider examples of business model innovation to explore how such processes unfold. Cellular Dynamics (CD) provides an example of configurational business model innovation in the regenerative medicine space.

Cellular Dynamics was originally formed as two entities spun out of University of Wisconsin-Madison based on the research of James Thomson. As a stand-alone firm, CD was a world leader in supplying mass produced stem cells as drug discovery research and development tools. The company also maintained long-term research to develop cell-based treatments, with special interest in blood therapeutics. This configuration of separate entities aligned with traditional strategic frameworks that separate business models based on risk-reward profiles [37]. After

Table 12.3 Evolution of Censo Biotechnologies business model

Business model categories	Focused stem cell sourcing	Hybrid regenerative medicine product and services provider
Cell model	Allogenic	Allogenic
Vertical integration	Niche product	Vertically integrated
Resource differentiator	Research innovation	Research innovation and operational efficiency
Customer type	Therapeutic developer	Therapeutic developer
Manufacturing specificity	Customer scale	Small and customer scale
Distribution scale	Regional (Europe)	Regional (Europe) and global
Value form	Product and service	Product, service, and solution
Value type	Not-for-profit	For-profit and impact

licensing iPSC technology from WARF, the distinct entities were merged in 2008 to pursue both tools and therapeutics.

In a prior study, we used neural network analysis to simulate possible business models for the entity [26]. Possible options included a "null" solution in which all business model elements were inactive, as well as a focused therapeutics business and a variant of the two-entity solution. While these outcomes were coherent and plausible, they emerged in relatively few of the simulation runs.

Table 12.4 shows CD's business model change, including two of the plausible business model innovations identified by the simulation.

The change process selected by management (and by the neural network) created a coherent platform-based business model incorporating partly inconsistent organizational elements. The internal inconsistencies created resource allocation challenges across the tools and therapeutics operations of the combined organization. Despite these "flaws," the new configuration was internally coherent, which facilitated fundraising and helped management prioritize scale-up manufacturing, partnering, and marketing activities. CD was named the most innovative company in the world in 2011 by *The Wall Street Journal*. In 2015, CD was acquired by FujiFilm for USD $315 Million.

As previously noted, the typology hints at hundreds of possible business models that have yet to be formalized or tested. Possible business model innovation examples include not-for-profit treatment systems targeted at poor populations, disease-specific long-term treatment centers that align with the US reimbursement system, and public-private partnerships that link the cell culturing infrastructure of large research institutions with specialized, localized delivery. Cooperation among disparate economic participants, including government, philanthropy, and national health agencies, suggests that innovative solutions aligned with both physical delivery and payment systems could achieve rapid adoption. Developing business model innova-

Table 12.4 Realized and unrealized business model innovation at Cellular Dynamics

Business model categories	Original parallel stem cell entities business model	Unrealized parallel iPS entities business model	Realized iPS platform business model
Cell model	Allogenic	Allogenic	Allogenic
Vertical integration	Niche product	Niche product	Platform technology
Resource differentiator	Research innovation and operational efficiency	Research innovation and operational efficiency	Operational efficiency
Customer type	End user and therapeutic developer	End user and therapeutic developer	Therapeutic developer
Manufacturing specificity	Moderate and batch scale	Moderate and batch scale	Industrial scale
Distribution scale	Global	Global	Global
Value form	Product	Product	Solution
Value type	Profit	Profit	Profit

iPS induced pluripotent stem cells

tion types include Lonza's strategic manufacturing relationship with NIH CRM and Life Technologies development of a horizontally integrated platform of research and manufacturing tools. Again, it is too early to be sure whether these represent commercially viable business model innovations.

12.5 Facing Reality in Regenerative Medicine Entrepreneurship

To date, the promise of regenerative medicine science has not yielded significant advances in human health care and outcomes. While the general consensus of scientists and businesspeople remains positive, the near- and mid-term reality requires pragmatism.

12.5.1 The Flawed Narrative of Regenerative Medicine Entrepreneurship

In a developing industry like regenerative medicine, where technological innovation confers significant value, strategic analysis can be misleading ([13, 26]). In this context, entrepreneurs succeed by accomplishing what other firms find impossible:

> *Entrepreneurship is a process by which individuals—either on their own or inside organizations—pursue opportunities without regard to the resources they currently control* [60].

Entrepreneurship inspires heroic narratives precisely because it strives against the odds. Similarly, regenerative medicine science aims to radically improve healthcare outcomes. The combination of entrepreneurial action and regenerative medicine potential has proven irresistible to scientists, institutions, entrepreneurs, financiers, and governments, as a narrative of hope and promise. Narratives of emotion and cognition are important sensemaking devices [16]. In regenerative medicine, the narrative structures that address venturing uncertainty encourage entrepreneurs to perceive their efforts within a heroic context, precisely because of unrealistic expectations for commercial and societal impact.

Despite sometimes glowing depictions in the media, however, entrepreneurship is not generally a mythical, heroic endeavor. Most risk capital-funded technology ventures fail within 5 years of founding. The significant majority of venture capital-funded life science ventures will not bring a product to market.

12.5.2 The Role of Failure in Regenerative Medicine Venturing

Failure is an unavoidable aspect of entrepreneurial activity. Industry-level advances almost always induce a high failure rate during innovation phases. In fact, high levels of experimentation and concomitant failure are directly linked to higher levels of community-level learning and evolution [7]. Even well-funded, proven competitors may struggle.

> *Every [regenerative medicine] venture cannot possibly be successful* [47].

The challenges of regenerative medicine commercialization were exemplified by failure at Geron, a pioneer in the field [54]. Regenerative medicine ventures are exposed to significant risks, which can be quantified and managed, and uncertainties, for which firms can only prepare and hope to adapt [35]. The reality of most regenerative medicine venturing activity is a constant struggle with both real and perceived failure. As one venture founder and CEO explained to us:

> *I went to a meeting in India with the company, I did some seminars and things there; I've done all sorts of things… but things are not progressing very well this year, and really if things don't really start picking up I really need to think about perhaps doing something else.*

The problem is that regenerative medicine venturing carries high costs in research infrastructure, patent prosecution, and venture support. This has led some universities, TTOs, and even funders to reject venture failure as an option. In these cases, institutions and individuals continue to support regenerative medicine ventures long after the technological innovation or business model has proven infeasible.

We have heard institutional stakeholders defend this approach with cluster economics theory, based on the idea that a critical mass of regenerative medicine commercial activity will become self-sustaining [48]. This is an understandably attractive argument, in which the key ingredients for a thriving commercial regenerative medicine ecosystem are sufficient finance and staying power. But, keeping "dead" ventures alive is problematic for regenerative medicine ecosystems. Valuable resources become locked into ventures that could otherwise be redeployed within the ecosystem to better purpose [5, 59]. This includes capital, people, and technology.

Worse, facilitating regenerative medicine venturing activity will not automatically lead to large, successful businesses tied to the local community [42]. The only guaranteed outcome of facilitating more ventures is more failed ventures. Overemphasizing the importance of local ventures may also be misguided. Since robust entrepreneurial ecosystems have porous boundaries, protecting boundaries too rigorously will only hinder the development of a healthy and resilient ecosystem. The development of viable industry clusters or ecosystems requires a complex set of factors not easily reproduced via extra-market programs and incentives [41].

Scientists choosing to participate in the commercialization of their regenerative medicine research should carefully assess their own motivations. Researchers pri-

marily interested in financial gain, responding to institutional or peer pressure or high levels of technology affinity, are likely to experience disappointing results. On the other hand, scientists interested in the commercialization *process*, or engaged in addressing specific medical problems, are likely to build and extend their own capabilities. Researchers may further their academic career regardless of the ultimate disposition of the specific technology or venture.

12.5.3 The TTO Funding and Returns Problem

It is unlikely that new models for funding will significantly impact licensing or spinout activities. Since pharmaceutical partnering and venture capital thrives on economies of scale and informal networks, a limited number of ventures from a select set of university research programs will likely continue to receive the lion's share of early-stage investment. In addition, being first to market is a dramatically different proposition than being first to the FDA with a novel therapeutic treatment. This suggests that most TTOs supporting first generation regenerative medicine technologies (with the possible exception of institutions with key foundational patents) will achieve low long-term financial rewards. The returns on second-generation regenerative medicine medicines may be significantly higher.

Ultimately, the challenge facing TTOs commercializing regenerative medicine innovations is the selection of success metrics. Even the most efficient, effective TTOs will struggle to support extensive development activities spanning discovery to monetization. Setting up non-profit distribution entities such as Roslin Cells and WiCell may provide a partial solution to the commercialization challenge but is unlikely to generate the long-term yields associated with upfront, high-risk investments. The perceived social value of regenerative medicine has challenged the presumption that TTO returns should primarily accrue to the TTO and the institution. In the long run, TTOs may find that balancing regenerative medicine patenting, licensing, and venturing outcomes requires more explicit prioritization, especially as pricing and distribution models for disease treatment evolves.

12.5.4 Focusing on the Ecosystem Rather than the Innovation

Our findings suggest that universities, TTOs, and policymakers should focus attention on the ecosystem when evaluating commercialization outcomes [12]. This is especially important because the *direct* economic impact of technology transfer, especially NTV activity, is often overstated (e.g., [15, 21, 42]). Further, efforts by universities and policymakers to copy practices from mature successful ecosystems tend to be unsuccessful. While some best practices may be imitable [29], transplanting entire programs or structures ignores critical, localized idiosyncrasies in culture, entrepreneurial norms, and institutional context. In other words, TTOs and

policymakers need to understand the nature of the local and regional ecosystem to effectively develop metrics of success that incentivize and reinforce problem-based coping and knowledge collaboration activities. Supporting one innovation at a time appears to be the shortest path to commercial success, but ultimately it is the health of the ecosystem that will determine long-term economic outcomes.

12.6 Conclusions

Regenerative medicine venturing at the U-I boundary is complex, difficult, and uncertain. Conflicting motivations across individuals and organizations create tensions for scientists, entrepreneurs, and institutions. In the short term, firms that focus on tools, services, and diagnostics present more viable business models than ventures based on novel regenerative medicine therapeutics. Resolution of regulatory, delivery, pricing, reimbursement, and exit uncertainty will determine whether innovative business models favor upstarts or incumbents. In the meantime, inventing entrepreneurs, TTOs, and policymakers should approach regenerative medicine venturing with realistic and appropriate metrics for success and with an eye toward the development of healthy entrepreneurial ecosystems.

References

1. Aboody K, Capela A, Niazi N, Stern JH, Temple S. Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta Stone. Neuron. 2011;70(4):597–613.
2. Afuah A, Tucci CL. Internet business models and strategies: text and cases. New York: McGraw-Hill Higher Education; 2000.
3. Amit R, Zott C. Value creation in e-business. Strateg Manag J. 2001;22(6/7):493–520.
4. Barringer BR, Ireland D. Entrepreneurship: successfully launching new ventures. Saddleback: Prentice-Hall Pearson; 2011.
5. Baumol WJ. Entrepreneurship: productive, unproductive and destructive. J Polit Econ. 1990;98:893–921.
6. Bergman K, Graff GD. The global stem cell patent landscape: Implications for efficient technology transfer and commercial development. Nat Biotechnol. 2007;25:419–24.
7. Bernardo A, Welch I. On the evolution of overconfidence and entrepreneurs. J Econ Manag Strateg. 2001;10(3):301–30.
8. Bigliardi B, Nosella A, Verbano C. Business models in Italian biotechnology industry: A quantitative analysis. Technovation. 2005;25(11):1299–306.
9. Bock AJ, George G. The business model book: design, build and adapt business ideas that thrive. Harlow: Pearson; 2018.
10. Bock AJ, Johnson D. Entrepreneurial ecosystems: fixing the triple helix. Eur Bus Rev, November–December. 2015;73–76.
11. Bock AJ, Johnson D. A comparative study of ecosystem development in regenerative medicine. In: Phan P, editor. Academic entrepreneurship: translating discoveries to the marketplace. Cheltenham: Edward Elgar; 2016. p. 218–50.

12. Bock AJ, Johnson D. The uniqueness of stem cell ecosystems: lessons in matching local culture. Coller Venture Rev. 2016;4:42–53.
13. Bock AJ. Technology transfer. In: Marvel, editor. Encyclopedia of new venture management. Thousand Oaks: SAGE; 2012.
14. Bock AJ, Opsahl T, George G, Gann DM. The effects of culture and structure on strategic flexibility during business model innovation. J Manag Stud. 2012;49(2):279–305.
15. Breznitz SM, Feldman MP. The larger role of the university in economic development: Introduction to the special issue. J Technol Transf. 2012;37(2):135–8.
16. Byrne O, Shepherd DA. Different strokes for different folks: entrepreneurial narratives of emotion, cognition and making sense of business failure. Entrep Theory Pract. 2013;39(2):375–405.
17. Calderini M, Franzoni C, Vezzulli A. If star scientists do not patent: The effect of productivity, basicness and impact on the decision to patent in the academic world. Res Policy. 2007;36(3):303–19.
18. Cyranoski D. Patients seek stem-cell compensation. Nature.com, July 6, 2012. 2012. Accessed online on 17 Sept 2012 at http://blogs.nature.com/news/2012/07/patients-seek-stem-cell-compensation.html.
19. Denoon A, Vollebregt E. Can regenerative medicine save Big Pharma's business model from the patent cliff? Regen Med. 2010;5(5):687–90.
20. Etzkowitz H. The norms of entrepreneurial science: cognitive effects of the new university–industry linkages. Res Policy. 1998;27(8):823–33.
21. Feldman M, Feller I, Bercovitz J, Burton R. Equity and the technology transfer strategies of American research universities. Manag Sci. 2002;48(1):105–21.
22. Fink DW. FDA regulation of stem cell–based products. Science. 2009;324(5935):1662–3.
23. Frangioni JV. The impact of greed on academic medicine and patient care. Nat Biotechnol. 2008;26(5):503–7.
24. George G, Bock AJ. Inventing entrepreneurs. Saddleback: Prentice Hall Pearson; 2008.
25. George G, Bock AJ. The business model in practice and its implications for entrepreneurship research. Entrep Theory Pract. 2011;35:83–111.
26. George G, Bock AJ. Models of opportunity. New York: Cambridge University Press; 2012.
27. George G, Kotha R, Zheng Y. Entry into insular domains. A longitudinal study of knowledge structuration and innovation in biotechnology firms. J Manag Stud. 2008;45(8):1448–74.
28. Giebel LB. Stem cells – A hard sell to investors. Nat Biotechnol. 2005;23(7):798–800.
29. Graham R. Creating university-based entrepreneurial ecosystems: evidence from emerging world leaders. Cambridge, MA: Massachusetts Institute of Technology Report; 2014.
30. Heirman A, Clarysse B. How and why do research-based start-ups differ at founding? A resource-based configurational perspective. J Technol Transf. 2004;29(3–4):247–68.
31. Hill CWL, Rothaermel FT. The performance of incumbent firms in the face of radical technological innovation. Acad Manage Rev. 2003;28(2):257–74.
32. ISIS Innovation website, Oxford University. Accessed on 14 Sept 2012 at http://www.isis-innovation.com/about/index.html.
33. Jain S, George G, Maltarich M. Academics or entrepreneurs? Investigating role identity modification of university scientists involved in commercialization activity. Res Policy. 2009;38(6):922–35.
34. Johnson D, Bock AJ. Coping with uncertainty: entrepreneurial sensemaking in regenerative medicine venturing. J Technol Transf. 2017;42(1):33–58.
35. Knight FH. Risk, uncertainty and profit. Boston: Houghton Mifflin Co; 2006.
36. Ledford H. In search of a viable business model. Nature Reports Stem Cells, October 30, 2012. 2008. Accessed online on 17 Sept 2012 at http://www.nature.com/stemcells/2008/0810/081030/full/stemcells.2008.138.html.
37. Markides C, Charitou CD. Competing with dual business models: A contingency approach. Acad Manag Perspect (1993–2005). 2004;18:22–36.
38. Mason C, Brown R. Entrepreneurial ecosystems and growth entrepreneurship. Background paper prepared for the workshop organised by the OECD LEED Programme and the Dutch

Ministry of Economic Affairs on entrepreneurial ecosystems and growth orientated entrepreneurship, The Hague, Netherlands, 7th November 2013. 2014.
39. McCormick JB, Owen-Smith J, Scott CT. Distribution of human embryonic stem cell lines: who, when, and where. Cell Stem Cell. 2009;4(2):107–10.
40. McKernan R, McNeish J, Smith D. Pharma's developing interest in stem cells. Cell Stem Cell. 2010;6(6):517–20.
41. Menzel MP, Fornahl D. Cluster life cycles-dimensions and rationales of cluster evolution. Ind Corp Chang. 2010;19(1):205–38.
42. Miner AS, Eesely DT, DeVaughn M, Rura T. The magic beanstalk vision of university venture formation. In: The Entrepreneurship dynamic: origins of entrepreneurship and its role in industry creation and evolution. Stanford: Stanford University Press; 2001.
43. Nature Biotechnology. "Burning Bridges" – Editorial. 2007. Nat Biotechnol. 2007;25:2.
44. Nosella A, Petroni G, Verbanoc C. Characteristics of the Italian biotechnology industry and new business models: the initial results of an empirical study. Technovation. 2005;25(8):841–55.
45. O'Connor S. The use of MTAs to control commercialization of stem cell diagnostics and therapeutics. Berkeley Technol Law J. 2006;21(3):1017–54.
46. Patzelt H, Knyphausen-Aufsess DZ, Nikol P. Top management teams, business models, and performance of biotechnology ventures: an upper echelon perspective. Br J Manag. 2008;19(3):205–21.
47. Perkel JM. The evolving regenerative medicine business model: concept and funding. Harvard Stem Cell Institute. 2011. Accessed online Sept 2012 at http://www.stembook.org/node/713.
48. Porter M. The competitive advantage of nations. Cambridge, MA: Harvard Press; 1990.
49. Porter ME. Competitive advantage: creating and sustaining superior performance. New York: Free press; 1998.
50. Powell WW, Koput KW, Smith-Doerr L. Interorganizational collaboration and the locus of innovation: networks of learning in biotechnology. Adm Sci Q. 1996;41(1):116–45.
51. Resnik D. Commercialization of human stem cells: ethical and policy issues. Health Care Anal. 2002;10:127–54.
52. Rockoff J, Tam PW. Biotech funding gets harder to find. Wall Street J, March 16, 2012. 2012. Accessed online on 17 Sept 2012 at http://online.wsj.com/article/SB10001424052970203961204577267814201399918.html.
53. Rothaermel FT, Thursby M. Incubator firm failure or graduation?: the role of university linkages. Res Policy. 2005;34(7):1076–90.
54. Scott C, Huggett B. Geron's quixotic fate. Nat Biotechnol. 2012;30:497.
55. Siegel DS, Phan PH. Analyzing the effectiveness of university technology transfer: implications for entrepreneurship education. In: Advances in the Study of Entrepreneurship, Innovation & Economic Growth, vol. 16; 2005. p. 1–38.
56. Siegel DS, Veugelers R, Wright M. Technology transfer offices and commercialization of university intellectual property: performance and policy implications. Oxf Rev Econ Policy. 2007;23(4):640–60.
57. Siegel DS, Waldman DA, Atwater LE, Link AN. Toward a model of the effective transfer of scientific knowledge from academicians to practitioners: qualitative evidence from the commercialization of university technologies. J Eng Technol Manag. 2004;21(1–2):115–42.
58. Speser PL. The art and science of technology transfer. Hoboken: Wiley; 2006.
59. Stam E. Entrepreneurial ecosystems and regional policy: a sympathetic critique. Eur Plan Stud. 2015;23(9):1759–69.
60. Stevenson HH, Jarillo JC. A paradigm of entrepreneurship: entrepreneurial management. Strateg Manag J. 1990;11(5):17–27.
61. Taylor L. Stem cell market growth "facing thorny challenges." PharmaTimes online, June 18, 2012. 2012. Accessed on 17 Sept 20012 at http://www.pharmatimes.com/article/12-06-18/Stem_cell_market_growth_facing_thorny_challenges.aspx.
62. Tripsas M. Unravelling the process of creative destruction: complementary assets and incumbent survival in the typesetter industry. Strateg Manag J. 1997;18(S1):119–42.

63. Trounson A, Thakar RG, Lomax G, Gibbons D. Clinical trials for stem cell therapies. BMC Med. 2011;9:52.
64. U.S. Code. Title 35, Part 2, Chapter 18. 1980. Accessed online at http://uscode.house.gov/download/pls/35C18.txt.
65. Van Looy B, Callaert J, Debackere K. Publication and patent behavior of academic researchers: conflicting, reinforcing or merely co-existing? Res Policy. 2006;35(4):596–608.
66. WARF. Wisconsin Alumni Research Foundation website. 2012. Accessed at http://www.warf.org/news/index.jsp?cid=20.
67. WiCell. 2012. WiCell website accessed at http://www.wicell.org/home/about-wicell/about-wicell.cmsx on 1-November 2012.
68. Willemstein L, van der Valk T, Meeus MTH. Dynamics in business models: an empirical analysis of medical biotechnology firms in the Netherlands. Technovation. 2007;27(4):221–32.
69. Wolfinger NH, Mason MA, Goulden M. Stay in the game: gender, family formation and alternative trajectories in the academic life course. Soc Forces. 2009;87(3):1591–621.
70. Zucker LG, Darby MR. Star scientists and institutional transformation: patterns of invention and innovation in the formation of the biotechnology industry. Proc Natl Acad Sci. 1996;93(23):12709–16.
71. Teece DJ. Business models, business strategy and innovation. Long Range Plan. 2010;43(2–3):172–94.
72. Zott C, Amit R, Massa L. The business model: recent developments and future research. J Manag. 2011;37(4):1019–42.

Index

E. G. Schmuck et al. (eds.), *Cardiac Extracellular Matrix*, Advances in Experimental Medicine and Biology 1098, https://doi.org/10.1007/978-3-319-97421-7