The extracellular matrix (ECM) is a complex and dynamic entity that drives the formation and development of the cardiovascular system, determines critical aspects of cardiovascular performance, and plays key roles in the initiation and progression of abnormal cardiovascular function with aging and disease. Microscopic studies in the late 1700s identified fibrous structures surrounding cells, which led scientists to conclude that the ECM was primarily a foundational unit to provide support. Unfortunately, this historic view that the ECM is a static scaffold is still to this day held by many who are not in the field. Using molecular, cell based, and dynamic imaging systems, however, experts now recognize that the ECM is an ever-changing component that responds to normal and abnormal molecular and biophysical cues and, in turn, drives changes in overall cardiovascular structure and function.

The ECM contains structural and nonstructural proteins, interacts dynamically with unique and differentiating cell types, serves as a reservoir and processing site for signaling molecules, and forms communication corridors for both protein and genetic information. Thus, the ECM is a diverse entity that presents a novel and exciting research frontier that could yield improvements in diagnostics, prognostics, therapeutics, and prevention of cardiovascular disease. A recent Pubmed search (accessed August 23, 2016) using the terms ECM and cardiovascular, cardiac, or vascular revealed that this field has been growing annually since the 1990s (Figure). Driving forces for the increased interest in ECM research include the recognition of biological and pathophysiological importance, improvements in biochemical, cellular, and molecular techniques by which to study this complex unit, and advances in the capacity at microscopic and macroscopic levels to provide greater insight into the dynamically changing ECM. Using combinatorial approaches, ECM research can be explored at greater depths than previously possible. This editorial forms a coalescence of discussions by an ad-hoc panel motivated by a call by the American Heart Association to define important cardiovascular ECM research topic areas.

Significance

There is no question that the ECM plays a defining role in the initiation and progression of major cardiovascular diseases, including ischemic heart disease, hypertensive heart disease, valvular disease, pediatric and adult cardiomyopathies and cardiovascular malformations, arteriosclerotic and vascular disease, and cerebrovascular disease. Advances in our understanding of ECM roles will yield new approaches toward the prevention, detection, and treatment of cardiovascular disease states.

ECM abnormalities form a milestone event in aging and increased susceptibility to cardiovascular disease. Thus, new discoveries can yield improvements in approaches to maintain a healthy ECM that contributes to prolonged longevity and health span. Moreover, as an abnormal ECM is a common defining point in several diseases, including neurodegenerative disorders, cancer, and inflammatory, autoimmune and fibrotic conditions, new ECM discoveries within the setting of the cardiovascular system will transcend to overall health and disease concepts.

Current Knowledge Gaps in ECM Roles in Cardiac Physiology and Pathophysiology

The table summarizes several areas that require thorough investigation for a full understanding of ECM roles, both in the healthy aging environment and after pathology. Note that the areas are synergistic, as defining what the ECM is at the cellular and molecular scales and developing more selective ECM imaging tools will provide the knowledge on which to build computational models. This will, in turn, generate novel hypotheses about how cell and ECM interactions and signaling interact during aging and after pathology, such as myocardial infarction (MI; heart attack), chronic pressure overload of the heart (hypertension), or diabetes mellitus—all of which will reveal how ECM influences physiology and pathology of the left ventricle.
Cardiac ECM research is in a growth phase. Source: PubMed.

**Collagen Production as a Driver of Remodeling**

Fibrosis is defined as the excessive accumulation of ECM. In cardiac pathology, fibrosis is a generic term that encompasses different types of ECM deposition, all of which impair or even obliterate heart function, for example, by replacing cardiomyocytes causing dilation, suppressing heart pumping, and disrupting electric transmission between cardiomyocytes. In fibrosis in response to hypertension, collagen fibers intertwine between viable cardiomyocytes and surrounding coronary arteries, and this pattern is characterized by interstitial and perivascular ECM accumulation that slowly impairs cardiac function over time and is known as reactive fibrosis. Cardiac aging generates an ECM environment that is characterized by replacement interstitial fibrosis. Fibrosis in response to MI is scar ECM that replaces dead myocytes (reparative fibrosis), and this postinfarction scar displays notable variability in collagen content and arrangement.

Collagen accounts for a majority of the cardiac ECM protein volume, yet our fundamental understanding of how collagen is produced, assembled, and aligned is limited. Outside of the fibrillar assembly and mechanical properties provided by collagenous ECM, understanding how an initially isotropic ECM can develop regional variation because of local cell–ECM interactions and influences how other ECM proteins with structural, signaling, and accessory functions play critical roles in disease states remain to be elucidated. Although fibrosis is often considered a negative event, the absence of an ECM scar in the setting of MI would obviously be disastrous, resulting in a significant loss of myocardial structural integrity and increased risk for rupture. We need to understand how to guide myocardial cells to generate a scar that has the right balance of structural support but also one that does not contribute to continuous abnormalities in local biomechanics to induce feed-forward processes of MI expansion, left ventricular dilation, and pump dysfunction. Moreover, understanding what critical structural proteins and cell types drive favorable remodeling will also guide new avenues of research using injectable bioengineered biomaterials and ECM scaffolds strong enough to prevent rupture and stiff enough to minimize systolic dyskinesis, without impairing filling or the function of remaining viable myocardium.

The adult mammalian myocardium has negligible endogenous regenerative capacity, and MI can result in death of up to a billion cardiomyocytes, overwhelming the limited cardiac regenerative reserve. Inflammatory leukocytes, vascular cells and fibroblasts control deposition, and remodeling of ECM needed to repair the heart and to form scar. Matricellular proteins transduce signals to inflammatory and reparative cells. These cells are enmeshed within a dynamic network of ECM proteins that modify their phenotype and function and may modulate cell differentiation and survival. Whether ECM modulation and activation of specific matricellular pathways can activate a regenerative program in the myocardium has not been investigated.

**ECM Degradation and Turnover**

An extensive body of information on the inhibition of ECM degradation and ECM production has been made available from studying compiled data from MI studies—especially related to degrading collagen by inhibiting matrix metalloproteinase (MMP) activity. These studies, despite their overall failure to ameliorate post-MI outcomes, taught us that small molecules can be deployed to modify the ECM. Understanding what the optimum ECM is to allow repair without promoting further development of dysfunction will provide insight into the new homeostatic balance that is achieved between ECM degradation and production after injury.

Of note, over half of the MMPs have not been evaluated in the heart. Furthermore, other protease families such as a disintegrin and metalloproteinase domain–containing proteins (ADAMs) and ADAMs with thrombospondin motifs are understudied. How the different protease families compete or work in concert to degrade ECM, both in physiological and pathological conditions, remains to be clarified. Likely, different portfolios of enzymes are produced or activated dependent on the stimulus. How proteases work with their endogenous inhibitors, the tissue inhibitors of metalloproteinases, remains to be fully evaluated. Although the imbalance in MMP/tissue inhibitors of metalloproteinase ratio is

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**Table. Fundamental Knowledge Gaps**

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<th>We need to:</th>
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<tr>
<td>define the composition of the ECM in the healthy and post-MI heart (Cardiac ECM Atlas).</td>
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<tr>
<td>understand the effects of the ECM on inflammatory, reparative, and progenitor cells to coordinate cardiac wound healing.</td>
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<td>develop better ECM imaging tools.</td>
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<td>develop computational models that use knowledge gained to understand the time evolution with aging, pathology, and aging superimposed on pathology.</td>
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<td>understand cell–cell and cell–ECM interactions that drive cardiac remodeling.</td>
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<tr>
<td>understand the complex interaction between ECM and cell-signaling growth factors.</td>
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<tr>
<td>dissect ECM-mediated actions that may increase myocardial stiffness and contribute to heart failure with preserved ejection fraction in aging and diabetes mellitus and with reduced ejection fraction in MI and pressure overload.</td>
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ECM indicates extracellular matrix; and MI, myocardial infarction.
an important factor in ECM remodeling, the complex in vivo protease and antiprotease system is likely more complicated. The cardiac metalloproteinase actions postulates have been developed to serve as a template for exploring protease roles in the post-MI setting.\textsuperscript{11}

In addition to enzymes that contribute to ECM remodeling by degradation, other protein classes such as transglutaminases, lysyl oxidases, lysyl oxidase–like enzymes, and lysyl hydroxylases control the cross-linking degree of the ECM. Crosslinking provides higher mechanical stability (stiffness), but excess cross-linking could render ECM less susceptible to controlled degradation in an irreversible manner.\textsuperscript{17} Although proteolytic enzymes and their cell sources represent attractive targets for therapeutic strategies, we need to understand which cells, and in which activation state, promote beneficial or detrimental heart repair.

### Cardiac Fibroblast

The cardiac fibroblast is the key cell that produces and organizes the ECM, yet this cell type has been understudied both in vivo and in vitro because of technical limitations (eg, limited ability to define this cell type and its heterogeneity) and variability introduced by cell type examined (eg, neonatal versus adult) and culture conditions used (eg, passage and plate coating).\textsuperscript{18} Activated fibroblasts have enhanced ECM production and have been termed myofibroblasts based on their high ECM-contracting capacity because of increased \(\alpha\)-smooth muscle actin synthesis.\textsuperscript{19,20} Fibroblast and myofibroblast are generic labels, however, for a heterogeneous groups of cells that have limited molecular and phenotypic characterization. Although the fibroblast cell family has common features among its members, these cells likely derive from a variety of sources and respond to a diverse range and combination of stimuli that are present in the heart under physiological and pathological conditions, including differences in mechanical loading.\textsuperscript{19,21} New genetic models that trace cell origins in the pathological conditions, including differences in mechanical payment, including evaluations of groups across age and Pediatric Cardiac Disease

**Common and Distinct Mechanisms Between Adult and Pediatric Cardiac Disease**

Comparative studies are needed to provide insights into common denominator and disparate mechanisms and signaling pathways, including evaluations of groups across age and pathology spectrums. In patients with congenital heart disease, abnormal ECM accumulation is prevalent but not well understood. Malformations in ECM organization may be more detrimental than abnormalities in ECM proteins. For patients with congenital heart disease, a change in disease
status or re-emergence of disease may be expressed as ECM malformations.

Advances in Approaches to Understand the Cardiac ECM

As described below, a wide array of molecular and biological tools now exist that can be harnessed and turned toward new discoveries in the physiology of cardiac ECM.

Multiomic Evaluations

Cardiac ECM research, and ECM research in general, has not been easily amenable to genomic, proteomic, and metabolomics approaches in the past, because of several logistical issues. These include solubility issues that make sampling inconsistent and a relative lower abundance of ECM compared with easily soluble intracellular proteins in the myocardium. Recent advances have provided research templates on which cardiac ECM omics studies are now highly feasible and informative. ECM is regulated primarily at the post-translational level, which means that proteomics is more informative for understanding ECM than genomic approaches. For example, in the absence of pathology, ECM proteins have relatively long half-lives, which increases their chances of being post-translationally modified (eg, advanced glycation end product accumulation). After MI, the robust increase in proteases exposes ECM proteins to increased enzymatic processing.

Computational Approaches

Bringing big data into the cardiac ECM community serves two purposes. First, this technology allows the integration of results from multiple dimensions, which provides a template for novel hypothesis generation and allows a deeper understanding at the systems level. Consolidation of vast amounts of information to distill the most informative components (big data to knowledge) streamlines future data collection for more efficient application. Second, generating computational models that can be used to predict outcomes has implications for both basic science and clinical translation. Computational tools have been applied to various fields including cardiac electrophysiology and to cardiac fibroblasts to identify potential mechanisms driving a phenotype (eg, electric conduction or tissue remodeling) or potential therapeutic targets against a detrimental phenotype. The construction of fibroblast signaling networks will provide insight into how fundamental molecular pathway changes translate to cell physiology. Similar approaches will be needed to understand the complex interactions of matrix components, proteases, and growth factors in the extracellular space. Multiscale models and models that integrate cross-talk among cell types provide the ability for in silico evaluations to predict how therapies will affect properties and function at the tissue and organ level.

In Vivo Dissection of the Role of ECM Components in Cardiac Pathophysiology

The development of strategies for genetic targeting in rodents has revolutionized biomedical research, providing unique opportunities to test a pathophysiological paradigm in vivo. Progress in understanding the role of the ECM in cardiac homeostasis and disease is dependent on the development of robust in vivo models to dissect specific ECM-dependent actions in the pathogenesis of cardiac repair, remodeling, and fibrosis. In terms of cardiac pathophysiology, this means generating models that modify inflammatory leukocytes and fibroblasts.

Cell Biological Approaches

The development of directed therapeutic intervention strategies focused on one cell type will depend on the identification of molecular targets, which is often done in vitro to reduce system complexity. Validity of these systems will depend on our ability to isolate key individual cell components, namely inflammatory leukocytes (neutrophils, macrophages, and lymphocytes) and fibroblasts that have defined inflammatory and reparative polarization phenotypes. Cell culture systems that support cell growth in a setting that sufficiently resembles the in vivo environment will provide insight into cardiac cell physiology within a mechanically active environment. This may include the use of mechanically stimulated engineered constructs with physical and chemical properties comparable to the endogenous microenvironment. With the advent of miniaturized systems with defined mechanical conditions, higher throughput testing of cardiac cells relevant to ECM will soon be available. In addition, protocols that couple cell isolation with flow cytometry provide the ability to determine the relative abundance of cell types in the myocardium.

Imaging

Imaging of ECM has largely been limited to visualizing the fibrotic ECM structure, such that fibrosis can be detected, but details on quantity and quality of various proteins within the fibrotic environment cannot be readily assessed. MMP activity can be imaged, particularly in the post-MI setting where MMPs are elevated. The emergence of elastography methods may provide important information on ECM construction and stiffness, and the use of biomedical imaging using second and third harmonics provides some information about the organizational state of collagen in biopsies. Overall, imaging of ECM is an area with great potential that remains to be developed.

Conclusions

Insights into ECM roles in cardiovascular disease are highly relevant for understanding the pathophysiology of a wide range of conditions in other systems. Alterations in the ECM network play a central role in a variety of conditions involving many different organs, including idiopathic pulmonary fibrosis, systemic sclerosis, liver cirrhosis, and renal failure. In all conditions, dysregulated expression or altered function of ECM proteins contribute to organ dysfunction. Moreover, ECM proteins play a crucial role for cell proliferation and invasive potential in cancer and contribute to the pathogenesis of neurological disorders. Understanding the fundamental effects of the ECM on cellular phenotype in the heart can generate new ideas for understanding and treatment of conditions involving other systems. Therefore, a better understanding of how the cardiac ECM coordinates, and is coordinated, will feed forward to inform and benefit other systems as well.

The cardiac ECM field has made remarkable progress, particularly in the past 20 years. The field is poised to answer
significant outstanding questions that are fundamental to cardiovascular research. Advancing our understanding of cardiac ECM has the potential to cross-translate to other diseases, because understanding how normal and abnormal cardiac ECM processes occur informs on a broader scale.

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Crossing Into the Next Frontier of Cardiac Extracellular Matrix Research
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