Matrix as an Interstitial Transport System

Dong Fan, Esther E. Creemers, Zamaneh Kassiri

Abstract: The extracellular matrix (ECM) is best known for its function as a structural scaffold for the tissue and more recently as a microenvironment to sequester growth factors and cytokines allowing for rapid and localized changes in their activity in the absence of new protein synthesis. In this review, we explore this and additional new aspects of ECM function in mediating cell-to-cell communications. Fibrillar and nonfibrillar components of ECM can limit and facilitate the transport of molecules through the extracellular space while also regulating interstitial hydrostatic pressure. In turn, transmembrane communications via molecules, such as ECM metalloproteinase inducer, thrombospondins, and integrins, can further mediate cell response to extracellular cues and affect ECM composition and tissue remodeling. Other means of cell-to-cell communication include extracellular microRNA transport and its contribution to gene expression in target cells and the nanotube formation between distant cells, which has recently emerged as a novel conduit for intercellular organelle sharing thereby influencing cell survival and function. The information summarized and discussed here are not limited to the cardiovascular ECM but encompass ECM in general with specific references to the cardiovascular system. (Circ Res. 2014;114:889-902.)

Key Words: extracellular matrix ▪ extracellular matrix metalloproteinase inducer ▪ microRNAs

The extracellular space, or the interstitium, can be broadly divided into a fluid phase (water, electrolytes, nutrients, and some plasma proteins) and the extracellular matrix (ECM). In the heart, the ECM comprises structural proteins (primarily collagen types I and III) that form the fibrillar structure of the matrix; the basement membrane that forms an interface between the cardiomyocytes and the interstitial space as well as the molecules that mediate the cell–ECM connection; and the nonstructural proteins such as proteoglycans. Molecules traveling in the extracellular space come in contact with different ECM components, and these interactions determine the direction, speed, and distance of their transport, whereas the ECM can also provide a reservoir for several growth factors and cytokines. Moreover, the cell–ECM connection allows for transmission of signals across the cell membrane. Therefore, in addition to the classical role of ECM in providing structural support, it contributes to a multitude of intra- and extracellular events. Several thorough reviews have been published recently on the structural properties of the ECM and its remodeling in heart disease.1–3 In this review, we will discuss less explored aspects of ECM function, such as its conductivity in extracellular transportation, its role in cell–cell interactions, and different modes of cell–cell communication through the extracellular space.

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Nonstandard Abbreviations and Acronyms

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<td>Ago2</td>
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<td>ECM</td>
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<td>EMMPRIN</td>
<td>extracellular matrix metalloproteinase inducer</td>
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<td>FGF</td>
<td>fibroblast growth factor</td>
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<td>HSPGs</td>
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<td>TGF</td>
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<td>TNTs</td>
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Cell–Cell Communication Across the Matrix

Mechanisms of Extracellular Transportation

Intercellular communication is critical in the development, homeostasis, and pathological remodeling of different organs and can occur in an autocrine, juxtacrine, paracrine, or endocrine fashion. During development, morphogens, the signaling molecules that mediate tissue development, are secreted from their source cells and diffuse in the interstitial space toward their target cells where they control cell fate such as proliferation, differentiation, migration, and turnover. The mechanism of morphogen diffusion has been investigated extensively in developmental biology, although several described principles could also apply to interstitial transportation of growth hormones and cytokines in different stages of life. This interstitial transportation can be divided broadly into 2 categories: extracellular diffusion based and cell based. Here, we will focus on different modes of extracellular diffusion–based transportation, for which 3 models have been proposed: (1) free diffusion, where molecules disperse freely from the source to the target cell(s). This is the simplest form of dispersal and occurs based on a concentration gradient of the signaling molecule and only holds for freely diffusible molecules assuming no obstacles in the diffusion path; (2) hindered diffusion, where extracellular diffusion of the molecules can be hindered by obstacles, such as cell packing, or by transient binding interactions with molecules such as the ECM components; and (3) facilitated diffusion, which is an extension of hindered diffusion model where movement is enhanced by interactions with positive regulators that facilitate the diffusion.

Nonstructural ECM Proteins and Extracellular Transportation of Signaling Molecules

During development, proper transportation of key morphogens is essential in normal development and growth. The 4 chambers of the heart in mammalian embryos derive from distinct precursor pools under strict spatial and temporal regulations. The elongation of the linear heart tube is mediated by several morphogens and signaling pathways, including sonic hedgehog, canonical Wnt, and fibroblast growth factor (FGF). Members of the transforming growth factor β (TGFβ) superfamily, such as Nodal, are required for establishment of the left–right axis in the embryo, whereas decapentaplegic is required to prevent overgrowth of the heart. In a mature heart, under normal physiological conditions, activities of these pathways are markedly subdued compared with during development; however, one of the characteristics of heart disease is recapitulation of fetal gene expression. Wnt signaling is enhanced after myocardial infarction, implicating its role in tissue repair, whereas hedgehog signaling is upregulated in ischemic myocardium to facilitate angiogenesis. Therefore, modes of extracellular transportation for these molecules during embryonic development can provide insight into their transportation across the ECM during physiological and pathological remodeling of the heart.

Heparan sulfate proteoglycans (HSPGs), a family of proteoglycans that comprise the nonstructural ECM, contain a core protein (glypicans, perlecans, or syndecans) to which ≥1 HS chains are attached. HS chains are dominant molecules in the pericellular matrix surrounding the plasma membrane and can bind to a broad range of ligands, thereby HSPGs can mediate extracellular transportation of several morphogens and growth factors. Extracellular diffusion of Wnt and Hedgehog, which are important players in heart development, can be hindered by noncovalent binding to cell surface HSPGs. In addition, Wnt and Hedgehog proteins are hydrophobic molecules, which require transportation via lipoprotein particles in the matrix. Association of HSPGs with lipoproteins facilitates the extracellular long-range movement of Wnt and Hedgehog, which are then recruited by the target cells through their cell surface HSPGs and lipoprotein receptors. Therefore, HSPGs can play both passive (transient binding) and active (promoting movement) roles in extracellular transportation of these signaling molecules. HSPGs can also function as coreceptors to promote the signal transduction of Wnt and Hedgehog. However, several HSPGs, such as glypican-3, can inhibit Hedgehog signaling by inducing its endocytosis and degradation. This is an important regulatory mechanism for the hedgehog signaling pathway as loss-of-function mutations of glypican-3 result in Simpson–Golabi–Behmel overgrowth syndrome, and glypican-3 null mice demonstrate developmental overgrowth because of increased Hedgehog signaling.

Nodal gene, a member of the TGFβ superfamily, is essential in setting up the embryonic left–right axis during development. Defective establishment of left–right axis is a major cause of congenital heart defects in humans. Nodal travels through the matrix via hindered diffusion or binding-mediated hindered diffusion. Transiently binding to extracellular diffusion regulators decreases its diffusivity, whereas interaction with HSPGs and chondroitin-sulfate proteoglycans in the basement membrane further mediates its transportation to the target site. Decapentaplegic, another member of the TGFβ superfamily, is involved in heart development during germ-band extension. Defective decapentaplegic signaling results in heart overgrowth and reduced cardiac output in Drosophila. Studies in Drosophila have revealed that decapentaplegic transportation can occur through multiple mechanisms including free or hindered diffusion (by binding to HSPGs) and cell-mediated transport.
collagen type IV, a basement membrane protein, can bind to decapentaplegic and regulate its extracellular shuttling, short- and long-range movement, and gradient formation in *Drosophila* embryo.\(^6\)\(^{29}\) Collagen type IV can also increase decapentaplegic-mediated signaling by promoting its binding to its receptor.\(^29\) The amino acids in *Drosophila* collagen type IV that are required for this interaction are conserved in human as well as mouse, worm, and mosquito,\(^29\) whereas human collagen IV can also bind to bone morphogenetic protein-4 (a vertebrate ortholog of decapentaplegic in *Drosophila*), indicating that this mechanism is conserved in humans.\(^29\)

Therefore, although the network structure of the ECM is often the focus of investigations, it is critical to acknowledge that the nonstructural components of the ECM are equally essential in extracellular transportation of key signaling molecules contributing to heart development and possibly in pathological states.

### Multiple Components of the Interstitium Can Influence Extracellular Transportation

The ECM is highly hydrated as the proteoglycans and glycosaminoglycans in the ECM have a great capacity to bind water and form hydrated matrices resistant to compressive forces.\(^30\)\(^{31}\) The pattern of movement of particles through the ECM has been described as Brownian random walks through the spaces between network structures influenced by components of the matrix in 3 ways: (1) steric interactions: collision with matrix fibers, (2) hydrodynamic interactions: restricted thermal motion of water molecules and slowed diffusion as the particles diffuse near fibers, and (3) electrostatic interactions with charged components of the ECM, which is an additional force for charged particles.\(^32\) It has been long known that conductivity of the matrix correlates negatively with its glycosaminoglycans and collagen content.\(^33\) Because ECM composition alters during pathological remodeling, the impact of parameters such as ECM fiber volume fraction, organization, and fiber diameter become important factors in determining the trafficking of particles through the matrix. A mathematical model proposes that when the fiber size is comparable with the Debye length of a charged particle (the distance that a charge carrier’s net electrostatic effect persists), electrostatic forces between the fibers and the particles result in slowed diffusion. However, as the fiber diameter increases or as fibers accumulate (eg, in fibrosis), the repulsive forces become less important.\(^32\) For smaller ECM molecules, such as glycosaminoglycans, the repulsive electrostatic interactions are significant but less significant for larger fibers such as collagen. In addition, when the particle size is small compared with the fiber diameter, hydrodynamic interactions are less important,\(^34\) whereas when the particle size is comparable or larger than the fiber diameter, hydrodynamic hindrance can slow down the mobility of the particle by many folds.\(^35\) ECM can also serve as a selective filter to control the local distribution of the transported macromolecules, where permeability is determined by both size and surface charge of a particle. ECM functions as an electrostatic bandpass, which suppresses the diffusive motion of charged particles, whereas uncharged particles can diffuse easily through the matrix. The localized charged patches in the ECM are responsible for its highly unspecific but strongly selective filtering effect.\(^36\) Proteoglycans provide a permeability barrier in the basement membrane that blocks the passage of negatively charged macromolecules.\(^37\)\(^ {38}\) Therefore, the structure, density, and integrity of fibrillar and nonfibrillar ECM components are major factors in interstitial transportation of growth factors, cytokines, proteins, ions, drugs, etc, which mediate cellular responses during physiological and pathological states.

In addition to the ECM protein components, the fluid phase of the interstitium can also influence the flow of molecules through the extracellular space via a balance between hydraulic conductance and hydraulic resistance. Hydraulic conductance is defined as the flow of a fluid per unit pressure drop across unit area of material (ie, interstitial fluid), whereas hydraulic resistance is the opposite of conductance. Hence, composition of the fluid phase of the interstitial space is as important as the ECM components in flow and transportation of molecules in the extracellular space. Interstitial convection forces are potentially another important mechanism for distribution or transportation of molecules through the ECM. In vivo models have demonstrated that the size of the molecules dominates molecular convection (transportation) through the interstitial space, as larger particles are slowed down by mechanical hindrance, and proteins convect slower than linear molecules of equal mass.\(^39\)\(^ {40}\)

### ECM as an Extracellular Reservoir

Growth factors generally are not freely diffused in the interstitial space on their production, but instead are localized to ECM basement membrane by binding to HSPGs. FGFs are the most extensively studied growth factors, comprising a large family critical in cell proliferation, survival, migration, and differentiation during development, growth, and disease.\(^41\)\(^ {42}\)\(^ {43}\) FGFs are essential in embryonic development of the cardiovascular system\(^46\)\(^ {47}\) and can be protective in cardiomyopathies.\(^48\)\(^ {49}\)\(^ {50}\)\(^ {51}\) Therefore, their optimal transportation to the target cells is essential. Binding of FGFs to their receptors is orchestrated through their binding to HSPGs within the ECM.\(^41\)\(^ {42}\)\(^ {52}\)\(^ {53}\) whereas HSPGs also mediate controlled movement of several other growth factors (insulin-like growth factors, FGFs, and TGFs) in the pericellular matrix.\(^54\)

Growth factors can move beyond the length of a single HS chain, translocating from one HS-binding site to another.\(^55\) Binding of growth factors to HSPGs prevents their proteolytic degradation,\(^56\) hinders their passive diffusion,\(^6\) and serves as a storage that allows for their rapid release on demand (Figure 1). Disruption of this association can greatly enhance diffusion and transport of growth factors through the interstitial space,\(^57\) which if not controlled, can result in excess downstream signaling and adverse outcomes. Shedding of syndecan-4 (an HSPG) from myocardial ECM correlated with the inflammatory response in failing human heart and in an experimental model of heart disease.\(^58\) Moreover, deletion of syndecan-4 prevented compensatory hypertrophy after pressure overload, which led to accelerated left ventricular dilation and heart failure.\(^59\) Therefore, HSPG-mediated transportation of growth factors is essential in compensatory hypertrophy and remodeling of the heart.
In addition to HSPGs, other components of the ECM can bind to and retain cytokines and growth factors in the interstitial space, to be released in response to a trigger. For instance, TGFβ on secretion is sequestered within the ECM by binding to latent TGFβ-binding peptide, an ECM protein, and can be released in response to proteolytic or mechanical triggers. Therefore, the ECM provides a microenvironment for growth factors and cytokines, whereby the bioavailability of these molecules and activation of their downstream signaling can be regulated rapidly and locally, and independent from their de novo synthesis. This should also be considered in studying the activity of these growth factors because their

Figure 1. Multiple functions of heparan sulfate proteoglycans. Heparan sulfate chains serve as a coreceptor for growth factors on the same cell, or on an adjacent cell, and also serve as a reservoir for growth factors in the extracellular matrix.

Figure 2. Extracellular matrix metalloproteinase inducer (EMMPrin) in the myocardium. A, EMMPrin structure; (B) EMMPrins form homo-oligomers. Homo-oligomerization and N-glycosylation induce matrix metalloproteinase (MMP) synthesis through an unknown mechanism (1), whereas an EMMPrin monomer (2) or nonglycosylated dimer (3) cannot trigger gene expression. (4) EMMPrin can induce expression of membrane-bound membrane-type (MT)-1-MMP, soluble pro-MMPs, and itself. (5 and 6) EMMPrin can be cleaved by MMPs and released into the interstitial space where it can induce MMPs in distant cells. MT1-MMP cleaves EMMPrin between extracellular domain (EC)-I and ECII, whereas other MMPs could cleave the entire extracellular domain. These mechanisms need to be confirmed in cardiomyocytes. C, Normal human cardiomyocytes stained for EMMPrin (green) and sarcomeric protein (red). Adapted from Spinale et al93 with permission of the publisher. Copyright © 2000, American Heart Association. D, Cardiac overexpression of EMMPrin led to dilated cardiomyopathy with aging. Adapted from Zavadzskas et al101 with permission of the publisher. Copyright © 2008, The American Physiological Society. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
total mRNA or protein levels do not accurately reflect their activity within the tissue.

**Transmembrane Interactions**

**Extracellular Matrix Metalloproteinase Inducer, an Extracellular Inducer of MMPs and Other Cellular Responses**

In addition to secreted proteins that trigger signaling responses in proximal and distant cells, transmembrane interactions between neighboring cells can be mediated by proteins such as ECM metalloproteinase inducer (EMMPRIN, CD147, or basigin), a transmembrane glycoprotein. EMMPRIN consists of 2 extracellular domains (ECI and ECII), which have the glycosylation sites, a transmembrane, and a short cytoplasmic domain (Figure 2A). EMMPRIN is essential during development because EMMPRIN-deficient mice exhibit defective matrix metalloproteinase (MMP) regulation, impaired lymphocyte responsiveness, neurological dysfunctions, and infertility. EMMPRIN can also trigger angiogenesis by inducing vascular endothelial growth factor27 and can mediate inflammatory responses by acting as a signaling receptor for cyclophilins A and B,73.75 chemotactic agents for various immune cells that interact with HSPGs, whereas EMMPRIN selectively associates with syndecan-1.77

EMMPRIN can induce production of several MMPs, including MMP1, MMP2, MMP3, MMP9, MMP11, MMP13, 67,78–81 and membrane-type MMPs, membrane-type 1 (MT1)-MMP and MT2-MMP,82,83 MMP induction occurs through homophilic interactions between adjacent EMMPRIN molecules mainly through ECI of each EMMPRIN molecule.67,80,84 This homophilic adhesion is essential for the EMMPRIN-mediated induction of MMPs, but not sufficient by itself and also requires glycosylation of EMMPRIN for this process.80 Interestingly, deglycosylated EMMPRIN can function as an antagonist of MMP induction through an unknown mechanism.86 The intracellular signaling pathways mediating the EMMPRIN-induced MMP production are the subject of ongoing investigations although phospholipase A2, 5-lipoxygenase and p38 mitogen-activated protein kinase have been proposed to be involved in this process.87,88 The membrane bound and soluble EMMPRIN can equally induce MMP production,89,90–91 while EMMPRIN can also induce its own expression through a positive feedback mechanism.90 Activated MMPs can cleave EMMPRIN and generate soluble EMMPRIN that can further induce MMP and EMMPRIN production in a paracrine fashion.90,92 This could give rise to a vicious cycle of aberrant proteolysis. Studies in tumor cells have shown that EMMPRIN can be cleaved by MT1-MMP, and to a lesser extent by MT2-MMP, at the link between ECI and ECII.92 EMMPRIN and MT1-MMP are coexpressed in several cell types, which could facilitate this process.92,93 Other MMPs, such as MMP2 and MMP9, have also been suggested to cleave EMMPRIN ectodomain, at the same or a different site.90,94 A proposed mechanism for the activity of EMMPRIN in the myocardium is illustrated in Figure 2B. The ECII domain of EMMPRIN is required for its association with caveolin-195,96; however, the impact of this interaction on EMMPRIN function is controversial.95,97 Significance of EMMPRIN–caveolin association remains to be determined in the cardiovascular system.

**EMMPRIN in the Cardiovascular System**

It was first reported in 2000 that EMMPRIN is expressed in cardiomyocytes (Figure 2C), and its levels are elevated in the myocardium of patients with ischemic and nonischemic dilated cardiomyopathy, which correlated with upregulation of MT1-MMP expression in the cardiomyocytes from these hearts.91 EMMPRIN was also shown to be further elevated after left ventricular assist device support.98 Increased EMMPRIN levels have been reported in the myocardium99 and in monocytes100 from patients with acute myocardial infarction, which induced MMP9 in monocytes and MMP2 in smooth muscle cells.100 Cardiac-specific overexpression of EMMPRIN in mice resulted in elevation of several MMPs in the heart, left ventricular dilation, and dysfunction (Figure 2D).101 EMMPRIN downregulation by nitric oxide has been proposed in ischemia reperfusion, whereas haploinsufficiency of EMMPRIN reduced myocardial ischemia–reperfusion injury by suppressing the inflammatory response.102 EMMPRIN was elevated in patients with inflammatory and noninflammatory cardiomyopathies, whereas cyclophilin A was only elevated in inflammatory cardiomyopathy, highlighting EMMPRIN as a marker of pathological cardiac remodeling independent of the inflammatory component.104 Although the molecular mechanisms of EMMPRIN function in the myocardium and the involved intracellular signaling pathways require further investigation, the growing list of EMMPRIN partners highlights involvement of this membrane protein in diverse aspects of physiological and pathological remodeling.

**ECM–Cell Interactions Are Critical in Cell Response to Extracellular Cues**

Although composition and integrity of the ECM are critical for optimal function of an organ, it is equally important for the ECM to interact with the cells that comprise the organ. Among the molecules that mediate the cell–ECM interactions, we will discuss thrombospondins and integrins, which have been shown to play key roles in the myocardium. Thrombospondins are a multi-domain, multimeric, and multifunctional extracellular family of proteins that mediate ECM synthesis and deposition, cell–matrix interactions, and tissue remodeling, particularly in disease.105–107 Thrombospondins are considered matricellular components of the ECM and bind to multiple components of the ECM, such as collagen, fibronectin, laminin, and HSPGs, as well as cell surface receptors, such as integrins, CD36, and CD47.108–111 Thrombospondin-1, thrombospondin-2, thrombospondin-3, and thrombospondin-4 are increased during hypertensive cardiomyopathy and contribute to cardiac remodeling.112–118 Thrombospondin-1 can trigger ECM synthesis by activating TGFβ in vitro and in vivo by releasing the ECM-bound latent TGFβ.119–121 Inhibition of thombo(spondin-1–mediated TGFβ1 activation improved myocardial fibrosis and function after pressure overload,119 highlighting the central role of thrombospondin-1 in this process. In addition to triggering ECM synthesis, thrombospondins promote matrix
assembly, particularly thrombospondin-1 and thrombospondin-2. Thrombospondin-2–deficient mice exhibit a significant rate of cardiac rupture after angiotensin II infusion indicating the critical role of thrombospondin-2 in optimal assembly of ECM in the myocardium. Thrombospondins can inhibit a broad spectrum of proteases, including MMP2 and MMP9, which could underlie their ability to protect and stabilize newly formed ECM during tissue remodeling. This could also underlie their antiangiogenic function, although the N-terminal fragment of thrombospondin-1 (25 kDa) is proangiogenic through its interaction with syndecan-4 of endothelial cells. Thrombospondins also affect cell behavior and interactions. Thrombospondin-1 deficiency prevents fibroblast differentiation into myofibroblasts and impairs TGF-β-mediated ECM synthesis, whereas lack of thrombospondin-2 prevents their adhesion to vitronectin, collagen type I, and fibronectin and impairs their ability to spread and organize their cytoskeleton. Thrombospondin-4 has been shown to function as a myocyte-Interstitial mechano-signaling molecule because thrombospondin-4–deficient hearts fail to augment contractility after pressure overload, whereas thrombospondin-4–deficient myocytes respond normally to stretch. These studies highlight the significance of thrombospondins in mediating cell–matrix interaction and its impact on cell behavior, myocardial remodeling, and function.

Integrins are a large family of heterodimer (α and β subunits) transmembrane cell surface receptors that bind to ECM proteins (fibronectin, laminin, and vitronectin) and link the ECM and the intracellular cytoskeleton. Integrins transmit signals across the cell membrane, in inside-out and outside-in fashions, and thereby can regulate cell differentiation, adhesion, migration, proliferation, survival, and apoptosis in response to extracellular signals. It is well documented that in response to mechanical stress, expression of cardiac integrins (α1β1) is elevated concomitantly with enhanced activation of hypertrophy-related intracellular signaling pathways. In a healthy myocyte, integrins are primarily localized to costameres anchoring the sarcomeric z-discs, whereas in disease this colocalization is lost. Integrins and their interaction with ECM are critical during development and in disease. Deletion of integrin β1, the cardiac isofrom, impaired differentiation of cardiac muscle cells and their sarcomeric arrangement. Cardiomyocyte deletion of integrin β1 severely compromised the ability of the heart to withstand pressure overload, whereas with aging these mice developed dilated cardiomyopathy. Proteolytic degradation of integrin β1 in tissue inhibitor of metalloproteinase 2–deficient mice led to dilated cardiomyopathy and nonhomogeneous ECM remodeling after pressure overload. More recently, integrins have been recognized to contribute to stretch-induced opening of several ion channels and to mechanoelectrical coupling. Integrins can also interact with the ECI domain of EMMPRIN, which can further form a complex with CD98 to promote cell aggregation. Moreover, integrin–laminin interaction can be regulated by EMMPRIN, which can in turn affect basement membrane formation, cell adhesion, MMP induction, chemotaxis, and cell proliferation.

Therefore, the cell–ECM interaction allows for communication between the intra- and extracellular environments. This is critical for homeostasis and optimal remodeling of the cellular and noncellular (ECM) components during physiological and pathological remodeling.

Transport of Intracellular Molecules and Organelles in the Interstitial Space

The lipid bilayer that forms a boundary separating the intracellular components and organelles of a cell from the extracellular space can be crossed in several fashions. Extracellular transport of cytoplasmic components, such as microRNAs (miRNAs), mitochondria, and other organelles, through the ECM mediates communication between distant cells.

MiRNAs and ECM Remodeling

MiRNAs are functional but noncoding RNAs, which have emerged as powerful regulators of a wide range of biological processes by inhibiting protein translation. Several miRNAs have been identified to regulate ECM turnover in multiple organs. MiR-21 is elevated in pressure overload and ischemic cardiomyopathy and reported to trigger myocardial fibrosis because its silencing prevented fibrosis in pressure overloaded cardiomyopathy. Consistently, miR-21–deficient mice were protected against renal injury and fibrosis. MiR-29 is another miRNA linked to ECM synthesis by directly regulating the expression of ECM genes, such as collagen type I and III, elastin, and fibrillin. The reported downregulation of miR-29 in several cardiac pathologies suggests that loss of inhibitory function of this miRNA could in fact underlie the excess production of ECM structural proteins and fibrosis. Consistently, overexpression of miR-29 decreased bleomycin-induced pulmonary fibrosis in mice.

Extracellular Transport of miRNAs

It was first reported in 2008 that miRNAs can be released from cells and that they are remarkably stable in the extracellular fluids despite the presence of ribonucleases. MiRNA-mediated intercellular communication relies on several critical steps, such as selective miRNA secretion, miRNA transport through the ECM, uptake of the miRNAs by recipient cells, and ability of the miRNA to target and suppress specific miRNAs within the recipient cells. Cells can indeed select some miRNAs for extracellular release, whereas others are retained. MiRNAs can be released from cells via several mechanisms: (1) passive leakage through disrupted cell membrane in damaged cells (secondary to necrosis or apoptosis); (2) active secretion through microparticles such as exosomes and microvesicles; and (3) secretion on association with RNA-binding proteins, such as argonaute2 (Agou2), the key intracellular effector protein of miRNA-mediated RNA silencing. The impermeability of miRNA-containing microvesicles and exosomes to RNases and high stability of Ago2 proteins have been proposed to protect the miRNA in the extracellular environment. Interestingly, some miRNAs (eg, let-7a) are associated exclusively with vesicles, whereas others (eg, miR-122) are present exclusively in nonvesicle Ago2 complexes. It is currently not clear whether the Ago2–miRNA complexes are passively released from apoptotic cells or are exported through cell membrane transporters.
On release into the extracellular space, miRNAs travel through the ECM to reach their target cells. There is currently limited information on the mode and mechanism of miRNA transportation in the interstitial space, or if packaging the miRNA in exosomes and microvesicles provides an advantage in transportation efficacy compared with the Ago2-associated miRNAs. It also remains to be determined whether transportation of miRNAs is facilitated by binding to ECM proteins such as HPSGs, or whether it follows the Brownian random walk pattern. It is plausible to speculate that changes in ECM composition would influence this transportation process such that ECM disruption would exert less hindrance for miRNA diffusion, whereas fibrotic ECM depositions would limit the distance of miRNA transportation and the site of action.

It has been demonstrated that miRNAs can be taken up by the target cells; however, it remains to be determined whether microparticles are phagocytosed by recipient cells, fuse directly with the plasma membrane, or taken up by a receptor-mediated process. Nevertheless, the interaction between the miRNA and the target cell is highly specific and does not occur in random. This specificity is likely mediated by adhesion molecules such as integrins on the surface of the microparticle. Currently, there is no evidence that Ago2-associated miRNA enters the target cells in vivo, and it has been speculated that the Ago2-associated miRNAs simply serve as a silencing complex released by cells to regulate their own gene expression rather than a mode for transporting miRNA to target cells. Several in vitro studies have demonstrated that exosomal miRNAs may participate in cell–cell communications in viral infection, immune response, atherosclerosis, and cancer progression. More research is warranted to demonstrate validity of these mechanisms in physiological settings and their contribution to pathological states. Additional questions that remain to be answered include the form in which the miRNAs are released in microparticles (mature or pre-miRNA), physiological relevance of circulating miRNAs, the factors that influence the direction, speed, and distance of their transport in the interstitial space, and their functionality in the paracrine target cells.

**Tunneling Nanotubes**

It is well established that the ability of cells to communicate with one another is essential for development, growth, and survival of multi-cellular organisms. Cell–cell communication can take place through chemical signaling, which does not require physical contact between cells (hormones, neurotransmitters, and microparticles), through gap junctions or synapses in adjacent cells allowing passage of ions and small hydrosoluble molecules, and as more recently demonstrated, through formation of tunneling nanotubes (TNTs) between distant cells. TNTs, also referred to as membrane nanotubes, intercellular bridges, or cytoplasmic bridges, are long nonadherent actin-based cytoplasmic extensions that mediate intercellular connections between a range of different cell types. TNTs were first found and described in neurons in 2004 and since then they have been reported in several different cell types. TNT formation is either through directed filopodia-like protrusions that extend from one cell to another cell and form a conduit between the 2 cells, or via dislodgement (less common) as cells previously in contact detach from one another but remain connected through a TNT. F-Actin is a major component of TNTs, and actin polymerization is central to the formation of these tunnel structures as depolymerizing drugs completely blocked TNT formation and intercellular organelle transport. However, once formed, the TNTs are no longer sensitive to depolymerizing drugs. There is considerable diversity in

**Figure 3. Connectivity between cardiomyocyte and cardiac fibroblasts.** A, Cultured neonatal rat ventricular myocytes and fibroblasts labeled for microtubules (α-tubulin) and F-actin (rhodamine-conjugated phalloidin). Arrows point to tunneling nanotubes (TNTs). B, Thin structures similar to TNTs in adult mouse heart tissue (arrow). Scale, 10 μm. A and B, Adapted from He et al with permission of the publisher. Copyright © 2011, Oxford University Press. C, Proposed structure for TNTs between a cardiomyocyte and a cardiac fibroblast comprising F-actin and microtubules. Mitochondrial transport along F-action is mediated by myosin and actin microtubule by kinesin molecules. Cytoplasmic vesicles and other materials (eg, lysosomes and endosomes) can also be transported through the nanotube. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. CM indicates cardiomyocyte; FB, fibroblast; and WGA, wheat germ agglutinin.
size and structure of different nanotubes. Thicker nanotubes (diameter>700 nm) contain microtubules in addition to F-actin, and their larger diameter allows bidirectional transportation as well as transfer of organelles of various sizes. Organelles, such as Golgi vesicles, late endosomes and lysosomes, and mitochondria, can move bidirectionally in thick, but not in thin TNTs, whereas bacteria can be trapped and transferred only in thin membrane nanotubes. The intercellular transport of vesicles in thick membrane nanotubes is ATP- and microtubule-dependent, but the transport via thin nanotubes is only ATP-dependent. Relatively thick membrane nanotubes have been found to mediate transportation of lysosomes and early endosomes between neonatal cardiac myocytes and fibroblasts while similar structures were also found in adult mouse myocardium (Figure 3A and 3B), suggesting that organelle sharing can take place among cardiomyocytes and other myocardial cells, which could influence cell survival and function. Formation of TNTs could be triggered by inflammation or cellular stress such as oxidative stress. Recently, cell adhesion molecules and receptor–ligand interactions have been shown to be essential for the initiation of TNT formation. M-Sec, a mammalian protein formerly known as tumor necrosis factor-α–induced protein 2, is reported to trigger de novo membrane protrusions, which could tether to neighboring cells and form TNTs. A schematic of TNT connection between a cardiomyocyte and a cardiac fibroblast is presented in Figure 3C.

**Table. Future Directions**

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<td>Impact of interstitial factors on delivery of therapeutic agents</td>
<td>The impact of all ECM components, structural and nonstructural as well as interstitial convection should be considered</td>
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<td>In vitro studies assessing interstitial transportation or cell migration should use 3-dimensional Matrigels comprising both fibrillar and nonfibrillar ECM components, which more closely represent the ECM in vivo</td>
</tr>
<tr>
<td></td>
<td>In designing therapies, it should be considered that the adversely remodeled ECM can interrupt (excess ECM deposition) or accelerate (disrupted ECM) transportation. Therefore, drug delivery systems should be tailored to the type of heart disease with consideration to the nature of tissue remodeling</td>
</tr>
<tr>
<td>EMMPRIN</td>
<td>Mechanism of EMMPRIN regulation and function in the myocardium requires further investigation. For instance, is EMMPRIN cleaved by membrane-bound or soluble MMPs, and will this augment or abrogate its functionality?</td>
</tr>
<tr>
<td></td>
<td>Complete profile of MMPs (or other molecules) induced by EMMPRIN</td>
</tr>
<tr>
<td></td>
<td>The primary site of action for EMMPRIN. Myocytes, fibroblasts, or other myocardial cells?</td>
</tr>
<tr>
<td></td>
<td>Temporal and spatial induction and function of EMMPRIN</td>
</tr>
<tr>
<td>MiRNA transport</td>
<td>Functional significance of extracellular transportation of miRNAs in the target cells needs to be validated</td>
</tr>
<tr>
<td></td>
<td>If proven that packaging of miRNAs in microvesicles and exosomes facilitates their transportation and uptake by target cells, similar strategies can be used for therapeutic delivery of miRNA or small molecule drugs</td>
</tr>
<tr>
<td>Organelle transport and exchange between myocardial cells</td>
<td>The influence and contribution of the ECM on formation of TNTs, and if compromised ECM integrity can interfere with TNT formation</td>
</tr>
<tr>
<td></td>
<td>Physiological significance of organelle sharing in vivo should be verified. This could be particularly important in cell therapy where stem cells are injected into the diseased myocardium</td>
</tr>
</tbody>
</table>

ECM indicates extracellular matrix; EMMPRIN, extracellular matrix metalloproteinase inducer; MiRNA, microRNA; MMP, matrix metalloproteinase; and TNT, tunneling nanotubes.
not through a passive transport system. A similar intercellular communication has been reported between human endothelial progenitor cells and neonatal rat cardiomyocytes through transiently formed TNTs, which interestingly only mediated a unidirectional transportation of the mitochondria from the cardiomyocytes to endothelial progenitor cells.\(^\text{190}\) TNT-mediated mitochondrial transport has also been reported between epithelial cells\(^\text{190}\) and macrophages.\(^\text{172}\) Although in tumor cells TNTs are thought to promote tumor growth and invasion, and inhibition of TNT formation is proposed as an effective treatment,\(^\text{191}\) TNT formation between different myocardial cells is implicated to affect cardiac recovery from injury positively. Additional investigation is required to determine the exact function of TNTs in myocardial physiology and pathology.

Although recognition of TNTs has provided new insights into understanding cell-to-cell communications, several important questions remain to be addressed. It is important to determine the interstitial factors that influence formation of TNTs. For instance, do TNTs extend through the spaces in the ECM network, do the ECM proteins integrate into the TNT structure and contribute to its formation, or does the ECM network remodel to allow the formation of the TNT through the interstitial space.

**Matrix as a Hydrostatic Environment**

One more function of the ECM is regulating the interstitial hydrostatic pressure and subsequently the transcapillary exchange,\(^\text{192}\) which are also affected by the concentration of soluble molecules (e.g., cytokines, growth factor, and angiogenic factors) in the interstitial fluid phase.\(^\text{193,194}\) This function of ECM is particularly relevant to inflammation. Studies on dermal ECM have shown that during inflammation, elevated interstitial hydrostatic pressure exerts tension on ECM collagen fibers and microfibrils. This tension is reduced through a mechanism involving the collagen-binding integrin β1 and laminin, allowing ground substances (e.g., glycosaminoglycans, particularly hyaluronan) to expand and take up fluid, thereby relieving the interstitial hydrostatic pressure.\(^\text{195}\) Subsequently, the interaction of platelet-derived growth factor-BB with integrin β3 can normalize this decrease in hydrostatic pressure and restore ECM compaction.\(^\text{192,196,197}\) The increased interstitial hydrostatic pressure can, in turn, influence the function of the resident cells and the composition of ECM itself. In vitro studies have shown that exposure to elevated hydrostatic pressure increased production of collagen type I and aggrecan,\(^\text{198}\) decreased MMP2 and MMP3 expression,\(^\text{199,200}\) decreased collagen I, and increased sulfated glycosaminoglycans production.\(^\text{201}\) These changes further alter ECM composition influencing intercellular transportation. Integrins transmit extracellular tension and compression signals to the intracellular cytoskeleton.\(^\text{202,203}\) Integrin β3 levels were increased in response to hydrostatic pressure in human bone marrow–derived mesenchymal stem cells,\(^\text{204}\) whereas hydrostatic pressure–mediated elevation of vinculin-binding integrin (αv) in endothelial cells enhanced cell adhesion and proliferation.\(^\text{205}\) Blocking the integrins in mesenchymal stem cells abrogated hydrostatic pressure–enhanced sulfated glycosaminoglycans,\(^\text{206}\) indicating the key role of integrins and cell–ECM interaction in mechanotransduction of hydrostatic pressure.

**Conclusions and Future Perspectives**

It has become evident that ECM is not a passive and static network structural, but a highly complex and multifunctional network of any tissue or organ. In addition to providing structural stability, components of the ECM can hinder or facilitate movement of molecules in the extracellular space, in addition to regulating the interstitial fluid pressure. Because transportation of molecules through the ECM is a critical determinant of delivery efficacy for drugs or other therapeutic agents, a clear understanding of how different components of the ECM influence this process can help in optimizing the packaging of these molecules to enhance transportation through the dense or disrupted ECM in diseased myocardium and to markedly improve the therapeutic strategies. Although the past 2 decades have revealed new functions and properties of the ECM and its contribution to different pathologies, recent discoveries highlight the fact that more remains to be explored. As ECM has been the subject of vigorous investigations in the cancer field for much longer than in the cardiovascular field, valuable lessons can be learned from interdisciplinary application of findings, which could reveal additional novel aspects of ECM function in the cardiovascular system. Areas that require future investigation to expand our understanding of the ECM toward the goal of developing effective therapies for heart disease are listed in the Table.

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**Disclosures**

None.

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