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The Extracellular Matrix: At the Center of it All

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Introduction

Responding to stimuli is a hallmark of the definition of life, and we are constantly adapting to our surrounding environments. Individual cells also read and respond to various signals that surround them. Communication between cells is essential, and is profoundly influenced by the extracellular matrix (ECM). In addition to providing structural support, the ECM contributes to individual cellular and collaborative organ-level functions. In cardiac physiology, the ECM facilitates mechanical, electrical and chemical signals during homeostasis and the developmental process, as well as in response to physiological stress or injury. These signals modulate cellular activities and interactions such as cell proliferation, migration, adhesion and changes in gene expression during homeostasis and development. Furthermore, during post-myocardial infarction (MI) or hypertensive remodeling, components of the ECM can play agonistic and antagonistic roles that may contribute to progression towards a healthy recovery or heart failure. This paradoxical relationship and the array of distinct cellular and acellular signals that are involved in cell-ECM communication have made research in this area attractive at both the molecular and cellular levels. This review discusses several developmental processes that illustrate how cell-ECM communication is vital for proper cell-cell communication and cell behavior in the development of the heart, and how some of the same principles apply to cardiac disease and pathological remodeling. Because of the large degree of crosstalk between spatiotemporal and context-dependent signals of the ECM, it is imperative that we approach research involving cell-ECM communication collaboratively, and interpret results from as many angles as possible. We will discuss areas of focus that should help elucidate the cellular mechanisms involved in cell-cell and cell-ECM interactions in the heart, and likely other organ systems as well.

Studies involving the complexity of the ECM in the heart are directly applicable to a host of other physiological conditions. The cellular relationships and ECM factors discussed here are applicable to a wide range of physiological conditions that must be considered when studying any one system. Factors involved in the wound healing process, such as scar formation,

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collagen turnover, matrikines and cellular migration, are also found in cardiac development and response to injury [1–3]. In cancer progression and metastasis, epithelial-mesenchymal transition (EMT)-like transformations and interactions among tumor and stromal cells are similar to the cellular and acellular milieu seen in the heart [2,4]. Likewise, the basis of an appropriate immune response involves a conglomerate of autocrine, paracrine and hormonal signals, which are differentially released by particular cells, and include spatiotemporal receptor-ligand interactions and expression, just as in cardiac development and adult function. Furthermore, research pertaining to obesity and diabetes, vascular biology and the nervous system offer other areas of crossover, as patients with disorders in these areas present with conditions sufficient to alter homeostatic and pathologic cardiac function.

The Malleable ECM

It is important to regard the truly dynamic nature of the ECM and the variety of functions it is required to serve. Numerous studies reveal differences in the presence of specific ECM components during distinct physiological states (Figure 1). The diversity of the ECM composition is illustrated by the unique environments that are required for cell survival and proper function in the developing heart, a normal adult heart and a heart under pathological stimulation. Each cellular environment is subject to a host of autocrine and paracrine signals through cytokines, growth factors and hormones. Moreover, the different microenvironments within the developing or adult heart present with unique biochemical niches, largely driven by cell-cell and cell-ECM interactions.

Early cardiac development is largely dependent upon genetic integrity (i.e. mutations and DNA damage) and more immediate environmental signals (i.e. maternal hormones, mechanical and chemical signaling). Conversely, the adult heart is subject to a multitude of circulating factors and reciprocating signals from other organ systems within the body, especially in response to pathological conditions (i.e. from the kidneys, the central nervous system and the immune compartment). The presence of these “external” signals could alter the sensitivity of the myocardium and cardiac vasculature to more proximal signals from the surrounding cellular environment, during both homeostatic and pathological conditions. Although discussion of circulating factors and other organ systems are not within the scope of this review, they are an important element of the cellular microenvironment of the heart.

The ECM in the Developing and Adult Heart

During cardiac development, there is an array of environmental signals that ensures that cell migration and transformation occur in a specific spatiotemporal order. These signals include chemical and mechanical signals originating from both the cells and the ECM. During neonatal development, the ECM constitutes a thin layer within the epicardium, where epicardial mesenchymal cells and ECM thickening (collagen deposition) arise concomitantly [5]. These observations coincide with the differential functions of the fetal and postnatal heart, where ventricular wall thickness and tensile strength (stiffness) increase to accommodate changes in functional requirements following birth and the opening of the ductus arteriosis [6]. In addition, the developing heart tube is arranged as an endothelial cell (EC) layer surrounded by myocardial cells, with an acellular matrix composed of proteoglycans, collagens and glycoproteins found in between the inner EC layers [7]. This matrix serves to maintain tube structure and proper regulation of flow during embryologic heart development.

The composition of the ECM is a major factor in the process of EMT, which occurs during numerous developmental stages, as well as in response to pathology [4,8]. EMT is critical for organogenesis, specifically during cardiac cushion morphogenesis and valve formation. Secretions from myocytes and the surrounding ECM stimulate ECs to transform and invade the surrounding mesenchyme and ECM [7,8]. One component of myocyte (and EC) secretions

that promotes EC migration and EMT are members of the transforming growth factor-β (TGF-β) family of cytokines and their receptors [7]. Signaling via the TGF-β type 1 receptor, Alk5, seems to be essential for normal EMT and function of the epicardium during cardiac development [9]. Interestingly, Alk5 signaling is not essential for proper development of the myocardium, suggesting signal compartmentalization.

Early in cardiogenesis, the mouse heart tube is formed from endocardial and myocardial cell layers that surround the ECM. The heart tube subsequently loops around, and specific heart regions begin to develop into chambers [10]. Embryonic cushions, which proliferate and develop into the primordial heart valves following heart looping, differentiate from ECs in response to an increase in targeted, localized ECM synthesis by AV canal myocytes, which is then followed by an increase in ECM degradation as the cells undergo EMT and migrate through the mesenchyme to develop into cushions [11]. Because of their fibroblastic nature, these cushions are effectively formed from a localized expansion of the ECM. Only certain groups of cells within the heart tube respond to the paracrine and autocrine signals necessary for the initiation of cushion formation, and subsequent differentiation into a fibroblastic phenotype for valvulogenesis [12]. As mentioned, TGF-β is critical for proper EMT during all stages of development, including cushion formation and differentiation.

Another group within the TGF-β family of proteins that are critical for cushion differentiation, as well as subsequent valve formation, are the bone morphogenic proteins (BMPs). Different BMP proteins are expressed in specific regions of the developing heart at specific times [10]. Interestingly, BMPs can be retained within the ECM until subsequent activation, which makes it difficult to determine the origin of such signals. Because of their spatiotemporal expression, TGF-β and BMPs are considered possible inducers of cushion formation and differentiation [10]. Periostin, which will be discussed later in this review, is another ECM protein that plays a key role in EMT and cushion formation, as well as differentiation into the fibroblastic phenotype [12,13].

The organization and composition of the ECM is also critical to cardiac valve development and function. Following heart looping and the initiation of unilateral blood flow, endocardial cushions are formed from a localized expansion of ECM [14]. The proper transformation of a specific fraction of ECs, and their reception of signals from the surrounding mesenchyme and ECM, is critical for appropriate valve leaflet formation and subsequent valvulogenesis [14]. The composition and integrity of the ECM mediates critical cellular migration and compartmentalization that affects cardiac structure formation and heart function. The ECM is also integral to the spatiotemporal process of condensation and increased density of mesenchymal cells within valve leaflets [15]. Areas of condensation within the developing heart leaflet vary with embryonic and fetal stage, where the ECM factors involved (i.e. fibronectin and N-cadherin) characterize areas involved in condensation. The spatiotemporal expression (or ECM release) of BMPs also plays a role in valvulogenesis. Although traditional knockouts of single BMP proteins reveal no major cardiac defect, double-mutants result in severe cardiac deficiencies, and have enabled the investigation of these proteins in various tissues throughout development. A deficiency in BMP receptor type II (BMPRII), either throughout the embryo or localized to the endocardium, results in severe valve cushion and septal defects, as well as abnormal remodeling and positioning of the aorta [16].

The adult mammalian heart consists of a network of different cell populations that cooperate to maintain heart function under normal and pathological conditions. Structurally, the ventricular myocardium is organized into laminae of myocytes approximately 2–5 cells thick. These layers of myocytes are surrounded by an endomyosial collagen network, where cardiac fibroblasts interconnect with myocytes, and endothelial cells and vascular smooth muscle cells are largely confined to the coronary vasculature [17]. Resident stem cells and ‘transient’

circulating cells, such as platelets, monocytes and mast cells are found throughout the myocardium and interstitial space. In addition, the presence of a supportive fibrillar, connective network involved in cardiac cell physiology has been observed since the late 1970's [18]. This network is a critical component of the development and maintenance of cellular organization. The interconnections among cells and between cells and the ECM play critical roles in the physiology of the heart, as communication between different cardiac cell populations, specifically myocytes, fibroblasts and ECs, is important for proper heart function. Differences in the composition of the ECM, such as collagen content or enzyme expression, are directly involved in cell adhesion, migration and overall cellular communication.

The ECM is a dynamic collection of macromolecular proteoglycans, glycoproteins, proteases, collagens, growth factors and cytokines; modulation of these various components facilitates proper mechanical, chemical and electrical signaling between cells [19]. The signals provided by the ECM are either active or latent, and are critical in homeostasis and pathological remodeling. The different cell populations of the heart vary in their ability to make and secrete different ECM components (Figure 2). Generally, fibroblasts and vascular smooth muscle cells (VSMCs) are involved in fibronectin and collagen type I and III production, and VSMCs, myocytes and ECs produce type IV collagen and laminins [20]. Cardiac myocytes also produce collagen VI and proteoglycans [21]. Additionally, cardiac fibroblasts are the primary producers of matrix metalloproteases (MMPs) [19,22] and periostin [23], and also secrete various paracrine and autocrine factors, such as interleukin-6 (IL-6), TGF-β, endothelin-1 and tumor necrosis factor-α (TNF-α) [19]. Cardiac fibroblasts are sensitive to mechanical stimulation, depending on the environment provided by myocytes and the surrounding matrix [24]. The ECM composition and organization, along with the cellular components, affects force generation, receptor expression and chemical signaling which are important for proper cardiac function. Interestingly, many genes that are no longer expressed once development is complete are often re-expressed during remodeling, such as after MI [2]. Normally, most ECM proteins are primarily produced by the cardiac fibroblast; however, in pathological states such as MI, myofibroblasts, neutrophils, mast cells and macrophages also produce ECM proteins.

ECM in Cardiac Pathology and Remodeling

In response to MI, the process of cardiac remodeling includes angiogenesis, myocyte hypertrophy and fibroblast proliferation, which results in increased collagen deposition and alterations in ECM proteins and intracellular hormones, cytokines, matrikines and growth factors. These factors include IL-6, TGF-β, endothelian-1, TNF-α, fibroblast growth factor (FGF) and angiotensin II (Ang II) [19]. These chemical signals play roles in apoptosis, angiogenesis and cardioprotective responses following pathological insult [19]. Inflammation and scarring occurs at the site of injury, with a concomitant increase in laminins and β1-integrin at the myocardial interface of scar tissue [25]. Activated fibroblasts accumulate at the site of injury where they remain indefinitely, leading to an increase in local collagen deposition and fibrosis [3]. An imbalance in the synthesis and degradation of collagen and other ECM factors contributes to myocardial pressure increases and cardiac dysfunction. Ang II and TGF-β are secreted by fibroblasts, which alters cellular processes near and remote to the infarct, especially collagen turnover [3]. The secretion of TGF-β can trigger myocyte hypertrophy and reduce collagenase production, while leading to increases in tissue inhibitor of metalloprotease (TIMP) synthesis, which effectively decreases ECM degradation and leads to ECM accumulation. Stiffening of the myocardium via fibrosis and increased deposition of collagens and other ECM proteins can alter the mechanical and electrical dynamics of heart function, leading to cardiac dysfunction.

Following cardiac injury, surviving cardiomyocytes alter their cell-cell and cell-ECM interactions in response to surrounding cell death, which leads to ventricular remodeling [25].

These alterations occur both adjacent and distally to the site of the infarct [26,27]. The ECM can be temporarily remodeled, reversibly remodeled or fully adapt to the changes in biomechanical load; however, too large of a load increase over a long period of time results in detrimental collagen deposition, left ventricular dilatation and heart failure [28]. The composition and arrangement of collagen fibers within the heart determines the stress distribution across myocytes and movement of fluid within the extracellular compartments. Myocytes are highly sensitive to mechanical force that is transduced into chemical signals required for appropriate myofibrillar assembly and coordinated muscle contraction [29]. Due to hypertrophic conditions, transmission of mechanical load throughout the myocardium triggers a cascade of cellular events. Depending upon the nature of mechanical stimulation, ventricular wall thickening and increased contractility can be either beneficial, such as during exercise or development, or deleterious, as in response to pathophysiological signals [30]. In the latter condition, the heart undergoes maladaptive remodeling, such as fibrosis, possibly resulting in cardiac failure.

Variability of the ECM Depending on Species and Gender

On the cellular side, the ratios of different cardiac cell populations, namely myocytes, fibroblasts, ECs and VSMCs, change throughout development and into adulthood [31]. These observations are in accord with temporal relationships in alterations of ECM microenvironments of the heart. From heart tube formation to closure of the ductus arteriosis, as well as during cardiac development and pathological remodeling, changes in cellular phenotype confer reciprocating changes in ECM composition. However, although coordinated within a system, the functions of the ECM and effector molecules involved are not always consistent between different species, and gender-specific differences in ECM physiology are apparent.

Changes in the ratios of different cardiac cell populations, namely cardiac myocytes and fibroblasts, occur during development [31] and adult remodeling [28]. However, the balance of the different cell populations in the heart is species-specific in some instances, as studies show far more fibroblasts and fewer myocytes in the adult rat heart compared to the mouse heart, which is mostly composed of myocytes [31]. These differences can largely be explained by changes in biomechanical load requirements through increased body mass and circulatory demands. The law of Laplace states that the larger a vessel's radius, the larger the wall tension required to withstand a given internal pressure. Alteration in the radius of the heart in a trans-ventricular tension model designed to reduce the radius of curvature of the left ventricle has been shown to alter hemodynamic function and wall thickness [32]. As the radius of curvature of the left ventricle of a rat is larger than that in the mouse, this suggests that the wall tension on the heart would be increased in the rat when compared to the mouse. This suggests that the rat would need an increased amount of connective tissue; therefore, this would require an increase in the number of fibroblasts based on the radius of the heart given equal pressures, as it has been demonstrated that the mean arterial pressure in the mouse is almost identical to that observed in the rat. Thus, an increase in tension would require an overall increase in connective tissue and interstitial cells. In accord with this hypothesis, previous studies demonstrate that there are increased numbers of cardiac fibroblasts in the rat compared with the mouse [31]. Therefore, the conclusion is that the amount of ECM in the heart would be greater in the rat than in the mouse, indicating an increase in tension on the heart. This has been shown in studies assaying for hydroxyproline content in the heart [33]. In addition, previous studies from our laboratory have demonstrated that the adult rat heart contains more hydroxyproline compared with the mouse, 9.36 versus 5.95 μg collagen/mg dry heart wt, respectively [31]. Taken together, these previous studies suggest that larger mammals, which all display similar mean arterial pressures, would have greater wall tensions given their increased radius of curvature. Indeed, previous studies have demonstrated an increase in hydroxyproline content as seen in

a comparison between rats and dogs [34]. Thus, given that cardiac fibroblasts are the main producers of collagen in the heart, the rat heart may require more fibroblasts to provide the means to compensate for the increase in wall tension.

Species differences in ECM factors are seen in other aspects of heart development and remodeling as well. For example, in mouse and canine models of myocardial infarct, similar patterns of ECM deposition are seen following reperfusion; however, the time course of remodeling and scar formation is significantly shortened in the mouse, likely reflecting differential gene and/or protein expression patterns [35]. These differences illustrate the importance of dynamic cell-cell and cell-ECM interactions in responding to environmental stimuli to ensure proper heart function. They also lend to differences in responses to injury, as different signals would likely be required for healing processes in dissimilar cellular environments. The critical process of EMT and consequent cushion and valve formation requires the spatiotemporal expression of different TGF-β isoforms, as well as differential EMT progression, in avians when compared to mice [10]. Rather than ignoring these differences, we should further examine them in order better define the roles of a particular protein or pathway. Understanding the composition and signals of the ECM during remodeling could be beneficial towards regeneration of tissue at sites of defects or wounds.

Also relevant to the current discussion is the gender specificity of cardiac function and response to injury. Recent studies examining aortic valve stenosis demonstrated gender differences in cytokine and ECM expression during ventricular remodeling [36]. Collagen I was upregulated more than any other ECM component in women, and different branches of the TGF-β signaling pathway were activated in women versus men. Aside from gaining insight into gender-specific therapies, these studies ascertain that, in knockouts and other models of cardiac development and dysfunction, care should be exercised when combining data from male and female animal subjects. Future studies should be focused on dissecting the functional roles of these pathways in the downstream responses to hormonal and mechanical factors.

Angiogenesis and the ECM

Formation of blood vessels depends upon environmental cues that regulate EC function. The ECM acts as a physical scaffold for cells and a storage area for cytokines and growth factors. In addition, the mechanical and chemical properties of the ECM transmit information to cells that influences their behavior through interactions with cell surface receptors termed integrins, which are discussed in further detail below. During angiogenesis, the perivascular ECM plays a critical role in determining the proliferative, invasive and survival responses of ECs to angiogenic factors. Dynamic changes in the ECM, as well as in the vascular cells (ECs and VSMCs) act together to regulate new vessel formation. Digestion of ECM components by MMPs is essential for invasion by ECs, but also creates ECM fragments, which can antagonize the function of integrins (Figure 3). Integrin interactions with ECM proteins potentiate the signaling events that are critical to angiogenesis. Indeed, the signaling activity of many receptor tyrosine kinases is dependent upon interactions between integrins and the ECM.

Besides interacting with the ECM, ECs interact with other cell types, including other ECs and pericytes. Additionally, we have observed interactions between cardiac fibroblasts and ECs both *in vitro* and *in vivo*, suggesting that fibroblasts are also important for blood vessel formation during development and possibly during disease. As mentioned above, fibroblasts play a role in the development, growth, and remodeling of tissues. They do so through synthesis and deposition of ECM, as well as through secretion of cytokines and growth factors, which allow them to modulate their environment through autocrine and paracrine signaling. In addition, fibroblasts can also secrete vascular endothelial growth factor (VEGF). VEGF acts on endothelial cells and is important for stimulating angiogenesis and coronary collateral

formation for restoring the blood supply to injured myocardium. To date, few studies have directly investigated how fibroblasts contribute to blood vessel formation and this should be an intense area of investigation for years to come.

Other factors that have been shown to play a role in angiogenesis are also produced by fibroblasts, such as MMPs and TIMPs. MMPs can regulate EC proliferation, adhesion and survival leading to activation or inhibition of angiogenesis. MMPs act by degrading the ECM, which can promote EC migration and sprouting, or MMPs can cleave and release anti-angiogenic factors that inhibit these events. TIMPs have been shown to inhibit angiogenesis, as well as promote vessel formation. Thus, like MMPs, it appears that the function of TIMPs is context dependent. Recent studies from Lilly and colleagues demonstrated that TIMP-1 is secreted by fibroblasts and increases vessel formation [37]. In these studies, they observed that activities of TIMP-1 were MMP-dependent. In addition, they observed that direct interaction between fibroblasts and endothelial cells is necessary for optimal vessel formation [37]. These studies underline the significance of fibroblast-derived factors and cell-cell interactions in regulating angiogenesis. Future studies need to be directed at defining the cell surface molecules that play a role in these cell-cell interactions, as well as the importance of the ECM in regulating this process, especially during remodeling following cardiac insult.

The ECM Players

Integrins

Integrins are essential for ECM interactions with cardiac myocytes and fibroblasts, and overall structure and cell communication in the heart. They mediate cell-ECM and cell-cell communication in a bidirectional manner – that is, extracellular events affect nuclear activity, while signals that originate in the nucleus affect cell surface protein expression and function. Integrins are large transmembrane ECM receptors that consist of alpha and beta subunits and have a large extracellular domain and small cytoplasmic signaling domain; splice variants and heterodimerizations of the subunits confer a wide range of ligands while also maintaining a high level of specificity [27]. They are critical for cell behavior, specifically cell migration and survival, during virtually all developmental stages, as well as pathological remodeling. Both external and internal signaling can affect integrin expression and clustering within the cell membrane, as well as integrin conformation and ligand affinity. The list of integrin ligands is expansive [38]; most commonly, integrins are receptors for collagen, laminin, fibronectin and thrombospondin, as well as tenascin-c, osteopontin and periostin.

The temporal and spatial localization of integrins is altered during developmental progression and normal heart function, and is highly coordinated with ECM composition. Their proper expression is necessary for the process of developmental and pathological hypertrophy in the heart. A large number of knockout studies have found both overlap in integrin function and specific roles for particular integrin subunits and heterodimers, and are elegantly reviewed by Ross and Borg [27]. Knockouts of specific isoforms of β1-integrin result in poor blastocyst development and implantation, embryonic death and altered perinatal heart function [27]. Recently, β1-integrin was implicated in differential neonatal and adult cardiac fibroblast function [39]. In these studies, it was demonstrated that embryonic cardiac fibroblasts promoted myocyte proliferation through the secretion of fibronectin, collagen and heparin-binding epidermal growth factor, all of which are mediated by the β1-integrin receptor [39]. Interestingly, these studies also showed that adult cardiac fibroblasts promoted myocyte hypertrophy through β1-integrin receptors, indicating a functional switch in fibroblast phenotype and/or integrin activation. These studies illustrate the critical elasticity of cell-ECM interactions, and how these interactions change in response to environmental demands. In cardiac remodeling following MI, an isotype switch from β1D to β1A integrin in myocytes has been shown to be regulated by TNF-α during certain stages of the healing process [40],

exemplifying the role of cytokines in the spatiotemporal regulation of integrins. In fact, the expression of several cytokines are associated with integrin regulation, including TGF-β and angiotensin II [40]. As such, integrins may provide an important link between circulating cytokines and/or growth factors and the response of cardiac cells to signals originating outside of the heart. This is especially relevant to preventative therapy or in diseases where there is a substantial risk for secondary heart disease, such as with diabetes.

A current molecule of interest that utilizes several integrin signaling pathways is integrin-linked kinase (ILK). This molecule localizes at focal adhesions and areas of mitotic spindle formation, and can function as both a kinase and adaptor protein that regulates a number of cellular processes such as EMT, angiogenesis, cytoskeletal arrangement and mitosis [rev in 41]. It largely acts through the PI3K, β-catenin and E-cadherin signaling pathways, and overexpression of ILK has been linked to cancer formation and progression. In fact, clinical trials are currently underway to examine how using ILK inhibitors as chemotherapeutic agents may attenuate cancer cell mitosis and cancer progression [41]. Further investigation of ILK and other adaptor and effector molecules will not only elucidate the specific pathways involved in integrin signaling and expression, but also precise methods of crosstalk between integrins and other signaling and cell survival pathways.

Fibronectins and Laminins

Secreted by cardiac fibroblasts, fibronectin is an adhesive glycoprotein that associates with multiple ECM factors, and whose substrate specificity is regulated by different splice variants [20,42]. Fibronectin is an essential component of vascular morphogenesis, the importance of which is demonstrated by the embryonic lethality of fibronectin knockout mice [42]. Both mechanical and chemical factors can induce fibronectin expression in development and cardiopathology, which is often preceded by an increase in collagen and TGF-β expression [20]. A glycoprotein found in the basement membrane of cardiac myocytes, laminin contains differential binding domains for several ECM components, including collagens and cell membrane receptors [21]. Laminin is largely produced by cardiac myocytes, VSMCs and ECs [20,21]. Knocking out various subunits of laminin and laminin receptor isoforms results in altered cardiac development and/or adult cardiac function [21]. As mentioned above, the ECM plays a critical role in angiogenesis and laminin may be a critical player in this process.

Collagens

Collagens are an indispensible component of ECM. Different types of collagen exhibit unique properties, such as tensile strength and fibril formation [28,43]; therefore, its synthesis and deposition is highly coordinated. Collagen deposition can be regulated by autocrine and paracrine hormones, such as Ang II, TGF-β, aldosterone and angiotensin-converting enzyme (ACE) [3]. In response to particular biomechanical and biochemical signals, cardiac fibroblasts increase collagen synthesis and deposition. Fibroblast secretion of Ang II stimulates the expression of TGF-β and collagen I synthesis [44]. Moreover, Ang II can increase cytokine production in myocytes and enhance fibroblast sensitivity to cytokines [44]. ACE inhibitors decrease TGF-β, fibrosis and collagen deposition, and improve outcome hypertrophy and fibrosis [21]. One mechanism of action seems to be through the inhibition of hydrolysis of *N*-acetyl-seryl-aspartyl-lysl-proline (Ac-SDKP) by ACE, which decreases fibroblast proliferation and collagen synthesis [45]. Ac-SDKP, in turn, has been shown to reduce Galectin-3 expression, which is an endogenous lectin involved in inflammatory cell recruitment and increased cytokine secretion, fibroblast proliferation and ventricular dysfunction. The inhibition of Galectin-3 results in reduced fibroblast proliferation, collagen deposition and inhibited macrophage activation and migration, leading to a decrease in cardiac dysfunction following Galectin-3 treatment [45]. In addition, chronic activation of the renin-angiotensin system is associated with the presence of inflammatory cells and fibroblasts that

precede changes in the vasculature and affect cell recruitment and collagen deposition, leading to fibrosis [3].

Some collagens are only expressed during specific stages of development or remodeling, depending on the required function of collagen expression, and genetic defects in different collagens generally results in pathologic heart valve structure and dysfunction [43]. Various types of collagen are found in different regions of the heart depending on the stiffness and contractility required for tissue function [43]. As different collagens are used for specific functions, the ratio of collagen types deposited in certain areas of the heart contributes to tissue flexibility and function; a balance of collagen degradation and synthesis is necessary, but can become maladaptive following injury if stiffness is exacerbated [28]. It is important to note that an increase in collagen deposition, per se, is not the only cause for collagen-induced decreased contractility in response to cardiac injury or hypertrophy, as maladaptive changes in the ratios and type of collagen expressed will have the same pathologic affect. In addition to these alterations in collagen deposition following hypertrophy, changes in the degree of collagen crosslinking have been associated with altered cardiac function [rev in 28]. The arrangement of the collagen network can affect the conductance of electrical signals and bridging of myocytes by cardiac fibroblasts [17]. During normal electrical conduction, cardiac fibroblasts and myocytes interact with each other and themselves via homotypic and heterotypic cell-cell signaling through connexins (Cx 40, 43 and 45) [17,46]. Connexins are critical components of intercellular communication and cellular adhesion that mediate electrical conductance, among other cell-cell interactions. As fibroblasts themselves are not electrically excitable but are primary producers of ECM [19], they contribute to cardiac myocyte function through avenues such as connexin receptor expression (signal diffusion) and collagen deposition (signal inhibition) [46]. Overall, myocyte-fibroblast coupling and increased collagen deposition have been shown to decrease electrical conduction velocity [47]. The ability to control collagen deposition by cardiac fibroblasts in a spatiotemporal manner, which would include ECM contributions, would target myocardial development, wound healing, cardiac fibrosis and a host of consequent disorders. Along the same lines, approaches for intervening in collagen metabolism need further study for potential preventative and therapeutic intervention strategies.

Periostin

Another secreted ECM protein, periostin (or osteoblast-specific factor 2), is a 90kDa protein that belongs to the fasciclin family of genes (with *fasciclin-1*, *bIG-H3*, *stabling-1* and *-2*), and is able to bind to several integrin heterodimers [11]. Periostin is most often secreted by fibroblasts or cells that acquire a fibroblast-like phenotype during development and following injury [23]. Periostin can directly interact with other ECM proteins, including collagen I, fibronectin and tenascin-c, as well as several integrins. *Periostin*-deficient mice display gross abnormalities in cell differentiation and valve formation, possibly through a TGF-β-dependent pathway [6], which illustrates a regulatory role of periostin in cardiac morphogenesis [18]. In the developing chicken and mouse, *periostin* is localized to the endothelium of the ventricular trabeculae and in the endothelium and mesenchyme of the outflow tract and AV cushions [48]. Later in cardiac development, *periostin* is expressed in the fibrous region of the heart, suggesting that shear stress and altered hemodynamic load affect its expression [48,49]. Over the course of embryonic development, *periostin* expression is observed at E10.5 and steadily increases until E14.0–17.0, an expression pattern that mimics that of the endocardial cushion to valve formation [50]. Indeed, recent studies have demonstrated that loss of *periostin* in mice results in valve defects and a marked reduction in postnatal survival [49]. In the developing heart, periostin is absent from cardiomyocytes, but is expressed in non-cardiomyocytes, specifically fibroblasts and VSMCs [51]. Following vascular injury, periostin expression is observed from VSMCs and has been shown to be associated with VSMC differentiation *in*

vitro and promote cell migration [52]. Interestingly, periostin expression is not regulated by FGF in VSMCs, which may useful in delineating smooth muscle and fibroblast lineages.

In addition to its role in cardiac morphogenesis and development, periostin's involvement in cardiac remodeling is under intense investigation. In adult mice, overexpression of periostin via adenoviral administration results in ventricular dilation, a decrease in myocytes, increased collagen deposition and decreased myocyte spreading and fibroblast adhesion [23]. Many reports have demonstrated a critical role of periostin in developmental and adult ECM remodeling, collagen fibrillogenesis, and overall cardiac hypertrophy and remodeling [18]. We recently reported that *IL-6* knockout mice have an increased fibroblast to myocyte ratio, collagen deposition and cardiac dilatation that was accompanied by a marked increase in *periostin* expression [53]. Although a direct causal effect of IL-6 on periostin cannot be concluded from these studies, it illustrates direct and/or indirect links between cell populations, ECM composition and different signaling factors.

Animals that undergo pressure overload hypertrophy show a significant increase in periostin expression. Upregulation of periostin via the PI3K pathway has been demonstrated, and leads to the induction of TGF-β, FGF and Ang II [13]. Other studies have confirmed the involvement of PI3K pathways during cardiac remodeling, even in the absence of periostin signaling, which has been demonstrated in both embryonic development and the adult heart [6,54]. Furthermore, loss of anti-hypertrophic factors such as atrial natriuretic peptide (ANP) results in an increase in *periostin* expression of 20–40-fold when compared to control mice. However, this effect was only seen in ANP knockout mice that had pressure overload hypertrophy, not in mice that were ANP-deficient without hypertrophy [55]. Taken together, these studies suggest that increased hemodynamic stress plays a role in the expression of periostin, and that periostin plays a critical role in the pathology of the heart.

While periostin clearly plays an important role in cardiac development and response to hypertrophy, the specific mechanisms by which it contributes to these processes are currently under debate. A recent study implicated the expression and secretion of periostin with activation of DNA synthesis and cellular proliferation in rat cardiac myocytes [54]. However, in another study, Molkentin and colleagues did not observe an increase in cardiomyocyte proliferation in response to periostin treatment [56]. While results from these studies are contradictory, they underscore the importance in understanding the role of periostin in the heart, given that induction of cardiomyocyte proliferation has serious implications in the treatment of patients following myocardial injury. Periostin has many clinical implications and further investigation into the ability of periostin to initiate EMT could be useful in bioengineering/*in vitro* systems. The ability of periostin to reduce cardiac scar formation following ischemia or hypertrophic insult could also be further examined.

MMPs/TIMPs

While MMPs and TIMPs are expressed by virtually all cell types in the heart [57], fibroblasts are the main contributors. Various interactions among MMPs and TIMPs affect their ubiquitination and degradation, induce signaling cascades through dimerization and alter gene expression and cellular localization, effectively altering the extracellular environment to promote or hinder cell signaling, migration and survival. MMPs and TIMPs have been implicated in an array of physiological responses following cardiac hypertrophy, and specific changes in left ventricular function have been correlated with MMP expression [58]. Following MI in rats, different MMP and TIMP isoforms are activated in a coordinated fashion during specific stages of remodeling [59], contributing to the spatiotemporal regulation of ECM signaling.

In the heart, MMP-7 is expressed by macrophages and myocytes [60], and appears to influence cleavage of Cx43, a critical factor in gap junction-mediated cell communication and electrical conduction involved in cardiac remodeling. Altered Cx43 expression, cardiac remodeling and survival have been observed in MMP-7 knockout mice following myocardial infarction [60]. These studies leave open the possibilities of MMP inhibitors or even focusing on the downstream substrates of MMPs as a therapeutic option following cardiac injury.

Matrikines

The term *matrikine* has been proposed to define those circulating and tissue-specific peptides that are produced by the partial proteolysis of ECM macromolecules, and are able to affect cellular function. Once cleaved, matrikines are free to interact with cell surface receptors, thereby affecting a wide array of biological processes including matrix degradation, cell adhesion, migration, proliferation, apoptosis and protein synthesis [61]. Examples of matrikines are those peptides derived from elastins, connective tissue glycoproteins, laminins and collagens. Additionally, *matricryptic sites* are thought to exist on insoluble ECM molecules in tissues, matricellular ECM proteins and plasma-derived ECM molecules [62]. These sites only refer to those not normally exposed in the intact, activated peptide. Current research involving matrikines is largely targeted toward their protective actions in tumor-stromal interactions, wound healing and angiogenesis [61,62]. However, recent studies have demonstrated that elastin peptides were able to influence nitric oxide production by cardiomyocytes and ECs [63], offering a potential cardioprotective role for matrikines.

Cytokines

Many cytokines are involved in cardiac development and physiology, especially in cardiopathologies [64]. In both humans and rodents, circulating and myocardial levels of inflammatory cytokines such as IL-6 and TNF-α are increased following cardiac injury or in response to a developmental defect [64,65]. We have recently found that loss of IL-6 results in numerous cardiac abnormalities, including an increased number of fibroblasts and interstitial collagen deposition, reduced capillary density, increased periostin expression and overall heart dilatation [53]. These and many other studies not only advocate the importance of cytokine signaling in cardiac function, but illustrate the cellular and biomolecular interrelationships in the heart, which includes the myocardial ECM.

Perspectives and Conclusions

This short review is not entirely comprehensive with regards to cell communication and ECM composition in the heart. Additional factors that affect cell-cell and cell-ECM interactions include contributions of the CNS, cytoskeletal rearrangements, osteopontin, hyaluronin, SPARC, RANKL, other activators of the PI3K/JAK/ERK/MAPK pathways, different disease and physiological states, and a host of other growth factors, hormones and cytokines (other reviews within this issue cover many of these topics).

Care should be taken when interpreting studies using different techniques (i.e. *in vitro* matrices, surgically-induced hypertrophy models and knockout models) because of the extreme sensitivity and interrelationships involved with cell-cell communication and cell-ECM remodeling; however, interdisciplinary approaches are necessary for properly studying these systems. Knockout animal models will continue to elucidate the roles of specific biomolecular interactions and signals in cardiac function. Studies utilizing knockout animals should include some sort of rescue of function, as well as tissue-specific and conditional or induced knockouts, so that developmental, pathological and acute dysfunctions can be thoroughly examined. *In vitro* systems are becoming more advanced to include testable 3-D biochemical and mechanical interactions and relationships observed in the *in vivo* environment, but there is room for

improvement. Advances in microprinting and tissue bioengineering provide a tremendous opportunity for further development of *in vitro* ECM systems. Microprinting employs the "printing" of materials onto a substrate, just as an inkjet prints on paper [see 66]. In fact, heterocellular aggregates, which would constitute a part of the organ such as the vasculature, have already been created [66]; the printing of entire organs is not far from being a reality. The concepts and techniques used for the microprinting of organs are directly applicable to the creation of an artificial *in vivo*-like microenvironment. For example, Kit Parker's group has begun to use a "printed" matrix to examine changes in myocyte shape and size in response to geometric cues from the ECM substrate [67]. Current limitations of cell-ECM *in vitro* systems arise from difficulties in obtaining pure cell populations, isolating specific components of the ECM and maintaining *in vivo* cellular and extracellular morphology. The microprinting of biological materials could greatly attenuate these issues. The creation of "custom" matrices that include specific, printed ECM components could model virtually any physiological system and would be a huge advance for biomedical research. Alternatively, different populations of living cells could be printed in a controlled fashion, and induced to produce an 'authentic' ECM. Any printed ECM matrix would not only include chemical signaling factors of interest, but would also be subjected to mechanical and electrical stimulation, as they could easily be monitored and manipulated via computer. Moreover, these technical advances could help transition from a "3D" to a "4D" matrix that includes the variable of time, which would provide additional models for the *in vitro* examination of developmental systems, remodeling following pathological insult and gradual physiological changes in response to disease. These various microenvironments would facilitate the examination of individual and collaborative cellular responses to a variety of chemical, mechanical and electrical signaling events.

The communication and organization within the cellular environment of the heart has a wide range of clinical applicability, which provides opportunities for a host of additional basic and clinical research. As we have discussed, communication between cardiac cells and the ECM are critical for normal development, homeostasis and response to pathological stress. Moreover, chemical, mechanical and electrical contributions to cell-cell and cell-ECM physiology are highly interdependent. Continuing to develop these avenues of research will allow for the targeting of specific molecules for pharmacological intervention in the prevention and treatment of a variety of cardiac pathologies, as well as a host of other biological processes. This research should provide further insight into the dynamic regulatory nature of the ECM and also promote the development of novel therapeutics.

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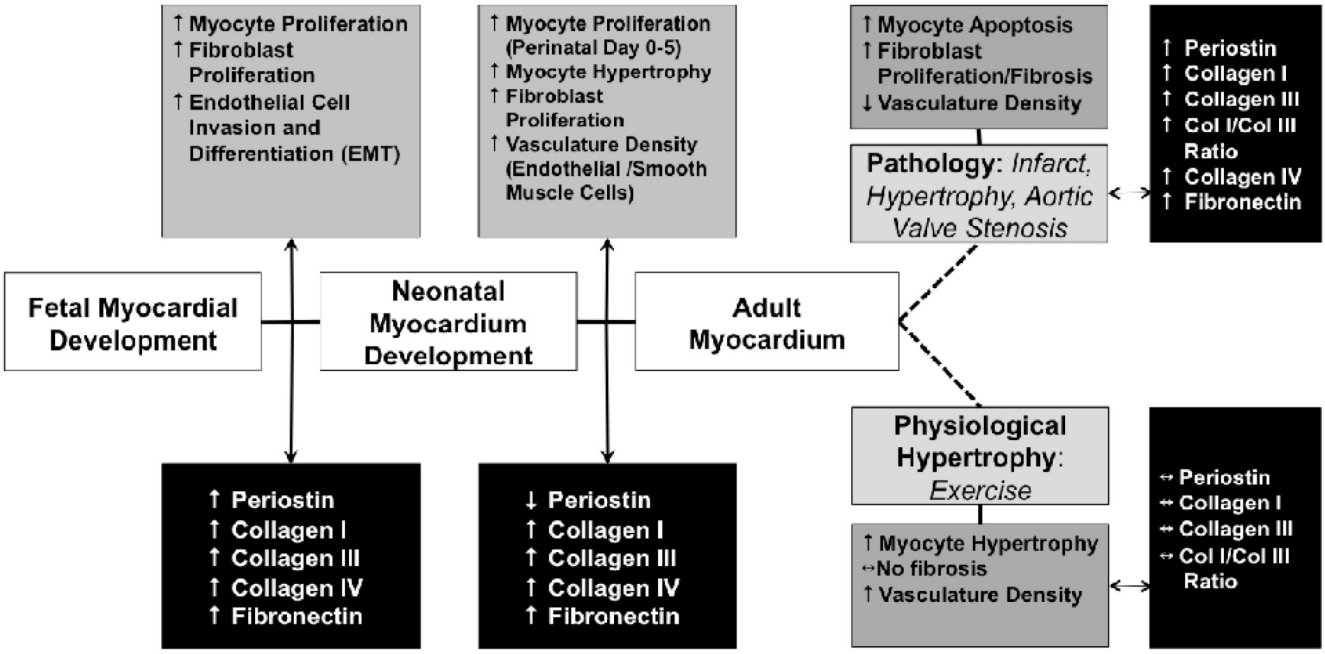

Figure 1.
Schematic of cellular changes (grey boxes) and ECM factor expression (black boxes) during various physiological cardiac states (white boxes).

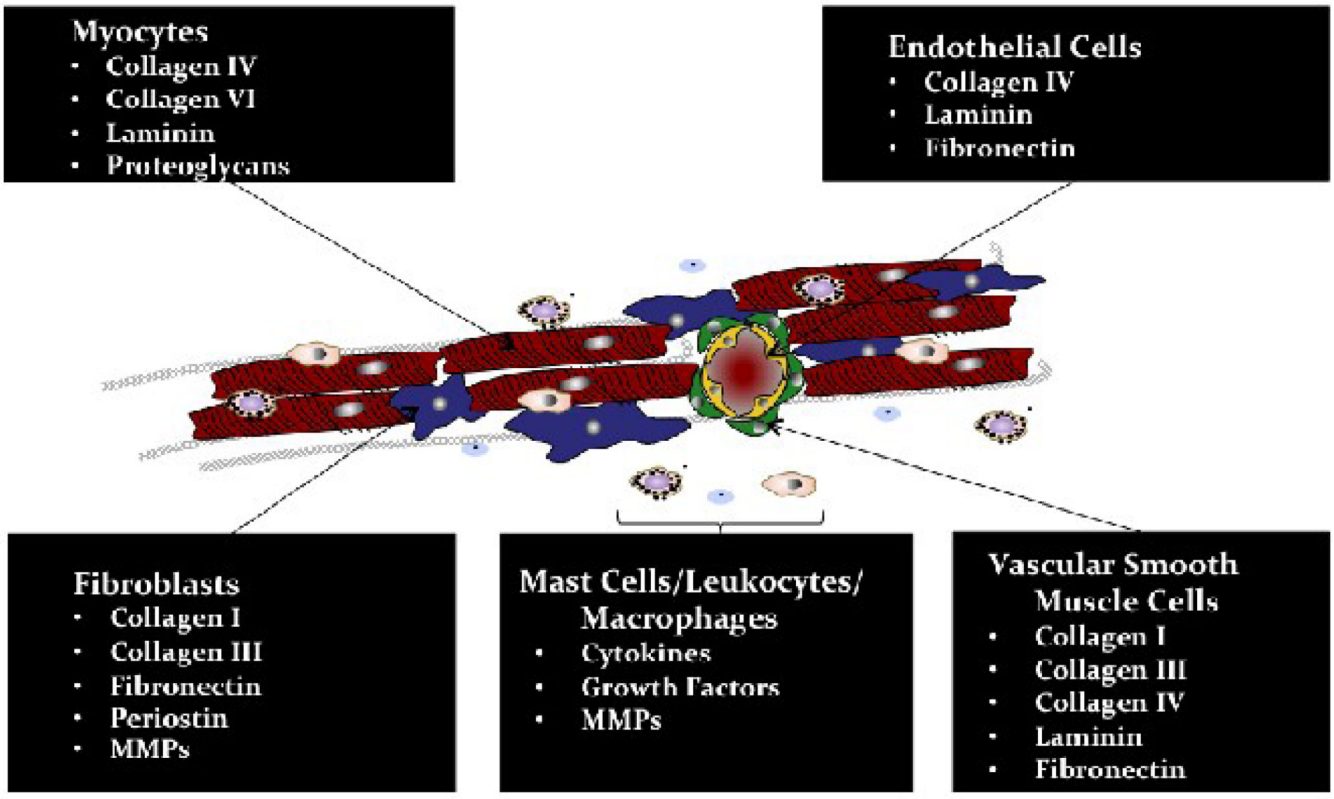

Figure 2.
Representative diagram of the cellular milieu within the myocardium. Note both individual and overlapping contributions of prominent matrix components by each cell type.

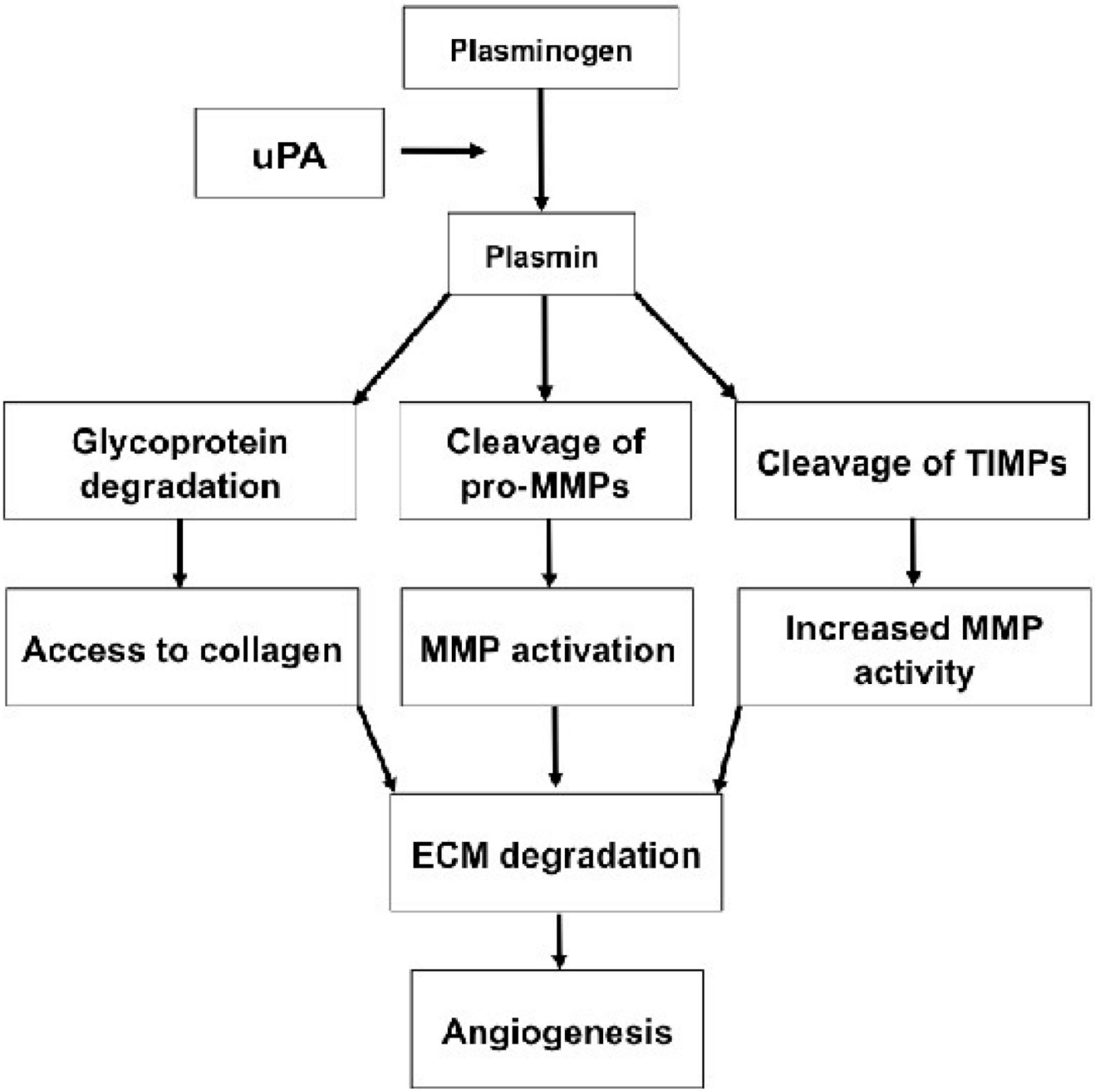

Figure 3.
The process of blood vessel formation. To initiate new capillary formation, endothelial cells of existing vessels must degrade the underlying basement membrane and invade into the neighboring tissue. These processes require the activities of urokinase-plasminogen activator (uPA) and matrix metalloproteinases (MMPs).