The relevance of extracellular matrix structure and composition in engineering the diseased cardiac microenvironment

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Review

The relevance of extracellular matrix structure and composition in engineering the diseased cardiac microenvironment

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Abstract

Introduction

Engineered cardiac tissues provide excellent tools to study cardiac (patho) physiology in vitro. These cardiac tissue models also a platform to create disease in a dish, which can be achieved by manipulating either the cells or the matrix. During disease, not only the cells are affected, but matrix organization and composition are also disturbed. In the healthy heart, the extracellular matrix guides cellular orientation and organization, thereby facilitating efficient contraction, force transduction and electrical transmission of the cells. Pathological alterations in matrix structure or composition will therefore affect cellular function which in the end may lead to reduced cardiac output and eventually heart failure. Our knowledge about the effects of different changes in matrix composition or structure on cardiomyocyte function is still limited. Understanding how cardiomyocytes respond to these different microenvironments will support the improvement of cardiac regeneration therapies and facilitate discovery of new possible targets to treat cardiac disease. In this review, we will discuss the main changes in cardiac structure and matrix composition that occur during heart disease and how these matrix properties are implemented in engineered cardiac tissue models.

Conclusion

Cardiac pathologies lead to alterations in structure and composition of the matrix, inhibiting normal cellular and tissue function. Systematically manipulating and quantifying these different matrix properties as well as their effects on cell and tissue function in vitro has revealed that both structure and composition provide important cues for cellular function. Analysis of diseased native tissue and improvements in the design of synthetic materials will lead to the development of the next generation cardiac tissue models.

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Introduction

Cardiac tissue engineering comprises the seeding of cardiac cells in a 3D environment and the subsequent culture of the resulting construct under specified conditions. It has been performed for two main reasons: to create functional tissues for regeneration of the diseased or injured heart; and to develop in vitro model systems to study normal and diseased cardiac physiology. In addition, these tissue models have been used for the development of new regenerative therapies and drug screening. They aim to bridge the gap between two-dimensional (2D) cell models and animal models of cardiac (patho) physiology, often used to test novel therapies. It is hypothesized and indeed plausible that engineered cardiac tissues more closely mimic the three-dimensional (3D) native cardiac environment 2D cell cultures. In addition, they produce real time information with a high degree of experimental control that would never be possible in animal or human studies. Furthermore, when designed correctly, cardiac tissue models allow for high throughput screening, and quantification of tissue contractility, which is the most important measure of heart function. Another advantage of engineered cardiac tissue models is that each of the composing elements of the tissue can be modified separately to create disease models (Figure 1).

For example, disease-specific cells can be used, or cells can be manipulated using soluble cues to induce disease. Additionally, the extracellular matrix (ECM), which can be either native or synthetic, can be engineered to manipulate the microenvironment of the cells. In this way cardiac disease in a dish can be created to study aspects of the underlying pathophysiological mechanisms (Figure 1).

Most of the currently used tissue models for cardiac disease focus on manipulating the cells by changing the culture conditions[1,2,3], adding pro-arrythmic drugs[4,5], or using genetically affected cells[6,7], while limited attention is paid to the cellular micro-environment.

However, in disease, not only the cells are affected, but the composition and structural organization of the microenvironment changes as well. Normally, the cardiac ECM provides an anisotropic structural scaffold to guide aligned cellular distribution and organization. This accommodates contraction and relaxation of cardiomyocytes and facilitates force transduction, electrical conductance, intercellular communication and metabolic exchange within the myocardial environment[8]. Despite the unequivocal influence of alterations in ECM structure and composition on cardiac function during disease, surprisingly little is known about the effects of matrix remodeling on cardiomyocyte function and survival.

Understanding how cells respond to differences in matrix organization and composition will increase our insights in disease mechanisms and aid in the development and optimization of (regenerative) therapies.

This review concentrates on the implementation of ECM structure and composition in engineered cardiac

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tissue models and their relevance for disease modeling. To accurately mimic ECM properties, detailed knowledge of the native ECM under healthy and diseased conditions is required.

Therefore, we first summarize the principle changes in structure and composition of cardiac ECM during disease development, followed by a discussion on current research related to ECM structure and composition in in vitro cardiac tissue models.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

In vivo ECM structure and composition of the diseased heart

In the majority of cardiac pathologies, the accumulation of ECM or fibrosis is an integral part of the compensatory and repair mechanisms leading to chamber remodelling and functional adaptation[9].

The distribution of myocardial fibrosis varies according to the underlying pathology. Replacement or scarring fibrosis occurs after cardiomyocyte loss, typically after a myocardial infarction and results in localized fibrosis. On the other hand, reactive fibrosis following myocardial stress or inflammation occurs in most cardiac diseases with pressure and volume overload.

The latter type of fibrosis has a progressive onset and is characterized by a diffuse distribution[10].

Fibrosis has profound consequences for cardiac function due to adverse cardiac remodelling, changes in ventricular stiffness, functional deterioration and the possible development of heart failure. Under normal conditions, mainly cardiac fibroblasts maintain the ECM by producing and degrading the ECM proteins that in a well-balanced manner. In heart disease, changes in biomechanical cues related to mechanical stress induce fibroblast transformation into myofibroblasts[11].

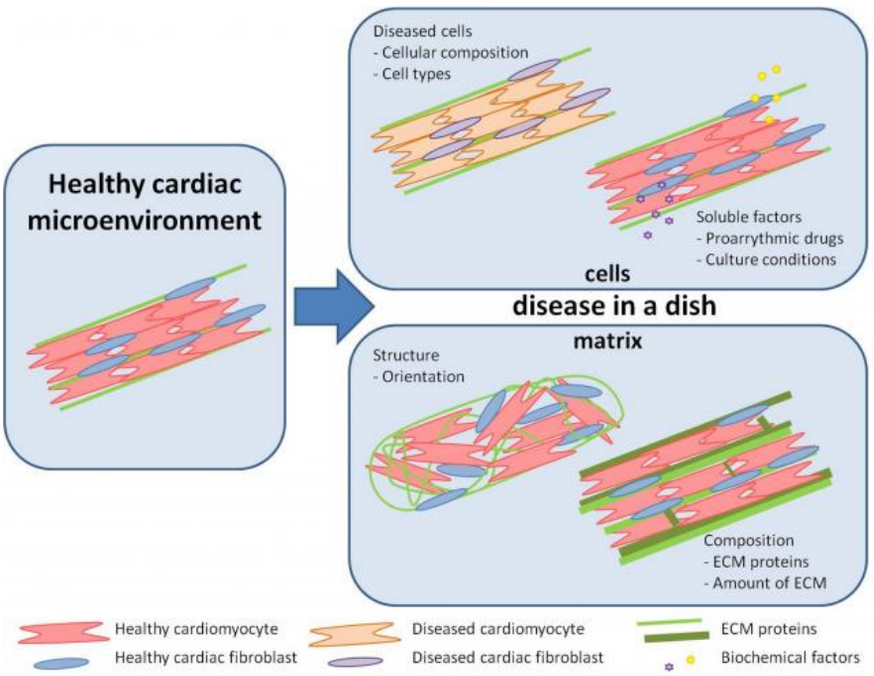

Figure 1: Engineering the cardiac microenvironment. Diseased cell types or changing cell ratios as well as biochemical cues can be used to manipulate the cardiac cells directly, while matrix structure and composition can be manipulated to study their effect on cardiac cell and tissue function.

Furthermore circulating macrophages, smooth muscle cells, endothelial cells and fibrocytes may also differentiate into myofibroblasts. These cells produce a different ECM, which is deposited in a chaotic network of collagen fibres, thereby altering the matrix architecture[8] (Figure 2 A, B).

Additionally myofibroblasts contribute to changes in collagen content, conformational changes in type of fibrillar collagen and an increase in cross-linking[11]. Collagen deposition in the extracellular compartment comprises mainly of collagen type I and III. Cardiac disease can cause a shift in the ratio of these collagen subtypes and thereby alter the composition of the matrix[12]. A shift in the ratio of these collagen subtypes was thought to be responsible for increased chamber stiffness, although changes in total myocardial collagen concentration or shifting phenotypes do not necessarily translate into increased myocardial stiffness. A loss of collagen support due to increased degradation of mature cross-linked collagen which in turn is replaced by newly synthesized collagen with decreased cross-linking will lead to breaks or tears in the myocardial matrix[12]. Other matrix components like fibronectin (Figure 2 C, D) and proteoglycans which are involved in cell-matrix interactions are also altered during cardiac disease and thereby affect cellular function and promote the formation of fibrotic tissue.

The change in dynamic interactions between cells and ECM due to fibrosis is detrimental for contractile synchrony and cardiomyocyte function[13]. The changes in ECM modify the microenvironmental signals that cardiomyocytes receive, leading to alterations in gene expression associated with cell morphology and contractile function[11]. Furthermore, ECM modification contributes to arrhytmogenesis through impaired conduction and subsequent generation of reentry circuits[9].

Interruptions of the fibrillar collagen matrix alter cell support, geometric alignment and coordination of myocardial excitation-contraction coupling and weaken attachment to the ECM. Change in MMP activity in fibrosis influences bidirectional signalling via integrins and dystrophin (Figure 2 E, F),

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thereby weakening the cell-matrix association and alter the response of cardiomyocytes to stress or pressure-overload[14].

Disruption of cell-matrix interaction also contributes to sliding displacement (slippage) of cardiomyocytes promoting cell death and leading to a decrease in the number of muscle layers[14]. Given the impact of ECM changes on cellular function in cardiac disease, these characteristics should also be incorporated in in vitro cardiac tissue models.

Structure and composition of the ECM in engineered cardiac tissue

Engineered cardiac tissues are usually created between two anchoring points to obtain an aligned tissue representing the healthy anisotropic cardiac architecture[4,5,6,15]. The composition of most currently used engineered cardiac tissues is a combination of either collagen type I, fibrin and/or matrigel[16].

Although mimicking essential parts of the ECM, this is off course a simplification of the complex native environment. In the next part, studies that specifically focused on mimicking at least part of the native cardiac structure or composition or studied the effect of changes in structure or composition on cardiac tissue function will be discussed.

Cardiac Structure

The architecture of the collagen network in the healthy heart induces proper alignment of the cells, thereby enabling end to end coupling of the cardiomyocytes which is essential for the myocardium to act as a syncytium. During disease, disarray of the cells and matrix may occur.

Systematic research using in vitro models has provided insight in the essential role for anisotropy in cardiac function. In studies with cardiomyocyte monolayers, anisotropy is mimicked by microcontact printed lines of ECM protein on a 2D substrate[17,18,19]. These printed lines act as extracellular cues to create anisotropy in monolayers of rat neonatal cardiomyocytes thereby improving calcium handling compared to cells seeded on homogenous protein layers[17]. Microcontact printing was also used on elastomeric thin films that bend upon beating of the cardiomyocytes, allowing for contractility measurements[18].

Rat neonatal cardiac cells seeded on these elastic substrates with anisotropic orientation exerted higher forces randomly oriented cells[19].

Wang et al. created alignment of cells in 2D by using wrinkled substrates to provide topological cues to human embryonic stem cell derived cardiomyocytes and showed that anisotropy significantly reduced the occurrence of arrhythmia in 2D cultures[20]. In 3D, matrix disarray which is frequently observed in diseased hearts has been mimicked by changing the anchoring points of the tissues. While anisotropic constraints on the tissue induce alignment of both cells and matrix, disarray can be achieved by using isotropic constraints.

Our group has previously used collagen/matrigel microtissues cultured between PDMS microposts as constraints to induce both alignment and disarray of the matrix[21]. We showed that alignment of mouse neonatal cardiomyocytes increased the homogeneity of force distribution in the tissues as compared to the isotropically constrained tissues. Another 3D study using fibrin gels created cardiac tissues in tubular moulds and showed that anisotropy increased the contractile forces of engineered cardiac tissues[22].

Furthermore, anisotropy in 3D network-like patches with rat neonatal cardiomyocytes also improved action potential propagation[23]. Together these studies indicate that an anisotropic

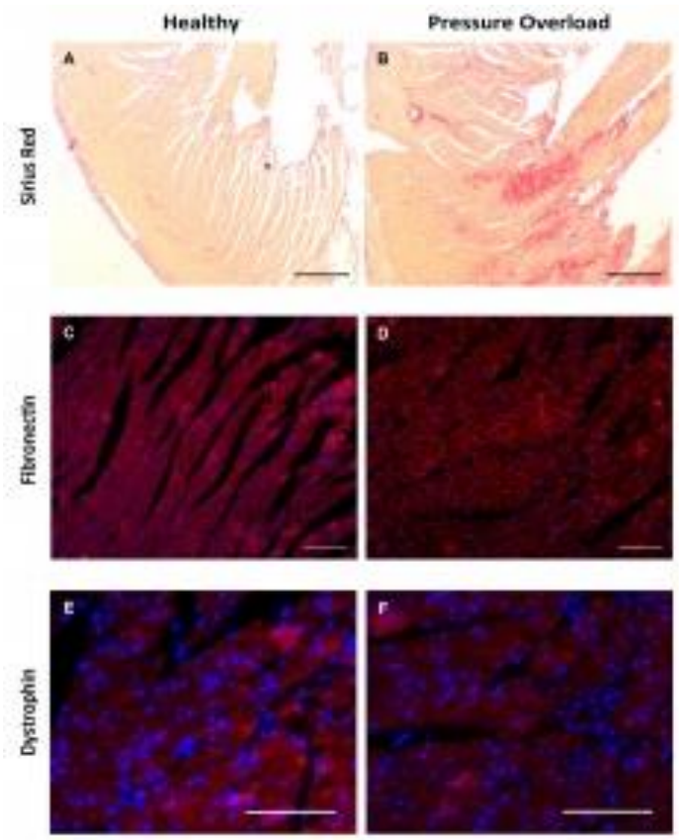

Figure 2: Overview of changes in matrix composition during cardiac disease in mouse hearts due to pressure overload (9 weeks of transverse aortic constriction). A,B) presence of collagen visualized with Sirius Red, shows increase in collagen content in hearts with pressure overload. C,D) Fibronectin is induced in the myocardial matrix during pressure overload. E,F) Cell-matrix interaction via Dystrophin is induced during pressure overload due to increase of Dystrophin. Scale bars: A,B: 500μm, C-F: 100μm.

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matrix structure is essential for proper cardiac tissue function.

Matrix Composition

Besides the structural role of ECM in guiding anisotropy, the complex composition of the cardiac environment plays an important nonstructural role in mediating cell-cell and cell matrix interaction.

The cardiac matrix consist of fibrillar collagens (Type I and III), fibronectin, proteoglycans and basement membrane proteins (laminin and Col IV) which contribute to cell adhesion, cell survival, proliferation, differentiation and function via cell-matrix interactions. Since these interactions are important in regulating stem cell behavior[24], cardiac tissue models have been frequently used to study the effect of different microenvironments on differentiation and maturation of stem cell derived cardiomyocytes or to study stem cell therapy. To mimic stem cell therapy for cardiac regeneration, Valarmathi et al. engineered cardiac tissues using embryonic stem cell derived cardiomyocytes seeded on collagen tubes in co-culture with bone marrow stromal cells[25]. The engineered cardiac environment induced differentiation of the stem cells into cardiomyocyte like cells.

Similarly, Dengler et al. used neonatal rat cardiomyocytes to engineer a cardiac microenvironment which was used to examine the injection of embryonic stem cells and cardiac progenitor cells[26]. After injection into the cardiac tissues, cardiac progenitor cells differentiated into the cardiomyogenic lineage, while the embryonic stem cells did not[26].

These in vitro models of stem cell injection recapitulate some of the in vivo results, but they lack a native like environment and more specifically a diseased environment. Researchers have tried to resolve this issue by the use of decellularised native matrices or matrix produced by cells from either healthy or diseased heart tissue. In a recent study performed by Castaldo et al. cardiac fibroblasts were isolated from healthy or end-stage heart failure cardiac tissues and used to deposit a layer of ECM proteins[27].

Cardiac stem cells were seeded on this Biomatrix in order to assess its differentiation potential. Although both healthy and diseased Biomatrix prevented cells from apoptosis, only the healthy Biomatrix was found to stimulate their proliferation and migration[27]. In another approach by Sullivan et al., decellularized healthy and infarct ECM was used as coating for 2D substrates[28]. These substrates were seeded with mesenchymal stem cells to test the cardiac differentiation potential of the different ECMs. The healthy ECM was shown to promote early cardiac differentiation when compared to infarct ECM[28]. In a 3D approach, native decellularised ECM powder mixed with collagen was used as a hydrogel to engineer cardiac tissues[29].

Hydrogels containing 75% cardiac ECM promoted better differentiation of human embryonic stem cells into cardiomyocytes when compared to a collagen gel without cardiac ECM but with supplemental growth factors.

While these studies show that a more native like ECM composition enhances the differentiation of stem cells into cardiomyocytes, the effect of composition changes on contractility of matured cardiomyocytes is largely unknown. Boudou et al. showed that collagen concentration influences cell contractility[15]. Cardiac microtissues were created using a collagen/ fibrinogen hydrogel, and to examine the influence of matrix composition on contractility, the collagen concentration was varied. Both dynamic and static stresses were significantly lower in tissues with higher collagen concentration[15]. While higher collagen concentration led to denser and stiffer matrices, it is unclear whether these lower stresses are due to changes in composition or stiffness of the matrix.

Future challenges

Current cardiac tissue models to study stem cell therapy mostly try to mimic the healthy cardiac micro-environment, while in vivo these cells will be injected in a diseased environment. Therefore, it would be relevant to mimic the diseased host environment in vitro when studying these processes.

As previously shown, the effect of ECM composition on cardiac tissue function has mainly been studied by using native decellularised matrices, although this decellularised native ECM is hard to control or manipulate. On the contrary, synthetic materials would provide the opportunity to manipulate all ECM components separately.

Unfortunately, currently used synthetic materials fail to mimic the complete native environment. Future improvements of synthetic materials that will enable researchers to mimic of the different component of the ECM will help to extend our knowledge on the effect of diseased matrix composition. Furthermore, analysis of native tissue structure and composition could provide additional input for disease specific cardiac tissue models.

Manipulating either the structure or composition of the microenvironment, may result in a changed matrix stiffness, which in turn will influence cardiomyocyte contractility[15,30,31].

Furthermore, it has been shown that changing only the constraints of the tissue to manipulate the anisotropy resulted in tissues with a different matrix composition after two weeks of culture[22], indicating that all these properties are interacting with each other. Studies that allow for systematic manipulation and quantification of different matrix properties, including matrix structure, composition and stiffness will aid in unravelling the complex dynamic interaction between cardiac cells and their micro-environment.

Conclusion

Both structure and composition of the native cardiac microenvironment provide important cues for normal cardiac function, which change upon development of heart disease. Research regarding the effect of changes in matrix structure and composition on cardiomyocyte functionality has highlighted some key functions of the different matrix properties. While the

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microstructure of the ECM was shown to be important for cardiomyocyte contractility, matrix composition seems to be essential for stem cell differentiation and cardiomyocyte maturation. Therefore, it is likely that regeneration of the heart can only be achieved when restoring both matrix structure and composition.

Improvement in the design of synthetic materials and better knowledge of the in vivo diseased microenvironment will provide input for development of the next generation cardiac tissue models.

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