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PERSPECTIVES

Cardiac Progenitor Cells: The Matrix Has You

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SUMMARY

Components of the cardiac extracellular matrix (ECM) are synthesized by residing cells and are continuously remodeled by them. Conversely, residing cells (including primitive cells) receive constant biochemical and mechanical signals from the ECM that modulate their biology. The pathological progression of heart failure affects all residing cells, inevitably causing profound changes in ECM composition and architecture that, in turn, impact on cell phenotypes. Any regenerative medicine approach must aim at sustaining microenvironment conditions that favor cardiogenic commitment of therapeutic cells and minimize pro-fibrotic signals, while conversely boosting the capacity of therapeutic cells to counteract adverse remodeling of the ECM. In this Perspective article, we discuss multiple issues about the features of an optimal scaffold for supporting cardiac tissue engineering strategies with cardiac progenitor cells, and, conversely, about the possible antifibrotic mechanisms induced by cell therapy. STEM CELLS TRANSLATIONAL MEDICINE *2018;00:000–000*

SIGNIFICANCE

Cardiac tissue is made of multiple cell types and of intercellular substance called extracellular matrix (ECM). These two components influence each other at multiple levels, but this balance is significantly altered in pathological conditions, such as heart failure, where the physiological composition and features of all tissue components are deeply disrupted. Any proposal for novel therapies based on regenerative medicine must consider the restoration of a healthy crosstalk between cells and the ECM, at both biochemical and biomechanical level.

INTRODUCTION

The myocardium, as all mammalian tissues, consists of parenchymal and supporting cells enclosed in a highly complex milieu that is mostly formed by the extracellular matrix (ECM). Just as in a famous sci-fi movie "The Matrix" could affect perception and demeanor, so the ECM has a significant effect on cell behavior. Although it is recognized that the ECM plays a prominent role in cardiac development and in cardiac adaptation to physiological and pathological stimuli [1], the composition of the cardiac ECM has not been comprehensively defined yet. Admittedly, the ECM is an extremely intricate framework in dynamic equilibrium with cells, responding to cellular demand or injury with changes in its composition and architecture [2, 3]. Specifically, components of cardiac ECM are synthesized by residing cells, like fibroblasts, cardiac myocytes, and endothelial cells [4], and are continuously remodeled by the same cells according to ever-changing conditions. Nonetheless, residing cells (including primitive cells) receive constant biochemical and mechanical signals from the ECM that affect cell survival, proliferation, migration, and differentiation [5–7].

The pathological condition of ischemic heart disease affects the survival and activity of cardiac myocytes and other residing cells, inevitably causing profound changes in ECM composition and architecture that, in turn, impact upon cell behavior [8–10]. Cellular therapy has been introduced about two decades ago as a therapeutic strategy to replace the dead myocardium after ischemic injury, and has slowly led to the recognition that the hostile ischemic microenvironment tends to oppose any attempt of succeeding in regenerating the heart [11].

Indeed stem/progenitor cell therapy for heart failure is gradually advancing toward more effective approaches. So-called "first generation therapies," based typically on unselected bone-marrow cells, are gradually being surpassed by "second generation therapies" with cardiogenic cell types, such as resident cardiac progenitor cells (CPCs), and higher repair potency [12]. Multiple protocols and criteria have been proposed to isolate resident CPC populations intrinsically committed to cardiovascular lineages from the adult mammalian heart [13, 14]. Nonetheless, only few among them, with very strong transcriptomic similarity [15], have been successfully applied to human cardiac tissue from advanced heart failure patients, and subjected to phase I/II clinical trials [16].

A key concept for the above-mentioned progress is "combination therapy," referring to either combining: (a) multiple

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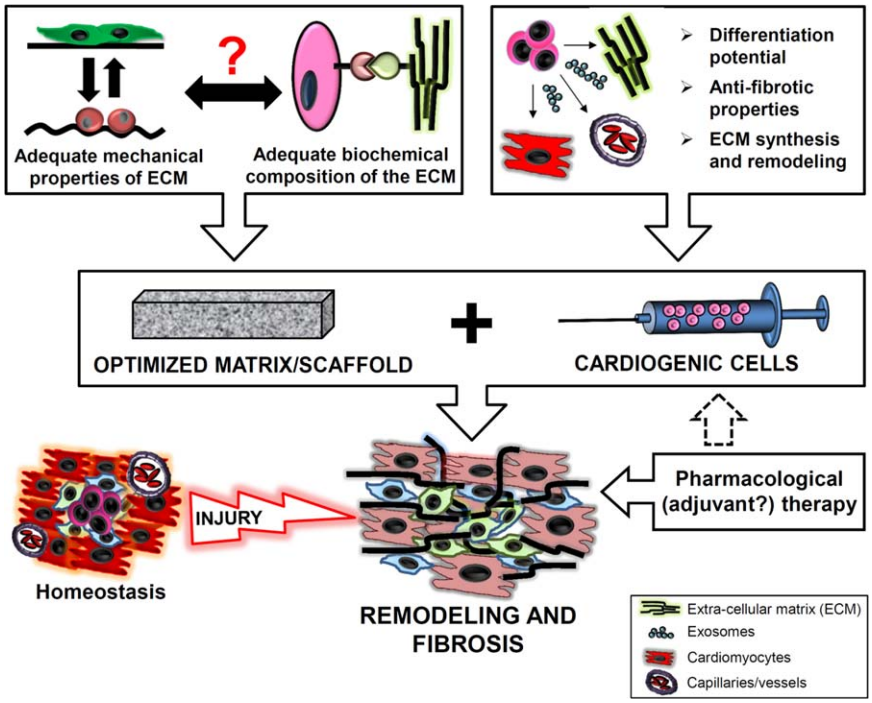

Figure 1. Representative scheme of a multiperspective approach to cardiac regeneration. Cardiogenic regenerative cells are selected not only for their differentiation potential, but also for their ability to affect endogenous remodeling and exert antifibrotic effects. Moreover, an optimized scaffold for cardiac tissue engineering should provide adequate mechanical stimulation and have the right composition to mimic the native ECM, in order to provide regenerative cells with the right cardiogenic microenvironment. Finally, the discovery of previously unknown mechanisms of action on cardiac regenerative cells of standard-of-care pharmacological treatments for heart failure, could introduce adjuvant strategies for cardiac cell therapy. Abbreviation: ECM, extracellular matrix

synergic cell types, (b) cardiogenic cells with an appropriate support for tissue engineering, or (c) cell therapy with optimal tissue conditioning. These concepts highlight the importance of a multifactorial perspective on cardiac regenerative medicine (Fig. 1), where the crosstalk between the host microenvironment and regenerative cell type(s) determines indeed the therapeutic outcome [7, 17]. It is of great importance to create and sustain microenvironment conditions that favor cardiogenic commitment of therapeutic cells and minimize pro-fibrotic or inflammatory signals, while conversely boosting the capacity of therapeutic cells to promote tissue protection and counteract adverse remodeling of the ECM.

Regenerating the Myocardium by the "Right" Matrix

One possible approach to repair the tissue in all its components is to provide an exogenous matrix as an ideal substrate and support for cell transplantation. Several biomaterials, either natural or synthetic, have been used as ECM substitutes thus far [18–20]. Undoubtedly, collagen, gelatin, alginate, and fibrin are the most used among natural polymers, as they are naturally charged with numerous cell binding sites while ensuring high biocompatibility and biodegradability. Nonetheless, they lack critical mechanical properties, such as strength, stiffness, or elasticity. On the contrary, synthetic polymers, like polyglycolic acid, polylactic acid, and the relatively new polyglycerol sebacate, are tunable in their physical properties, but poor in biological activity, even though they can be loaded with biochemical signals in the form of ECM protein fragments [21, 22]. Several cell seeded scaffolds of natural and/or synthetic biomaterials have been evaluated in vivo and their outcomes have been recently reviewed [23], leading to the conclusion that, although groundbreaking advances were made through tissue engineering, the optimal cardiac scaffold capable of meeting the requirements critical to support functional improvement of the failing heart still represents a demanding challenge. Indeed, the spatial organization of structural ECM components at its nanoscale and microscale, and the biochemical complexity of the ECM cannot be fully recapitulated by synthetic scaffolds, due to the still nebulous knowledge about physical properties and exact composition of cardiac ECM, as well as to the limits of currently available technologies.

Since only the native ECM itself could deliver the ideal mechanical and biological properties to therapeutic cells, several studies have investigated the possibility of engineering the myocardium for regenerative medicine purposes by combining decellularized cardiac ECM with stem/progenitor cells [24–26], speculating that the intrinsically perfect combination of mechanical and biochemical properties of native cardiac ECM may control and ensure cell engraftment while driving stem/progenitor cell fate. Despite the synergistic effect of all ECM components, a direct supporting mechanism has been already reported for some ECM molecules like fibronectin, proven to be necessary for endogenous CPC proliferation and activation for repair through focal adhesion signaling [27]. The ECM's influence on CPCs, though, may act at multiple levels: we have previously reported that human CPCs cultured short-term on cardiac fibroblast-derived ECM substrates from failing hearts release less trophic and anti-remodeling paracrine factors, such as tissue inhibitor of metalloproteinases 2 (TIMP2), compared to substrates from fibroblasts from healthy hearts [28]. Moreover, multiple studies have investigated several combinations of ECM

protein substrates (e.g., fibronectin, laminin, collagen) with variable mechanical stimuli (e.g., cyclic strain), suggesting that mechanical and biological signals can compensate and modulate each other in different proportions [29–32], making it very difficult to design an artificial matrix from scratch.

Therefore, the native cardiac ECM, as a whole, is by definition the ideal biomaterial for cardiac tissue engineering, but the shortage of donors, and the obvious priority to heart transplantation procedures, makes it difficult to obtain and prepare scaffolds of decellularized cardiac ECM. Although a prompt solution might be offered by xenografts of porcine cardiac ECM [33], whose structural and biomechanical properties have been also analyzed [34], comparative studies of porcine and human cardiac environment are urgently needed to comprehensively evaluate the feasibility of xenotransplantation. Additionally, a recent study provided substantiating evidence that xenogenic decellularized cardiovascular biomaterials elicit human immune response [35]. On this basis, finding an easily accessible alternative biological scaffold, capable of safely delivering biochemical cues and mechanical properties that are, at least partially, shared by the myocardium, is currently a top priority in cardiac tissue engineering (Fig. 1).

Regeneration Against Fibrosis

Even if locally providing optimal exogenous support to therapeutic stem/progenitor cells, the complex pathological process of cardiac remodeling and fibrosis in heart failure [10] may still hamper the overall efficacy of any regenerative strategy, due to detrimental cell-cell and cell-ECM crosstalk. Currently, there is still shortage of specific antifibrosis and anti-remodeling targeted therapies for heart failure. Although angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers, which are among the elective drugs for heart failure treatment, can indeed act directly on remodeling by reducing the detrimental effects of angiotensin II on cardiac fibroblasts activity [36], the discovery of novel targeted strategies would undoubtedly improve therapeutic efficacy. Multiple molecules are under clinical investigation, but many trials on antifibrotic drugs have been discouraging [37]. Interestingly, a majority of preclinical studies of cardiac cell therapy have evidenced how one of its main effects is the reduction of tissue fibrosis and remodeling, suggesting that a biological cell-based therapy may provide per se an effective antifibrotic strategy [38].

In fact, multiple studies have shown how transplanted therapeutic cells can oppose remodeling of the endogenous ECM, mediating a temporally and spatially regulated release of beneficial factors, and yielding reduced collagen deposition, increased collagen degradation, and matrix metallopeptidases (MMPs) levels [39]. Direct benefits on ECM remodeling have been reported with resident CPCs, which exert multiple basic beneficial effects in animal models of cardiac cell therapy [40]. In fact, CPC exosomes can paracrinally prime fibroblasts toward a cardioprotective and pro-angiogenic phenotype, simultaneously reducing adverse remodeling [41, 42] and fibroblast proliferation [43]. The pathways responsible for these mechanisms are largely unknown, but it has been reported that CPCs release many paracrine factors, including modulators of ECM remodeling (e.g., TIMPs) [44] and endoglin, which can inhibit TGF-β1/Smad signaling in fibroblasts [45].

Therefore, cell therapy can significantly act as an antifibrotic treatment for the heart, simultaneously helping to unravel key pathways for specific antifibrotic outcomes.

Moreover, under the above-mentioned perspective of combined therapies, a biological regenerative approach could be synergistically merged with existing standard care treatments that act on multiple pathways (Fig. 1). As an example, it has been suggested that beta-blockers (BBs) could represent an adjuvant therapy for cardiac regenerative protocols. BBs are an elective medicine for heart failure [46], and act at multiple levels [47], also indirectly affecting cardiac fibroblasts activation [48]. It has been reported that BBs can enhance mesenchymal stem cell therapy for myocardial infarction by increasing cell survival [49]. Moreover, they have been shown to sustain CPC viability [50] and their cardiogenic phenotype in human resident CPCs, while reducing their pro-fibrotic features, such as *collagen I*, *miR-21*, and *miR-29* expression levels [51]. Interestingly, BBs have been also associated to successful cardiac recovery, or "reverse remodeling," during left ventricular assist device (LVAD) support, that is near-normalization of the multiple myocardial abnormalities in advanced heart failure patients after mechanical unloading through LVAD implantation [52]. In fact, patients receiving BBs experience more frequently cardiac recovery [53], suggesting an intriguing mechanistic connection between reversing cardiac tissue adverse remodeling, and promoting antifibrotic and cardiogenic features of resident regenerative cells [54].

Conclusion

Tissue regeneration involves replenishment of lost parenchymal and stromal cells, and also ECM restoration to physiological composition, architecture, and biomechanical features. Optimal outcomes ideally require a productive crosstalk between cells and the ECM to boost the regenerative potency of progenitor cells while simultaneously providing the right ECM scaffold and minimizing fibrosis. First generation therapies for heart failure were based on the utopian expectation that a single injection of noncardiac primitive cells would be enough for effective cardiac regeneration. Almost two decades of preclinical and clinical research are now suggesting that a complex result requires a complex strategy, such as integrating the many aspects necessary for completely rebuilding tissue structure and composition. "The Matrix is everywhere," and every cell "develops a natural equilibrium with the surrounding environment" (freely edited from: Wachowski Lana and Andy, "The Matrix" motion picture, Warner Bros 1999), but the "right" cell on the "wrong" substrate may not behave as desired.

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The authors indicated no potential conflicts of interest.

Disclaimers

Authors have nothing to disclose.

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