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REVIEW PAPER

Predifferentiated Adult Stem Cells and Matrices for Cardiac Cell Therapy

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ABSTRACT

Stem cell therapy is a major field of research worldwide, with increasing clinical application, especially in cardiovascular pathology. However, the best stem cell source and type with optimal safety for functional engraftment remains unclear. An intermediate cardiac precommitted phenotype expressing some of the key proteins of a mature cardiomyocyte would permit better integration into the cardiac environment. The predifferentiated cells would receive signals from the environment, thus achieving gradual and complete differentiation. In cell transplantation, survival and engraftment within the environment of the ischemic myocardium represents a challenge for all types of cells, regardless of their state of differentiation. An alternative strategy is to embed cells in a 3-dimensional structure replicating the extracellular matrix, which is crucial for full tissue restoration and prevention of ventricular remodeling. The clinical translation of cell therapy requires avoidance of potentially harmful drugs and cytokines, and rapid off-the-shelf availability of cells. The combination of predifferentiated cells with a functionalized scaffold, locally releasing molecules tailored to promote in-situ completion of differentiation and improve homing, survival, and function, could be an exciting approach that might circumvent the potential undesired effects of growth factor administration and improve tissue restoration.

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KEYWORDS: Adult Stem Cells, Cell Transplantation, Heart Failure, Myocardial Infarction, Tissue Engineering

INTRODUCTION

Despite ongoing debate regarding the actual endogenous regenerative potential of human myocardium, progenitor cells or stem cells constitute a new approach to the treatment of cardiac disease. Several preclinical and clinical trials have shown exciting results, but the mechanism underlying cardiac improvement after stem cell injection is still not understood. True in-situ cardiac transdifferentiation has not yet been demonstrated, and cell viability and engrafting after administration have been found to be low, weakening the hypothesis of real tissue replacement or regeneration. Recent evidence also considers the paracrine effects exerted by the injected cells as playing a pivotal role in the improvement of cardiac function.[1,2] Moreover, issues concerning the best stem cell source and type for safe and harmonious

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functional engraftment with adequate electromechanical integration remain unresolved. A number of factors related to the patient's systemic condition and the microenvironment of the injection site hamper effective integration and differentiation of stem cells within the heart. Inducing partial differentiation towards a cardiac precommitted phenotype that expresses some of the key proteins of a mature cardiomyocyte could provide better integration into the cardiac environment. The predifferentiated cells would receive signals from the new environment, thus achieving gradual and complete differentiation. Our group developed a cytokine-free system to obtain stem and non-stem cell predifferentiation.[3,4] Furthermore, the combination of stem cells or predifferentiated stem cells with biomaterials organized into 3-dimensional structures replicating the extracellular matrix might obtain full tissue restoration and prevent ventricular remodeling. Biocompatible materials could be further functionalized with different growth factors to guide tissue regeneration and promote in-situ completion of the differentiation of precommitted progenitor cells.

PREDIFFERENTIATED STEM CELLS

Recently, research efforts on stem cell characterization and application in cardiology have exploded.[5,6] Cellular cardiomyoplasty is a stem cell-based regenerative or reparative therapy with the goal of limiting the consequences of myocardial infarction or non-ischemic cardiomyopathy. One unanswered question is how differentiated should the stem cells be for application in a sick heart. An intermediate option might result in better cell integration, higher environmental dependence, improved survival, and more efficient functional restitution. The predifferentiation process could let cells express in-vitro cardiac proteins that are the phenotypic expression of genetic changes toward the final cardiomyocyte stage. After injection, the precommitted cells would receive signals from the new environment, thus achieving gradual and complete differentiation. Avoiding signals associated with cell transformation, as many cytokines are known to be, a safer and ''clean'' predifferentiation model might be defined.[3,4] Stem cell predifferentiation results in several morphological changes including multinucleated cell formation and cytoskeleton rearrangement. The expression of cardiac proteins related to cell contraction and intercellular communication is also detected early. Therefore, cardiomyocyte predifferentiation of stem cells is a potentially powerful tool to obtain cells with oriented evolutive capacity and still able to respond to environmental signals. These cells exhibit phenotypic and metabolic changes that could facilitate their homing, adaptation, survival, replication, and function, with consequently improved therapeutic effects. Such predifferentiated stem cells could be useful in the generation of in-vitro and in-vivo tissue or organ substitutes by tissue engineering. This approach might be even more effective if it employed functionalized scaffolds that release molecules contributing to the completion of the predifferentiation process.

PREDIFFERENTIATED STEM CELLS: WHICH ONES?

The concept of ''stemness'' includes 2 main characteristics: the capability of self-renewal, and the potential to differentiate into mature cell types. Independent of the embryologic and developmental stages, stem cells can be distinguished by their age and differentiation potential. As the differentiation process progresses, cells lose evolutive capacity. It can be assumed that cells displaying major evolutive capacity will be more easily predifferentiated toward a defined phenotype.

EMBRYONIC STEM CELLS

Embryonic stem cells (ESC) derive from the morula stage or the epiblast tissue of the inner cell mass of blastocysts. They are characterized by totipotency or pluripotency, being able to give rise to all derivatives of the 3 primary germ layers, and thus to almost every mature cell type. Despite the enormous differentiation potency of ESC, the risk of tumorigenesis if the wrong differentiation pathway is taken, immunogenicity, and animal-derived pathogen transmission, limit their clinical translatability. At this point, despite their usefulness and potential benefits, ESC need to overcome numerous barriers before reaching the patient's bedside. Until now, human ESC application has seemed too complicated to be considered for general application.

ADULT STEM CELLS

Adult stem cells are lineage-restricted (multipotent) and classified as limited to differentiating into only a few types of mature cell. Down in the differentiation potential scale, progenitor or precursor cells are considered capable of differentiating into only one specific mature cell type. Adult multipotent or progenitor cells can be isolated from a number of different sources and tissues. Recent insights highlight the possibility of finding a particular stem cell niche in almost all human organs. However, bone marrow, blood, umbilical cord, adipose tissue, muscle, heart, and skin have been considered more useful for stem cell isolation.[7] In both the experimental and clinical fields, some beneficial effects of adult stem cell application have been reported already. In some cases, such as hematology, the use of adult stem cells has already had a definite impact on the treatment of disease. Beyond the application of hematopoietic stem cells in oncology, preliminary data have shown potential therapeutic effects of myoblasts in

cardiology, and fat-derived stem cells in plastic surgery.[8] A less conclusive but even more hopeful scenario is the use of mesenchymal stem cells in cardiac regeneration. Although the mechanisms underlying their effects have not been elucidated, adult stem cells could be beneficial in many pathological conditions, and they have been reported to improve patients' conditions.

Bone Marrow-Derived Mesenchymal Stem Cells

The bone marrow contains mesenchymal stem cells, multipotent cells, with proven ability to differentiate into osteoblasts, chondrocytes, and adipocytes, as well as cardiomyocytes under specific conditions in vitro.[9,10] Moreover, mesenchymal stem cells have elicited substantial attention because they can readily be made available for autologous application (by bone marrow aspiration), isolated, and expanded ex vivo, and they exhibit high plasticity. These cells have fueled an increasing amount of research and are an attractive therapeutic tool.

Adipocyte-Derived Stem Cells

Mesenchymal stromal cells are present in adipose tissue, in a perivascular niche. Adipocyte-derived stem cells can be easily isolated and cultured ex vivo, and express markers associated with mesenchymal and perivascular cells, including STRO-1, CD146, and 3G5, maintaining their characteristic multipotency, as shown by their capacity to form Alizarin Red-positive mineralized deposits, Oil Red O-positive lipid droplets, and Alcian Blue-positive proteoglycan-rich matrices in vitro. They also possess plasticity, under appropriate in-vitro culture conditions, to adopt cardiomyocyte and vascular cell phenotypic characteristics, and they have been shown in in-vivo preclinical studies to facilitate both myocardial repair and neovascularization.[11] This beneficial effect appears to be related to an indirect paracrine action rather than direct regeneration of endogenous cells by transdifferentiation, especially because current transplantation strategies achieve a quantitatively modest engraftment of cells into the host myocardium.[12]

Muscle Stem Cells

Mouse skeletal muscle has been found to contain a population of non-satellite cells that can differentiate into spontaneously beating cells with cardiomyocyte features.[13] Interestingly, the discovery of these cells arose from a few simple variations in the usual technique of skeletal muscle cell culture. It has been suggested that these cells are closer to true cardiomyocytes. Efficient homing to the heart in a model of acute ischemic injury, and the subsequent in-vivo differentiation into a cardiac phenotype at the border of an old infarct, predict possible further experimentation focusing on chronic heart failure or dilated cardiomyopathy.

Myocardial Stem Cells

Mammalian myocardium includes a small proportion of stem cells that express the cell-surface markers Kit or Sca-1.[14,15] These cells are unable to repair the myocardium after ischemia, but they can be isolated from human myocardial samples obtained using a minimally invasive procedure. These cells can be further expanded to an adequate number in a complex culture medium, with minimal risks of immune rejection or teratoma formation. However, no clinical data on the use of these cells are available yet.

Induced Pluripotent Stem Cells

Another recent strategy to overcome the restricted differentiation potential of adult stem cells is genetic reprogramming by incorporating an immaturity genome (e.g. from oocytes). The concept of nuclear transfer and cellular cloning has been applied in rodents, and may result in autologous pluripotent cell lines. Byrne and colleagues[16–18] were able to reprogram adult mammalian skin cells by nuclear transfer, obtaining a pluripotent cell line, and the transduction of only 4 defined transcription factors (Oct3/4, Sox2, Klf4, c-Myc) could induce pluripotency in an adult human dermal fibroblast cell line. These approaches might have value in future research in the cardiovascular field, despite being hampered by economic and technical issues relative to the need for expertise and genomic facilities, and the above-mentioned problems of controlling the final differentiation of pluripotent cells.

Stem Cells: A Translational Approach

To date, there have been 3 suitable physiological sources of stem cells identified following myocardial ischemia: the damaged myocardium itself, the peripheral circulation, and the bone marrow. Consequently, a wide variety of cell types derived from these tissues have been considered for therapeutic transplantation into humans. Bone marrow mononuclear fraction, hematopoietic and mesenchymal stem cells, cardiac stem cells, endothelial progenitor cells, adipose-derived stem cells plus embryonic stem cells, and skeletal myoblasts have been successfully employed in clinical trials.[19] A comprehensive index of clinical trials is available from the American Library of Medicine at http://www.the-scientist.com/supplementary/html/24104/.

The first clinical trials were performed using skeletal myoblasts, with encouraging results in terms of increases in regional wall motion and left ventricular ejection fraction.[20] Myoblasts are resistant to ischemia

and show ability to improve ventricular function in animal models, differentiating into myotubes in vivo. Unfortunately, a true cardiomyocyte phenotype could not be achieved, and myotubes do not integrate electrically with surviving cardiomyocytes, failing to contract synchronously with the surrounding myocardium. Several human trials using myoblasts in heart failure are ongoing, but some have been terminated because of lack of efficacy or recurrent malignant arrhythmias.[21]

Other progenitor cells have been assessed as therapeutic agents in conditions such as myocardial infarction or heart failure. Recent studies demonstrated that intracoronary infusion of bone marrow mesenchymal stem cells induces significant improvement in myocardial performance, with the most obvious benefits in patients who received the intervention more than 5 days after myocardial infarction. In addition to these positive findings, a number of additional clinical trials have yielded controversial results.[22] Although cell therapy has been suggested as an effective strategy to improve the performance of human myocardium, these results are not consistent across the literature, and the lack of confirmatory results from the most recent clinical trials puts on hold the initial enthusiasm regarding the clinical use of cell therapy in heart regeneration, questioning its actual effectiveness and safety.[23–25] The wide range of delivery techniques and different methodological approaches used by various research groups make the results of these studies difficult to interpret. There is an obvious need for methodological consensus or the creation of technical guidelines to draw reliable conclusions regarding the usefulness of cell therapy in human heart regeneration. Despite the myriad of cell types in several experimental studies, the ideal cell type has not yet emerged, and few studies have directly compared different stem cell types.[10] Phenomena related to cell plasticity or fusion have been claimed to be at the root of the stem cell contribution to improvement in cardiac function, but an insufficiently consistent amount of data supports this concept.

The actual beneficial effect of stem cell therapy could rely on a paracrine effect whereby growth factors, cytokines, and signaling molecules are produced and released by the infused stem cells. Considering the pharmacological potential of stem cells as a cytokine delivery system, it is also important to evaluate the characteristics and different clinical indications for each type of cardiac disease in which cellular therapy might be applied. Stem cells have been used in various clinical settings in largely nonuniform populations of patients.[26] This lack of standardization affects even the route of delivery, which poses another question regarding maximum survival and retention in such a specific area of the myocardium as the infraction zone. Sufficient collateral perfusion in the ischemic area is crucial for the survival of a high proportion of cells. Timing from the ischemic event to injection determines the selection of the delivery approach. Transvascular (intracoronary or intravenous) and direct injection into the ventricular wall (transepicardial, transendocardial, or trans-coronary-vein infusion) have been proposed. For intracoronary infusion, a high concentration of stem cells is delivered through the central lumen of an over-the-wire balloon catheter, allowing the cells to travel directly into myocardial regions in which blood, nutrient, and oxygen supply are preserved. However, occluded coronary arteries in the ischemic area prevent progenitor cells migrating out of the vessels into the perivascular heart tissue. Intravenous infusion is hampered by issues of nonspecific and noncardiac homing, which dramatically reduce the amount of cells engrafted and their therapeutic effectiveness.

Systems based on direct injection into the ventricular wall during open heart surgery allow direct visualization of the myocardium and superior engraftment, but require associated revascularization procedures to avoid grafting in areas of poorly perfused scarred myocardium, resulting in the formation of islands of cells with minimal perfusion and poor survival.[27] It is also possible to perform a trans-coronary-vein injection that delivers stem cells parallel to the ventricular wall through veins deeply within the myocardium. However, this system presents drawbacks related to the complexity of the injection technique and to the catheter system required.[28] Another approach involves transendocardial injection by means of a needle catheter advanced across the aortic valve and stabilized against the endocardial surface. Stem cells can be injected into viable regions of the myocardium detected by electrophysiological mapping of the heart.[29]

Stem Cell Therapy: Many Answers, Many Questions

Despite the enormous number of experimental efforts reporting positive results in terms of myocardial function and improvement in different pathological conditions, there has been no conclusive demonstration of the mechanism involved in tissue regeneration associated with adult stem cell treatment. Many different properties and presumed actions have been hypothesized, such as immunomodulation, paracrine activity, or the capacity to differentiate or transdifferentiate, but their role in the reported beneficial effects has not been clarified. No insights have been provided to dampen the dramatic loss of cells immediately after implantation. Trauma related to handling and the injection itself, together with the negative effects of the hostile environment in which the implant takes place, are most likely at the root of

this phenomenon. No precise data are available on the number of cells viable after injection and effectively homing to the affected site. These unanswered questions raise concerns about the actual effects of these cells, especially when an attempt is made to match the biological uncertainties with the evident improvement in cardiac function. Undoubtedly, obstacles to translating basic research into clinical use and the need to perform meticulous clinical trials are still at the forefront. Many issues have prevented the generation of useful and reliable data that could facilitate a potential clinical translation. In spite of its feasibility and attractiveness, cell therapy requires high quality and coordinated scientific, clinical, and technical efforts to validate the technique.

To date, no practical guidelines or scientific consensus regarding the technical aspects of the application of cell therapy in patients have emerged. The widespread application of this promising therapeutic tool is also hindered by some remaining clinical and biological questions. Patient selection, contraindications, timing of treatment, effective degree of benefit, safety, cell type, cell dosage, cell engraftment, and immunogenicity are some of the issues that need to be addressed. Understanding the mechanisms of normal and post-injury cardiomyocyte development and turnover will be crucial in guiding the direction of stem cell-based therapies. Evidently, cardiac regeneration requires a complex cascade of events beyond simple injection of the right type of cell into the right place. Thus characterization of the factors present in the hostile microenvironment of the injured myocardium, which hamper the survival and functional integration of transplanted cells, is also essential. Undoubtedly, along with the biology of stem cells, the actual effectiveness of therapy and safety in patients require to be further defined. Clearly, these issues of the best stem cell source and type, their harmonious and functional engraftment, and their safety, pose an even more paradoxical and provocative question: do we really need a stem undifferentiated phenotype? On the other hand, would a totally differentiated phenotype, the beating cell, achieve the necessary electromechanical integration to survive without generating ectopic autonomous contractile foci?

An intermediate partial cardiac precommitted phenotype, expressing some of the key proteins of a cardiomyocyte, might provide better integration into the cardiac environment. The predifferentiated cells would receive signals from the new environment, thus achieving gradual and complete differentiation. Expression of cardiac-specific gap junction-related proteins (such as connexin43) or fibrillar structural proteins (such as desmin or troponin) could potentially answer issues concerning defective electromechanical integration leading to arrhythmias. Avoidance of the potentially harmful drugs and cytokines involved in cell transformation and a rapid off-the-shelf availability of cells with reduced culture time are required. Recent advances demonstrated the role of cytokine-free biophysical stimulation systems in inducting cell differentiation.[30,31] Our group described a method to induce cardiac predifferentiation in both stem and non-stem cells by controlled delivery of electrical stimuli.[3,4] The basis of this approach was the observation that electrostimulation prevents immobilization-induced muscle atrophy. Mimicking physiological stimulation conditions provides the correct sequence of signals to maintain and improve muscle structure and function. Simulation of the cardiac cell environment via pacing and long-term electrostimulation mimics the electrical features of a beating heart, and could provide an additional stimulus to direct the cellular apparatus of stem cells toward a cardiomyocyte-type configuration. Interestingly, with this method, we described changes in the cell architecture of fibroblasts, the most abundant cell type in a myocardial scar, resulting in the formation of multinucleated myotubes. Thinking in terms of cell therapy, if these myotubes can be made to contract, systolic function could be improved. If they do not contract, as perhaps occurs with most of the cells injected into the myocardium, a passive cardiac improvement would still be expected through increased compliance and hence diastolic function of the infarcted myocardium.[4] We were able to reproduce similar results by applying this method of stimulation to both embryonic and adult cell lines, suggesting a non-pluripotential cell-restricted phenomenon.

The possibility of inducing a reorganization of the cell biosynthesis apparatus after a short period of stimulation allowed us to speculate on the early activation of mechanisms probably related to membrane transport modifications. Ion channel aperture timing could possibly be affected by electrostimulation, leading to temporary changes in intracellular pH with further nuclear transcriptional enzyme activation.[4] Interestingly, this system of predifferentiation could be applied to stem cell lines such as mesenchymal stem cells.[3] We further investigated potential mechanisms underlying the effect of electrostimulation, indicating growth factor and molecular pathways potentially accounting for this cardiomyogenic predifferentiation. We focused on an important member of the transforming growth factor-beta superfamily, follistatin (FST), a muscle differentiation regulator and a potential candidate for this process in stem cells.[32] FST is implicated in many biologic processes such as cell proliferation and differentiation, immune responses, various endocrine activities, wound repair, inflammation, and fibrosis. FST is considered to be a key modulator in muscle development, differentiation, and

regeneration, and it has been implicated in the repair of mesodermal- and endodermal-derived tissues, promoting cell proliferation and hampering fibrogenesis. The mechanism of action of FST concerns neutralization of activins. Those molecules are increased in the serum of heart failure patients and in cardiomyocytes after experimental myocardial infarction, suggesting the involvement of activins in heart failure pathogenesis. We could induce the production of FST by stem cells predifferentiated towards the cardiomyogenic phenotype by electrostimulation.[32] Considering the role of FST in the pathogenesis of heart failure, the possibility of producing a precommitted phenotype actively secreting a key regulator of mesodermal differentiation has potentially relevant therapeutic clinical applications.

TISSUE-ENGINEERED PREDIFFERENTIATED CELLS

Survival in the inflammatory environment of the infarcted myocardium presents a challenge common to all types of cells, regardless their differentiation state. Fibrotic tissue in the ischemic scar also limits cell engraftment. Survival and integration of transplanted cells can be improved by embedding them in a matrix such as collagen.[33] The concept at the root of tissue engineering is to generate a functional 3-dimensional environment suitable for cell culture to produce tissues in vitro, which are tailored in size, shape, and function, before implanting them into the body, or alternatively, using them directly at the site of damage. Several approaches have been developed and recent advances have been made in the engineering of cardiac tissue.[34] Biosynthetic and biodegradable materials have largely been investigated. A wide range of natural materials (collagen, fibrin, hyaluronic acid) and approaches involving tissue decellularization have been evaluated (Table 1).[35–53] A 3-dimensional collagen type I matrix, seeded with human umbilical cord blood mononuclear cells and grafted onto infarcted ventricles in an ischemic murine heart model, showed beneficial effects on left ventricular function and myocardial remodeling.[33] Recently, a similar approach was used successfully in a clinical trial (MAGNUM trial) with a bone marrow mesenchymal stem cell-seeded collagen sponge applied on top of the scarred area, with the aim of regenerating myocardial cells and restoring extracellular matrix function which is deeply altered following myocardial infarction.[54,55] The MAGNUM trial is an example of the possible association between stem cell therapy and tissue engineering.

Table 1. **Preclinical studies of cardiac tissue engineering**

Study	Material	Approach	Cell Type	Species	Effect
Christman[35] (2004)	Fibrin	In-situ tissue engineering	SMB	Rat	Infarct size↓ Angiogenesis
Ryu[36] (2005)	Fibrin	In-situ tissue engineering	BMMC	Rat	Angiogenesis
Chekanov[37] (2003)	Fibrin	In-situ tissue engineering	EC	Sheep	Angiogenesis
Thompson[28] (2003)	Collagen type I	In-situ tissue engineering	BMMSC	Swine	EF preserved
Zimmermann[38] (2006)	Collagen type I	In-vitro tissue engineering	Neonatal CM	Rat	EF↑, FS↑ (systolic + diastolic)
Kofidis[39] (2005)	Collagen type I	In-vitro tissue engineering	ESC	Rat	EF↑, FS↑, thinning↓
Kofidis[40,41] (2004/5)	Matrigel	In-situ tissue engineering	ESC	Rat	EF↑, FS↑
Zhang[42] (2006)	Matrigel + collagen	In-situ tissue engineering	Neonatal CM	Rat	EF↑, FS↑
Zimmermann[43] (2002)	Matrigel + collagen	In-vitro tissue engineering	Neonatal CM	Rat	EF preserved
Leor[44] (2000)	Gelatin	In-vitro tissue engineering	Fetal CM	Rat	Angiogenesis LV dilatation↓
Kellar[45] (2005)	Alginate	In-vitro tissue engineering	HDF	Mice	EF↑, FS↑
Krupnick[46] (2002)	PTFE + PLLA + collagen	In-vitro tissue engineering	BMMSC	Rat	EF↑, FS↑ (systolic + diastolic)
Siepe[47] (2006)	Polyurethane	In-vitro tissue engineering	SMB	Rat	EF preserved
Miyahara[48] (2006)	PNIPAAM	In-vitro tissue engineering	BMMSC	Rat	EF↑, FS↑, thinning↓
Davis[49] (2005)	Self-assembling peptides	In-situ tissue engineering	Neonatal CM	Mice	Angiogenesis
Dubois[50] (2008)	Self-assembling peptides	In-situ tissue engineering	SMB	Rat	No improvement
Matsubayashi[51] (2003)	PCLA	In-vitro tissue engineering	SMC	Rat	EF↑, FS↑

BMMC = bone marrow-derived mononuclear cells, BMMSC = bone marrow mesenchymal stem cells, CM = neonatal cariomyocytes, EC = endothelial cells, EF = ejection fraction, ESC = embryonic stem cells, FS = fractional shortening, HDF = human dermal fibroblasts, LV = left ventricular, PCLA = polymer of epsilon-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide, PLLA = poly(L-lactic) acid, PNIPAAM = poly(N-isopropylacrylamide), PTFE = polytetrafluoroethylene, SMB = skeletal myoblasts, SMC = smooth muscle cells.

Polymeric biomaterials have also been engrafted with growth factors, cytokines, and drugs, thus obtaining drug-releasing systems capable of focused and localized delivery of molecules, depending on the environmental requirements and the actual milieu in which the scaffold is placed.[34] Several techniques have been used for the fabrication of scaffolds for tissue engineering; the electrospinning technique is emerging as one of the most effective and functional approaches, with the possibility of combining with the newest techniques of cell seeding.[56] The electrospinning process allows control of the morphology of the fibers and utilization of a wide variety of polymers. The small fiber diameters produced by electrospinning have the advantage of a large surface-to-volume ratio, as well as high permeability and an interconnecting pore structure, both of which are desirable in a biological setting. Furthermore, this material can be functionalized with compounds that encourage cell survival, proliferation, and differentiation, to obtain a drug delivery system.

We explored the use of tissue engineering for enhancement of cell retention and proliferation purposes. We developed a 3-dimensional extracellular-matrix-like differentiating device containing the adequate signals locally compartmentalized inside the scaffold to promote differentiation of stem cells in an environment closely resembling the natural architecture of tissues. Previously, we developed a hydroxyapatite functionalized scaffold with the aim of reproducing the native histoarchitecture and molecular signaling of osteochondral tissue to facilitate cell differentiation towards chondrocytes. Poly-L-lactic/hydroxyapatite nanocomposites induced differentiation of human mesenchymal stem cells in a chondrocyte-like phenotype, with the generation of a proteoglycan-based matrix.[57] We further developed a scaffold tailored to cardiovascular structures. Cells seeded on this scaffold are not only exposed to growth factors promoting their differentiation but also encounter pre-constituted environments that differentially guide their commitment towards the phenotypes characterizing cardiovascular tissues.[58]

For myocardial repair, in-situ tissue engineering has been suggested as a potentially effective candidate; it involves injection into the infarcted wall of a mixture of biomaterials and cells.[59] As mentioned above, the electrospinning method is an attractive way of producing scaffolds that remarkably mimic the size and scale of the natural extracellular matrix. It enables the fabrication of structures with sub-micron pores and nano-topography, which is reported to be optimal for the realization of successful extracellular-matrix-like scaffolds for cardiac tissue engineering.[60]

Although tissue engineering has been shown to be a promising tool for cardiac repair, a number of technical issues need to be answered. The mechanical resistance of the constructs and their immunogenicity are key factors that could hamper effectiveness and delay the actual clinical translation.[61] In addition, production of a cardiac graft as well as a tissue-engineered vascular graft faces hurdles related to availability and accessibility of autologous adult differentiated cells to populate the underlying biomaterial. The possibility of combining the regenerative capacity of stem cells with the potential of polymeric scaffolds as a ventricular mechanical support, as well as a cell retaining and delivery system, has been explored. Recently, poly-co-caprolactone seeded with bone marrow-derived mononuclear cells has been used as a cardiac patch in rat myocardial infarction, achieving a reduction in left ventricular remodeling and preservation of systolic function.[38,62]

Considering the current limitations of both cell therapy and tissue engineering, the most amenable alternative could be a combination of the 2 approaches. The idea of a bridge between stem cell plasticity and the biomaterials that actively guide and provide the correct sequence of signals to allow ongoing lineage-specific differentiation of these pluripotent precursor cells is attractive, and it may represent a promising answer to the problems related to cardiovascular healing. Importantly, controlled delivery systems may provide an alternative means of releasing localized doses of bioactive molecules over sustained periods, minimizing undesired systemic effects, defining a favorable environment for cell engraftment and proliferation, and improving the post-implantation management of the grafts. The combination of predifferentiated cells with a functionalized scaffold locally releasing molecules tailored to promote in-situ completion of differentiation and improve homing, survival, and function, could be an exciting approach circumventing the risk of potential undesired effects of growth factor administration, and improving tissue restoration.

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REFERENCES

1. Li L, Zhang S, Zhang Y, Yu B, Xu Y, Guan Z. Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. Mol Biol Rep 2009;36:725–31.
2. Dai W, Hale SL, Kloner RA. Role of a paracrine action of mesenchymal stem cells in the improvement of left ventricular function after coronary artery occlusion in rats. Regen Med 2007;2:63–8.
3. Genovese JA, Spadaccio C, Chachques E, Schussler O, Carpentier A, Chachques JC, et al. Cardiac pre-differentiation of human mesenchymal stem cells by electrostimulation. Front Biosci 2009;14:2996–3002.

4. Genovese JA, Spadaccio C, Langer J, Habe J, Jackson J, Patel AN. Electrostimulation induces cardiomyocyte predifferentiation of fibroblasts. Biochem Biophys Res Commun 2008;370:450–5.
5. Leri A, Hosoda T, Kajstura J, Anversa P. Heart failure and regenerative cardiology. Regen Med 2006;1:153–9.
6. Stamm C, Liebold A, Steinhoff G, Strunk D. Stem cell therapy for ischemic heart disease: beginning or end of the road? Cell Transplant 2006;15(Suppl 1)S47–56.
7. Segers VF, Lee RT. Stem-cell therapy for cardiac disease [Review]. Nature 2008;451(7181):937–42.
8. Chachques JC, Herreros J, Trainini J, Juffe A, Rendal E, Prosper F, et al. Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. Int J Cardiol 2004;95(Suppl 1):S29–33.
9. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators [Review]. J Cell Biochem 2006;98:1076–84.
10. Wollert KC, Drexler H. Clinical applications of stem cells for the heart [Review]. Circ Res 2005;96:151–63.
11. Psaltis PJ, Zannettino AC, Worthley SG, Gronthos S. Concise review: mesenchymal stromal cells: potential for cardiovascular repair. Stem Cells 2008;26:2201–10.
12. Strem BM, Zhu M, Alfonso Z, Daniels EJ, Schreiber R, Beygui R, et al. Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. Cytotherapy 2005;7:282–91.
13. Winitsky SO, Gopal TV, Hassanzadeh S, Takahashi H, Gryder D, Rogawski MA, et al. Adult murine skeletal muscle contains cells that can differentiate into beating cardiomyocytes in vitro. PLoS Biol 2005;3:e87.
14. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–76.
15. Oh H, Chi X, Bradfute SB, Mishina Y, Pocius J, Michael LH, et al. Cardiac muscle plasticity in adult and embryo by heart-derived progenitor cells. Ann N Y Acad Sci 2004;1015:182–9.
16. Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature 2007;450(7169):497–502.
17. Mitalipov SM, Zhou Q, Byrne JA, Ji WZ, Norgren RB, Wolf DP. Reprogramming following somatic cell nuclear transfer in primates is dependent upon nuclear remodeling. Hum Reprod 2007;22:2232–42.
18. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
19. Mazhari R, Hare JM. Advances in cell-based therapy for structural heart disease [Review]. Prog Cardiovasc Dis 2007;49:387–95.
20. Menasché P, Hagège AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003;41:1078–83.
21. Cleland JG, Freemantle N, Coletta AP, Clark AL. Clinical trials update from the American Heart Association: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE [Review]. Eur J Heart Fail 2006;8:105–10.
22. Tousoulis D, Briasoulis A, Antoniades C, Stefanadi E, Stefanadis C. Heart regeneration: what cells to use and how? Curr Opin Pharmacol 2008;8:211–8.
23. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 2004;94:92–5.
24. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Forfang K. Autologous stem cell transplantation in acute myocardial infarction: The ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects. Scand Cardiovasc J 2005;39:150–8.
25. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199–209.
26. Cogle CR, Madlambayan GJ, Hubsher G, Beckman C, Speisman R, Tran-Son-Tay R, et al. Marrow cell therapies for cardiovascular diseases. Exp Hematol 2008;36:687–94.
27. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361(9351):47–9.
28. Thompson CA, Nasseri BA, Makower J, Houser S, McGarry M, Lamson T, et al. Percutaneous transvenous cellular cardiomyoplasty. A novel nonsurgical approach for myocardial cell transplantation. J Am Coll Cardiol 2003;41:1964–71.
29. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Silva GV, et al. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. Circulation 2004;110(Suppl I):213–8.
30. Kawahara Y, Yamaoka K, Iwata M, Fujimura M, Kajiume T, Magaki T, et al. Novel electrical stimulation sets the cultured myoblast contractile function to 'on'. Pathobiology 2006;73:288–94.
31. Yuge L, Kataoka K. Differentiation of myoblasts is accelerated in culture in a magnetic field. In Vitro Cell Dev Biol Anim 2000;36:383–6.
32. Genovese JA, Spadaccio C, Rivello HG, Toyoda Y, Patel AN. Electrostimulated bone marrow human mesenchymal stem cells produce follistatin. Cytotherapy 2009;11:448–56.
33. Cortes-Morichetti M, Frati G, Schussler O, Van Huyen JP, Lauret E, Genovese JA, et al. Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration. Tissue Eng 2007;13:2681–7.
34. Spadaccio C, Chello M, Trombetta M, Rainer A, Toyoda Y, Genovese J. Drug releasing systems in cardiovascular tissue engineering. J Cell Mol Med 2009;13(3):422–39.
35. Christman KL, Fok HH, Sievers RE, Fang Q, Lee RJ. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. Tissue Eng 2004;10:403–9.
36. Ryu JH, Kim IK, Cho SW, Cho MC, Hwang KK, Piao H, et al. Implantation of bone marrow mononuclear cells using injectable fibrin matrix enhances neovascularization in infarcted myocardium. Biomaterials 2005;26:319–26.
37. Chekanov VS, Nikolaychik V, Maternowski MA, Mehran R, Leon MB, Adamian M, et al. Deferoxamine enhances neovascularization and recovery of ischemic skeletal muscle in an experimental sheep model. Ann Thorac Surg 2003;75:184–9.
38. Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat Med 2006;12:452–8.
39. Kofidis T, de Bruin JL, Hoyt G, Ho Y, Tanaka M, Yamane T, et al. Myocardial restoration with embryonic stem cell bioartificial tissue transplantation. J Heart Lung Transplant 2005;24:737–44.
40. Kofidis T, de Bruin JL, Hoyt G, Lebl DR, Tanaka M, Yamane T, et al. Injectable bioartificial myocardial tissue for large-scale intramural cell transfer and functional recovery of injured heart muscle. J Thorac Cardiovasc Surg 2004;128:571–8.

41. Kofidis T, Lebl DR, Martinez EC, Hoyt G, Tanaka M, Robbins RC. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. Circulation 2005;112(Suppl I):173–7.
42. Zhang P, Zhang H, Wang H, Wei Y, Hu S. Artificial matrix helps neonatal cardiomyocytes restore injured myocardium in rats. Artif Organs 2006;30:86–93.
43. Zimmermann WH, Didie M, Wasmeier GH, Nixdorff U, Hess A, Melnychenko I, et al. Cardiac grafting of engineered heart tissue in syngenic rats. Circulation 2002;106(Suppl I):151–7.
44. Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, et al. Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? Circulation 2000;102 (Suppl II)156–61.
45. Kellar RS, Shepherd BR, Larson DF, Naughton GK, Williams SK. Cardiac patch constructed from human fibroblasts attenuates reduction in cardiac function after acute infarct. Tissue Eng 2005;11:1678–87.
46. Krupnick AS, Kreisel D, Engels FH, Szeto WY, Plappert T, Popma SH, et al. A novel small animal model of left ventricular tissue engineering. J Heart Lung Transplant 2002;21:233–43.
47. Siepe M, Giraud MN, Pavlovic M, Receputo C, Beyersdorf F, Menasché P, et al. Myoblast-seeded biodegradable scaffolds to prevent post-myocardial infarction evolution toward heart failure. J Thorac Cardiovasc Surg 2006;132:124–31.
48. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006;12:459–65.
49. Davis ME, Motion JP, Narmoneva DA, Takahashi T, Hakuno D, Kamm RD, et al. Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. Circulation 2005;111:442–50.
50. Dubois G, Segers VF, Bellamy V, Sabbah L, Peyrard S, Bruneval P, et al. Self-assembling peptide nanofibers and skeletal myoblast transplantation in infarcted myocardium. J Biomed Mater Res B Appl Biomater 2008;87:222–8.
51. Matsubayashi K, Fedak PW, Mickle DA, Weisel RD, Ozawa T, Li RK. Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. Circulation 2003;108(Suppl I):219–25.
52. Remuzzi A, Mantero S, Colombo M, Morigi M, Binda E, Camozzi D, et al. Vascular smooth muscle cells on hyaluronic acid: culture and mechanical characterization of an engineered vascular construct. Tissue Eng 2004;10:699–710.
53. Schaner PJ, Martin ND, Tulenko TN, Shapiro IM, Tarola NA, Leichter RF, et al. Decellularized vein as a potential scaffold for vascular tissue engineering. J Vasc Surg 2004;40:146–53.
54. Chachques JC, Trainini JC, Lago N, Masoli OH, Barisani JL, Cortes-Morichetti M, et al. Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM clinical trial): one year follow-up. Cell Transplant 2007;16:927–34.
55. Chachques JC, Trainini JC, Lago N, Cortes-Morichetti M, Schussler O, Carpentier A. Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM trial): clinical feasibility study. Ann Thorac Surg 2008;85:901–8.
56. Xu C, Inai R, Kotaki M, Ramakrishna S. Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. Tissue Eng 2004;10:1160–8.
57. Spadaccio C, Rainer A, Trombetta M, Vadala G, Chello M, Covino E, et al. Poly-L-lactic acid/hydroxyapatite electrospun nanocomposites induce chondrogenic differentiation of human MSC. Ann Biomed Eng 2009;37:1376–89.
58. Spadaccio C, Rainer A, Chello M, Covino E, Trombetta M, Genovese J. Drug releasing hybrid scaffold: new avenue in cardiovascular tissue engineering. Tissue Eng Part A 2008;14:691–943.
59. Davis ME, Hsieh PC, Takahashi T, Song Q, Zhang S, Kamm RD, et al. Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. Proc Natl Acad Sci U S A 2006;103:8155–60.
60. Ishii O, Shin M, Sueda T, Vacanti JP. In vitro tissue engineering of a cardiac graft using a degradable scaffold with an extracellular matrix-like topography. J Thorac Cardiovasc Surg 2005; 130:1358–63.
61. Stankus JJ, Guan J, Wagner WR. Fabrication of biodegradable elastomeric scaffolds with sub-micron morphologies. J Biomed Mater Res A 2004;70:603–14.
62. Piao H, Kwon JS, Piao S, Sohn JH, Lee YS, Bae JW, et al. Effects of cardiac patches engineered with bone marrow-derived mononuclear cells and PGCL scaffolds in a rat myocardial infarction model. Biomaterials 2007;28:641–9.