[Frontiers in Bioscience S3, 901-918, June 1, 2011]

Stem cells cardiac differentiation in 3D systems

Cristiano Spadaccio[1], Alberto Rainer[2], Juan C. Chachques[3], Elvio Covino[1], Jesus Herreros[4], Jorge A. Genovese[1]

[1]University Campus Bio-Medico of Rome, Department of Cardiovascular Science, Unit of Cardiovascular Surgery, Via Alvaro del Portillo 200, 00128 Rome, Italy, [2]University Campus Bio-Medico of Rome, Tissue Engineering Laboratory, Via Alvaro del Portillo 21, 00128 Rome, Italy, [3]Georges Pompidou European Hospital, 20 rue Leblanc, 75015 Paris, France [4]University Hospital Valdecilla, Unit of Cardiovascular Surgery, Avenida Valdecilla s/n, 39008 Santander, Spain

TABLE OF CONTENTS

1. ABSTRACT

Cardiac regeneration requires a complex cascade of events. Stem cell therapy and tissue engineering are newly emerging tools with promising potential for recover or replace of damaged cardiac tissue. There are many factors, most of them still no clarified, that limit the effectiveness of these treatments and their translation to the clinic. Cells should graft, survive and functionally integrate to the target organ in order to have a chance to restore its function. As in original tissues, a complex and well defined set of signals, many of them coming from the extracellular matrix, is required for normal cell physiology. Biomaterials science gives us important tools to build this extracellular matrix. Functionalized 3D systems can provide the correct environment and act as a delivery system for genes or gene products, guiding the therapeutic cells to the functional phenotype.

2. INTRODUCTION

Stem cell (SC) therapy is a leading field of research worldwide. It attracts a stunning amount of clinical applications and has a promising potential for recovery or replacement of tissues and organs, including those involved in cardiovascular pathology.

There is a huge amount of experimental data reporting improvement of myocardial function by the treatment with SCs. However, there is a lack in the conclusive demonstration of the mechanism involved in tissue regeneration associated with this adult SC treatment. Immunomodulation, paracrine activity or capacity to differentiate or transdifferentiate have been pointed out or hypothesized, but their relevance as playing a pivotal role, accounting for the reported beneficial effect, has not been definitely clarified (1, 2). A true *in situ* cardiac

transdifferentiation has not been demonstrated yet. The cell viability and engrafting following cell administration have been found to be low, weakening the concept of a real tissue replacement or regeneration (3). Moreover, issues concerning the best SC source, type, safety and their harmonious and functional engraftment with an adequate electromechanical integration, remain unanswered.

Clearly, cardiac regeneration requires a complex cascade of events that goes over the simple injection of the right type of cells in the right place. To this extent, the characterization of the factors present in the hostile microenvironment of the injured myocardium, hampering the survival and functional integration of transplanted cells, is also essential. Undoubtedly, along with biology of SCs, actual effectiveness of the therapy and safety for patients requires to be further defined.

A real regenerative medicine approach should consider the significance of the extracellular matrix (ECM), and the strong biological signals that it can provide. Connective tissue atmosphere and structural microenvironment in which cells are embedded affect cells function and support their proliferation and differentiation.

Proliferation of most mammalian cell types has been shown to be anchorage-dependent (4); therefore, to be successful, a tissue engineering (TE) and regenerative medicine approach cannot disregard the importance of the ECM as the main cellular support able to provide a strong biological signaling as guidance for tissue restoration. The creation of suitable structures (scaffolds), where the correct biochemical signals guide the growth of functional neo-tissue and provide an appropriate surface for cell attachment, proliferation, differentiation, and migration, results crucial (4, 5).

In the panorama of TE the idea of a biomimetic approach based on the simulation of the ECM along with the guidance of SC differentiation aided by a growth factor is emerging as a new perspective. The goal to concentrate and spatially organize a biological mediator within a three-dimensional environment mimicking the native tissue histoarchitecture has been recently pointed out (6-8). It allows exploiting the well known benefits of the use of SCs in regenerative medicine, enabling cell differentiation in a familiar and convenient 3D microenvironment and preserving cells from harmful factors present in the injured myocardium.

3. STEM CELL THERAPY: A STAND POINT

Stem cell based therapeutic strategies aiming to restore myocardial cellularity and regenerate the contractile tissue have raised considerable interest due to encouraging preliminary results (9, 10). However, a number of interrogatives concerning the best stem cells source, type, safety and their ultimate fate, harmonious and functional engraftment with an adequate electromechanical integration, remain unanswered. Many factors related with patient's systemic conditions and the microenvironment of the injection site hamper an effective integration and differentiation of SCs in the heart (11).

No insights have been provided to dampen the dramatic loss of cells immediately after implantation. Cell trauma related to the handling and injection itself, together with the negative effects of the hostile environment in which the implant takes place, are reliably at the root of this phenomenon. No precise data are reported about the number of cells effectively viable after injection and homing to the affected site. A recent study by Assis *et al.* demonstrated that, after injection of radiolabeled bone marrow mesenchymal stem cells (MSC) in the tail vein of infarcted rats, approximately 70% of the cells migrated mainly to the lungs and, in small amounts, to the heart, kidneys, spleen, and bladder (12). Additionally, injection of living cells in a beating heart is another concern. Zhang *et al.* performed intramyocardial injections of SCs in beating porcine infarcted hearts and in cardioplegic arresting hearts after cardiopulmonary bypass was set up. Even after intramyocardial injection, many cells migrated to the spleen, lungs and liver. However, percentage of cells retained in the heart was significantly higher in the group injected in the arresting heart with respect to the beating heart group (13). These unsolved questions limit the possibility to define a number of cells to be injected and also raise concerns on the actual effects of the cells into the heart and in other organs. This is especially relevant when an attempt is made to match these biological uncertainties with the evident improvement in cardiac function.

Undoubtedly, there are still significant obstacles to translate the basic knowledge to the clinics, evidencing the necessity to perform meticulous clinical trials that let obtain reliable data. In spite of its feasibility and attractive nature, cell therapy requires a coordinated scientific, clinical and technical effort. Additionally, up to date, no practical guidelines or consensus of the scientific community on the application of cell therapy in patients have been issued.

Several cell types have been shown as potential candidates in cardiovascular medicine. For cardiac repair, especially following myocardial ischemia, MSCs provide an attractive potential therapeutic approach (14). Furthermore, trials with skeletal myoblasts (15) showed encouraging results concerning increases in regional wall motion and in left ventricular ejection fraction. Myoblasts are resistant to ischemia, and showed ability to improve ventricular function in animal models differentiating into myotubes *in vivo*. However, current evidence suggests that skeletal myoblasts or bone marrow-derived adult stem cells fail to electromechanically integrate into the recipient heart with direct consequences on their survival and terminal differentiation (16). Thus, identification of cell types which can achieve efficient electromechanical integration is mandatory. Guidance of stem cells differentiation becomes therefore crucial to avoid unfruitful or even harmful outcomes. It is possible to induce differentiation of several cell types, including fibroblasts, bone marrow-derived stem cells, human mesenchymal stem cells and embryonic stem cells, into committed cells expressing markers characteristic of a cardiac phenotype (17-19). Unfortunately, these transdifferentiation protocols either rely on the use of powerful and potentially dangerous drugs

(19) or require periods of co-culture with human or rat adult ventricular cardiomyocytes. The former clearly prohibits translation to the clinic and the latter raises issues concerning cell survival and the effects of a prolonged *ex vivo* culture on cell immunogenicity and transformation (20). Preliminary data suggest that cells with a true cardiomyogenic phenotype, such as cardiac stem cells and cardiac-pre-committed embryonic stem cells may satisfy these criteria, potentially ensuring regeneration of dead myocardium (21). In fact, a partial differentiation or a pre-committement of stem cells towards the cardiomyogenic phenotype might be preferable to fully differentiated cardiomyocytes (CMs) in this context, as the cardiac environment could provide the necessary biochemical and mechanical signals for integrated differentiation. Following completion of their differentiation within the myocardium, the pre-committed cells could effectively and selectively reduce fibrosis and restore muscle function. The concept of a pre-differentiation has been recently theorized and described (22).

The pre-differentiation process can induce genetic switches toward CM that are phenotypically translated in the *in vitro* expression of cardiac proteins. After injection, the pre-committed cells will receive signals from the new environment thus achieving a gradual ongoing complete differentiation. Additionally, it has been demonstrated that pre-differentiation could be achievable using cytokine-free systems, such as *in vitro* electrostimulation, and that it can be reproduced in both embryonic and adult, stem and non-stem cell lines, suggesting the idea of a non pluripotential cell-restricted phenomena (22-24).

Alternative strategies and mechanisms for *in vivo* regeneration could be represented by cell fusion between damaged myocardium and stem-cells, with further cell reprogramming to show cardiac phenotype (25). Bone marrow-derived cells (BMDCs) have been shown to contribute to the regeneration of diverse adult tissues, including brain, liver and heart, following bone marrow transplantation. These unexpected events, that were initially considered a result of transdifferentiation of BMDCs, challenge the long-standing notion of cell fate determination in mammalian developmental biology. However, these reports suffered from a lack of definitive evidence and in early 2002, spontaneous cell fusion was proposed as an alternative mechanism to account for such phenomena of unexpected cell fate-switches in stem cells. Labovsky *et al.* concluded that the extract of neonatal rat cardiomyocytes could promote a nuclear modification of hMSCs to cardiomyogenic-like cells (26). Konayagi *et al.* demonstrated that fusion-induced cell reprogramming has its molecular basis on a transient transmembrane exchange of proteins and organelles between cells. They showed that ultrafine intercellular nanotubular structures of connections among cardiomyocytes and endothelial progenitor stem cells allowed the transport of organelles, responsible of the acquisition in stem cells of a cardiomyogenic phenotype independent of permanent cellular or nuclear fusion (27).

However, in light of an effective clinical application by means of pre-differentiated or reprogrammed cell delivery, survival in the inflammatory environment of an infarcted myocardium represents a challenge common to all types of cells. Moreover, the persistent ischemia dramatically limits cell engraftment. At the cellular level, sudden suppression of the oxygen supply triggers intricate molecular cascades, which modulate a series of critical biological events (28). Immediately after an infarction, the process of wound-healing begins, with active migration of inflammatory cells, recruitment of cardiac fibroblasts, and eventual remodeling of the extracellular matrix aiming to stabilize the region and restore ventricular wall function. However, function is seldom restored, and CM loss and replacement by fibrous elements results in a natural process evolving from progressive left ventricular remodeling to congestive heart failure (29). A strict interplay between cellular elements and ECM needs to be taken into account, and understanding the mechanisms of normal and post-injury CM development and turnover will be crucial for guiding the orientation of stem cell-based therapies.

4. THE TISSUE ENGINEERING STAND POINT

The idea of combining principles from cell biology and engineering of biocompatible materials in order to create biologic replacement structures that restore, maintain, or improve tissue function, is at the basis of the so called Tissue Engineering (TE) area and defines a different approach to the problem of cardiac regeneration. TE presents some overlaps, but is not synonymous with "cell therapy", which intends to ameliorate function of an existing tissue or to promote the formation of new tissue by isolated cells delivered to the organs by direct injection or infusion. Differently, the concept at the basis of TE is to generate a functional 3D environment suitable for cell culture and growth in order to produce *in vitro* tissue substitutes tailored in size, shape, and function before implanting them into the body or, alternatively, to use the biomaterials directly at the site of damage (*in situ* TE) Thinking of tissue regeneration, this approach acquires an additional value considering the possibility to reproduce, using a different kind of matrices and scaffolds, the extracellular matrix (ECM), crucial for a full tissue restoration. Additionally, considering the eventual ventricular remodeling which is subsequent to a myocardial infarction, a fibrous ECM surrogate would be amenable to limit geometrical and shape remodeling of the infarcted myocardium, providing a mean for ventricular restraint and promoting tissue restoration.

Several approaches have been developed and recent advances have been made in the engineering of cardiac tissue (6). Biocompatible materials have been largely investigated. A wide range of natural materials spanning from collagen (30-33), fibrin (34), hyaluronic acid (35), up to approaches involving tissue decellularization (36, 37) have been evaluated.

In situ TE has been investigated for myocardial repair. Most frequently, this has involved the injection of the infarcted myocardium with a mixture of biomaterials and cells (38-41). The biomaterials, such as collagen

hydrogel or Matrigel®, act as a supporting matrix for the cells, which should then repair or regenerate the infarcted region (32, 42). Hydrolytically degradable biocompatible polymers, composed of poly(lactide) (PLA), poly(glycolide) (PGA) and their co-polymer PLGA (43, 44), have been broadly used to fabricate tissue engineered cardiac grafts (TECGs) according to several processing techniques, as their mechanic and elastic properties can be engineered to match native heart characteristics. Figure 2 reports an example of a PLLA scaffold obtained by thermal induced phase separation (TIPS) process.

A good amount of research has been performed to develop scaffolds with an extracellular matrix-like structure and topography, either by using polymers alone or by incorporating collagen with biocompatible polymers (45, 46). The electrospinning method produces scaffolds that remarkably mimic the size and scale of the natural extracellular matrix. The nano-topography of these structures is reported as optimal in the realization of successful extracellular matrix-like scaffolds for cardiac TE (47-51). Ishii *et al.* have cultured primary CMs harvested from neonatal rats onto biodegradable electrospun, nanofibrous poly(ε-caprolactone) meshes with an average fiber diameter of 250 nm (48), using a cell layering technique (52) with encouraging results. Recently, different stem cell populations have been engrafted on commercially available PGA biodegradable patches (44, 53-55) or collagen matrices (56), as well. Additionally, survival and integration of transplanted cells can be improved by embedding them in matrices such as collagen (57) or Matrigel® (58) or by implanting cells as monolayer sheets (59). Biomaterials have also been engrafted with growth factors, cytokines and drugs (8, 60-63), thus obtaining drug delivery systems (DDS) capable of focused and localized delivery of molecules depending on the environment requirements (64). Genetically modified cells can be used to engineer tissues with differentiated capacities of regeneration, repair or secretion (65-68). The use of biopolymeric scaffolds functionalized as gene delivery systems is even more exciting, as it could represent an approach to modulate the environment and guide cell function (69, 70). Genes of implanted or host cells could be silenced by the delivery of small interfering RNAs with potential impact on an essential cell process as proliferation in cancer (71). In both cases, the possibility to obtain a transgene expression, associated to a TE device result in the generation of a DDS with promising applications in the regenerative medicine field. These concepts become even more relevant in cardiac TE, where use of artificial myocardial patches is desirable to both limit geometrical and shape remodelling of the infarcted myocardium and to promote tissue restoration. The main consequence of a myocardial infarction regards the proliferation of fibroblasts and the deposition of ECM with the aim to compensate the loss of functional CMs and to prevent ventricular dilation. This adaptive mechanism presents as a deleterious counterpart the loss of cardiac elastomechanical properties and the decline of its dynamic compliance with an increase in ventricular stiffness, eventually compromising diastolic function. This perpetrating circle, sustained by ECM deposition, and leading to diastolic heart failure, is considered the real pathogenic mechanism at the basis of the clinical evolution of a myocardial infarction. On the other side, the loss of the systolic component, represented by the working CMs, and even tolerated within certain ranges, can further aggravate heart function. In this scenario, engineered functionalized myocardium might represent an amenable alternative, as it could provide both a mean for mechanical restraint and prevention of ventricular dilation. (6). At the same time, it could generate an environment to maintain the proliferative capacity of the cells surrounding the infarct area providing a molecular pathway to promote cell differentiation (13).

The possibility to produce a scaffold suitable for stem cells seeding, containing the appropriate factors to induce a guided differentiation towards the desired phenotype has been described (3, 7, 63, 72). In this settings, differentiation would be realized within a three-dimensional ECM-like environment, closely mimicking the tissue native architecture and allowing a harmonious ongoing cell growth and differentiation for tissue regeneration.

Additionally, in light of the biomimetic inspiration acquired by modern tissue engineering, structure and ultrastructure of the scaffold itself achieve an important significance even as a potential drive for cell differentiation. Also, with the idea of an *in vivo* application, the biological weight of the physical stimuli and dynamic conditions applying on the graft once *in vivo*, demands further considerations.

Scaffold ultrastructure and architecture, dynamic conditioning by physical stimuli, and bioactive functionalization could be considered the main factors defining guidance of stem cells differentiation in 3D systems (Figure 1)

4.1. Tailoring scaffold structure

The biomaterial scaffold plays a key role in most TE strategies. The scaffold is responsible for guidance of cell organization, growth, and differentiation in tissue engineered constructs, providing not only a physical support for the cells, but also the chemical and biological cues required in forming functional tissues (73). Therefore, the biomaterial should be able of interacting with cells providing, at the molecular level, a biological crosstalk, similarly to the natural interactions existing between cells and the native ECM. Several studies demonstrated that the ECM milieu surrounding the cells has physical and structural features in the nanometer scale. This arrangement may affect several aspects of cell behaviour such as morphology, adhesion and cytoskeletal arrangements (74-76). The so-called “TE approach” in regenerative medicine exploits these concepts with the aim to reproduce a biocompatible ECM surrogate to host cells, providing a biological stimulus to support their survival and to guide their proliferation. Thus, a great effort has been made to tailor biomaterials into nanometer scale structures, in attempts to simulate the matrix environment in which seeded cells can be accommodated to proliferate and differentiate towards desired lineages (77-79).

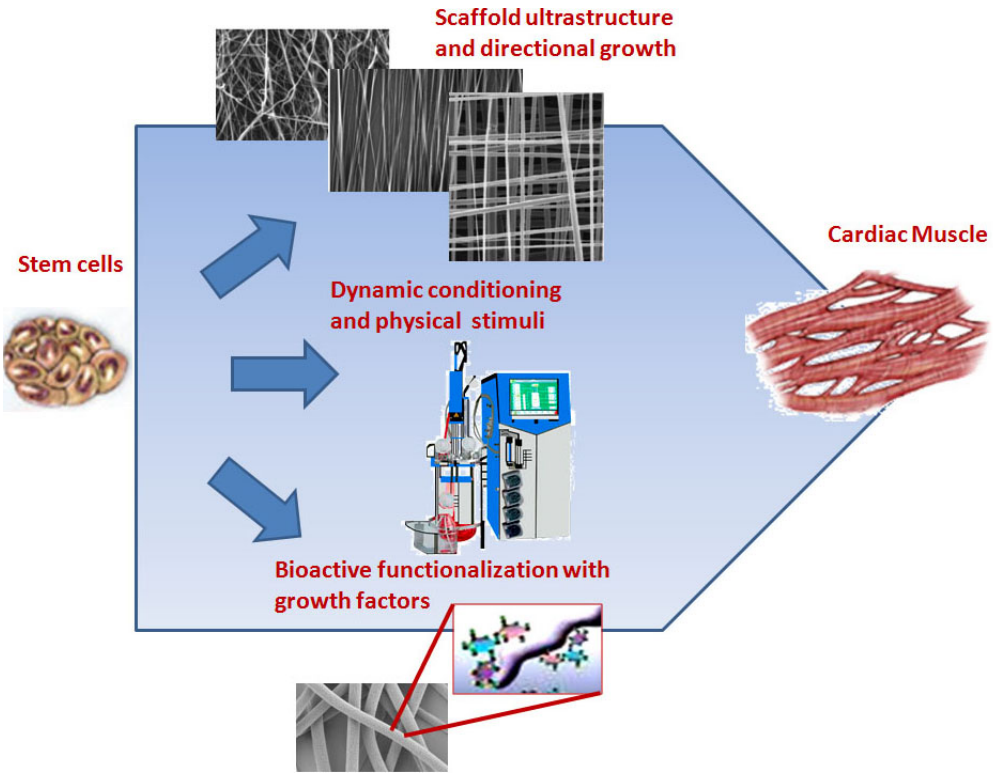

Figure 1. Different approaches for stem cell differentiation within 3D systems. Scaffold ultrastructure and architecture could be tailored in order to induce specific biological effects on stem cell phenotype. Dynamic conditioning by physical stimuli allows simulating the environmental conditions of a tissue fostering to differentiate changes in stem cells seeded in 3D scaffolds. Eventually, bioactive functionalization is considered one of the main factors defining guidance of stem cell differentiation in 3D systems.

Requirements for simple cell attachment and proliferation might be different from those demanded to induce cell differentiation. A large number of studies have demonstrated that the microarchitecture of the scaffold may guide cell functions by regulating the interaction between cells, and the diffusion of nutrients and metabolic wastes throughout the three-dimensional (3D) construct (80). Topographical features of the scaffold, in particular surface-to-volume ratio and pore size and interconnectivity, have a profound influence on cell proliferation and on cellular responses, especially in sensitive phenotypes as mesenchymal stem cells (81). Taking MSCs as an example, the microarchitectural features of the scaffold significantly influence cell morphology, cell binding and phenotypic expression, but also control the extent and nature of nutrient diffusion and tissue ingrowth (82). Therefore, maximizing surface-to-volume ratio is considered an important goal to enhance cell colonization and fluid transport. Even if the use of dynamic cell seeding and cultivation devices, in particular perfusion bioreactors or spinner flasks, has been shown to enhance proliferation and distribution of stem cells into the scaffold (83), in the absence of an appropriate scaffold microarchitecture this approach could result unsuccessful in ensuring the ingrowth of new tissue. Explanations accounting for this issue need to be found in the diffusion constraints into the interior of the construct, inducing the development of a necrotic core. An optimal combination of pore size and shape is essential to promote and guide the colonization of cells in 3D, and the pore size has been shown to directly affect some biological events, with different tissues requiring optimal pore sizes for their regeneration (80). Pore size represents an important requirement for cell proliferation inside the scaffold, as cells preferentially invade larger pores (with diameter in the hundreds of micron range), while micron and sub-micron pores, additionally coupled to the hydrophobic nature of the majority of polymeric ester-based matrices used in tissue engineering, may hinder hMSC colonization. However, one of the most important limitations of tissue engineering scaffold is that cell proliferation and extracellular matrix deposition may progressively occlude the entire porosity of the scaffold and, consequently, reduce nutrient delivery to, and metabolic waste removal from, the interior of the construct (84). Therefore, even if a large number of studies have pointed out the importance of an uniform cell infiltration in 3D, the presence of a pore network not accessible for cells could be very important as it might ensure the transport of fluids necessary for cell biosynthesis. To this extent, Salerno *et al.* proposed a novel μ-bimodal approach in the realization of a PCL scaffold using a combination of gas foaming and selective polymer extraction from co-continuous blends obtaining a multiscaled microarchitecture. The results showed high cell

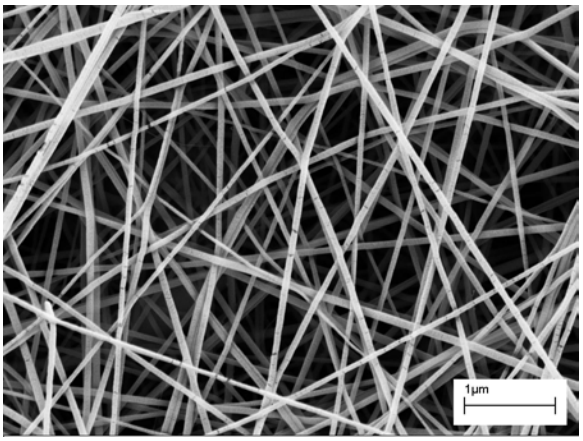

Figure 2. Scanning electron micrograph of a nanofibrous gelatin electrospun scaffold. Scaffold was obtained starting form a gelatin solution in formic acid.

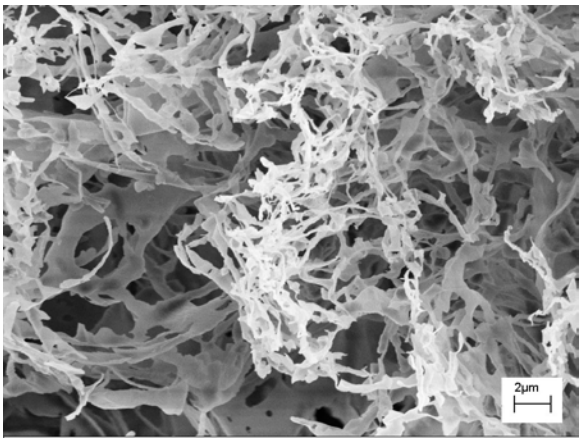

Figure 3. Scanning electron micrograph of a poly(L-lactide) (PLLA) scaffold obtained by thermal induced phase separation (TIPS) using liquid naphthalene as a solvent for PLLA. Phase separation occurs upon solidification (at room temperature) of naphthalene, that is then vacuum-extracted from the scaffold.

seeding efficiency and the ability of the scaffold to promote and guide selective 3D hMSC colonization and proliferation into the macroporosity, while ensuring the presence of a separate small size pores network for fluid transport (85).

On the other side, the further step of differentiation of stem cells inside the scaffold is guided by a myriad of factors that, beside the known effects of cytokines and other biologically active compounds, embricate with the functional and structural architecture of the scaffold itself. For instance, Lovett *et al.*, using a modular bioreactor capable of enhanced and fine tuned oxygen and nutrient delivery to the construct, demonstrated changes in the expression profiles of hMSCs differentiated under varied oxygen tensions and tissue-specific oxygen requirements for both adipogenic (20% O_2) and chondrogenic (5% O_2) differentiation (86).

Cell guidance by the scaffold, from cell-material construct to the new engineered tissue, requires a complex balance between chemical, biochemical and biophysical cues able to mimic the spatial and temporal microenvironments of the natural extracellular matrix (87).

Electrospinning is one of the approaches that allow the fabrication of several natural and synthetic materials into fibrous and porous structures in the micro- and nanometer scale, just by controlling few process parameters (88, 89) (Figure 3)

Thus, in cardiac tissue engineering the trend has been to design bioactive materials, which on one hand have the appropriate mechanical strength and the degradation kinetics of synthetic polymers, and, on the other hand, have the biological specificity of collagen, fibronectin, and laminin, which are the major ECM components.

Cardiac tissue presents naturally a high degree of hierarchical organization and architecture that define a particular functional environment, characterized by a precise geometry and dense cellularity. Therefore, the primary aims in the production of a cardiac substitute are focused on the possibility to accommodate a high number of working cells maintaining a resistant structure compliant to different working loads. Interconnected porous structures appear to be the most amenable candidates to fulfil this role, allowing for the hosting of a large number of cells.

A particular macro-feature in cell organization, often found *in vivo*, i.e. directional growth or anisotropy, can be effectively addressed using the process of electrospinning. An example was recently demonstrated by employing a spinning disk in combination with electrospinning to fabricate oriented non-woven scaffolds (90). For myocardial tissue, anisotropy carries functional importance and has been the focus of extensive research to orient the ECM or the cell growth in a 2D setting. Electrospun oriented fibers can effectively help in solving this problem (91). Applying a simple post-processing step, i.e. mechanical stretching of the electrospun membranes, Zong *et al.* achieved oriented scaffold texture, enforcing anisotropic cell growth. CMs interacted with the provided nano and microfibrous network, trying to organize their growth in order to follow the scaffold-prescribed direction, demonstrating that CMs are very sensitive to the composition of the electrospun PLGA-based scaffolds, with a preference to relatively hydrophobic surfaces (92). Apparently, hydrophilic and faster degrading electrospun scaffolds allow for a lower cell density. On the other hand, poly(L-lactide) (PLLA) scaffolds promoted better CMs adhesion and mature cytoskeleton structure with well-defined periodic units in the contractile machinery (sarcomeres) CMs reestablished cell-to-cell contacts and intercalated disks, typical for mature adult cardiac tissue on nano- and microstructured electrospun non-woven scaffolds. PLLA scaffolds are recognized to exert a superior behaviour on cells compared to PLLA copolymers with poly(glycolide) (PGA) and poly(ethylene glycol) (PEG), providing both flexibility and guidance for CMs growth (92).

Another further insight in understanding how scaffold architecture affects cell behaviour has been added by Fromstein *et al.* who generated large numbers of embryonic stem cells (ESC)-derived CMs in bioreactors, and compared results of seeding on porous, 3-dimensional scaffolds prepared using 2 different techniques: electrospinning and thermally induced phase separation (TIPS) (93). The effect of material macro-architecture on the adhesion, viability, and morphology of the seeded cells was studied. Results suggested that material macrostructure plays a large role in modulating ESC-derived CM morphology. Scaffolds fabricated by electrospinning were found to promote an elongated cardiac phenotype whereas the TIPS macrostructure retained a rounded morphology. Cellular distribution within TIPS scaffolds was limited, as most of the cells resided within the first 200 mm from the surface. On the contrary, cells were detected throughout the entire volume of electrospun scaffolds. Percentage of viable cells decreased with culture time within TIPS scaffold, whereas electrospun scaffolds showed consistently high proportions of live to dead cells. The percentage of dead cells in the TIPS materials also increased with the distance from the surface. This was not a concern in electrospun scaffolds because nutrients and oxygen diffusion was sufficient to maintain the cells in a constant aerobic state. Interestingly, on both materials, the seeded cells demonstrated functionality by contracting and staining positive for sarcomeric myosin heavy chain and connexin 43. This study represents a further confirmation that the interactions between cells and the extracellular matrix play a significant role in cell migration, differentiation, cell cycle, and proliferation. In this case, considering the use of ESCs, it is important to note that, during embryogenesis, not only soluble matrix factors guide different developmental pathways, but also cues from the extracellular matrix play a vital role in deciding cell fate. It is possible that the 3D nature of scaffolds produced by electrospinning technique could result in different protein deposition patterns within the matrix, potentially exposing different binding motifs to the cells, and ultimately modulating the phenotype. Then, the scaffold itself comes to be part of the signals set that will define the biology of the cell.

The electrospinning is also useful to process natural components of tissue ECM, as fibrin and fibrinogen. It provides a powerful device for cardiac TE, given their innate ability to induce improved cellular interaction and subsequent scaffold remodeling in comparison to synthetic scaffolds. McManus *et al.* successfully electrospun fibrinogen highly porous scaffolds with fiber diameters as small as 80 nm, showing good penetration of cardiac fibroblasts that efficiently remodelled the matrix (94).

In this context, even the alignment of scaffold fibers could represent an important clue for cell differentiation. Zhu *et al.* demonstrated that cells cultured on macroscopically oriented fibers generated by electrospinning aligned along the direction of fibers and showed changes in their phenotypes (95).

4.2. The dynamic conditioning approach

Load-bearing soft tissues present a structured extracellular matrix, organized to perform the tissues specialized functions. This extracellular matrix (ECM) is synthesized and organized by the cells under the influence (guiding) of external *stimuli*, such as tissue loading directions. Load-bearing soft tissues are composed of a network of fibrous proteins, predominantly collagen and elastin, embedded in a gel of proteoglycans, glycoproteins, and water and exhibiting anisotropic, non-linear viscoelastic behavior, A major challenge in cardiovascular TE is mimicking the native structural organization and hence biomechanical tissue behavior, especially when creating tissues with high biomechanical demands, such as blood vessels or heart valves. Also, simulations of the mechanical loading conditions represent another important factor modulating both cells and scaffold behavior. The effect of different mechanical loading conditions on the collagen organization in engineered tissues has been variedly studied (96).

A good piece of evidence is pointing at elucidating the relationships between mechanical conditioning and resulting tissue structure and mechanical properties. Cell-seeded biodegradable rectangular scaffolds were constrained or intermittently strained in longitudinal direction. Intermittent straining regimes were demonstrated to be favorable in terms of cell proliferation (97), matrix production (98), collagen crosslink density, and mechanical properties (99), as compared to continuous straining (100). In addition, intermittent straining resulted in stronger tissues after shorter culture periods in engineered heart valve tissue (101). Molecular mechanisms underlying the effects of dynamic physical stimulation on cell function are not fully understood; however, it has been recently shown that mechanical loading of cell-seeded biodegradable rectangular scaffolds, either constrained or intermittently strained in longitudinal direction, resulted in changes of the collagen fibers alignment present in the neo-ECM produced. The alignment shifted from oblique at the surface of the construct towards parallel to the straining direction in deeper tissue layers. Additionally, intermittent straining improved and accelerated the alignment of the collagen fibers, as compared to constraining the constructs (96).

The biomimetic paradigm of cardiac TE has driven the development of strategies involving the application of physiologically relevant chemical and physical *stimuli* to cultured cells in order to obtain cardiac differentiation. Biomimetic approaches to cardiac TE recapitulating the aspects of the actual *in vivo* environment, including the convection of blood through perfusion (102, 103), the presence of hemoglobin via oxygen carriers in the culture medium (104), and the exposure of cells to a cyclic stretch (105) or to electrical field stimulation (106, 107) have been attempted.

In recent studies, a system designed to deliver electrical signals mimicking those in native heart tissue resulted in the progressive development of conductive and contractile properties characteristic of cardiac tissue,

Table 1. Biomaterials functionalized with drugs and growth factors used in tissue engineering for cardiovascular purposes.

Source	Biomaterial	Drug/Growth Factor	Reference
Natural	Fibrin	VEGF	(156)
		bFGF	(157)
	Collagen	VEGF	(158)
		TGF-β	(159)
	Gelatin	VEGF	(160)
		bFGF	(161)
		TGF-β	(162)
		HGF	(163)
		Erythropoietin	(164)
	Alginate	VEGF	(165)
		b-FGF	(114)
		Heparin	(166)
	Chitosan	Heparin	(167)
		bFGF	(168)
	Hyaluronic acid	Heparin	(169)
		Erythropoietin	(170)
Synthetic	Poly-L-Lactic Acid (PLLA)	GCSF	(72)
		Heparin	(7)
	poly (D,L-lactic-co-glycolic acid) (PLGA)	Dexamethasone	(171)
		VEGF	(172)
		PDGF-BB	(117)
		bFGF	(173)
		Heparin	(130)
		plasmid DNA encoding platelet-derived growth factor (PDGF) gene	(174)
	poly (epsilon-caprolactone) (PCL)	Erythropoietin	(175)
		Heparin	(61)
	poly (ester urethane)urea (PEUU)	bFGF	(122)
	Polyurethane	Diazeniumdiolates NO donor	(176)
		VEGF	(177)
	poly (ethylene glycol) (PEG)	RGD peptide	(178)
		Stromal-derived factor 1alpha (SDF-1alpha)	(179)

including cell alignment and coupling, increased amplitude of synchronous construct contractions and a remarkable level of ultrastructural organization (104, 106, 108). The application of electrical stimulation to cardiac constructs markedly enhanced the contractile behavior with cells showing a high level of ultrastructural differentiation, comparable in several respects with that of the native myocardium. Electrical stimulation also increased the amounts of mitochondria and glycogen, and induced the formation of well aligned registers of sarcomeres that closely resembled those in native myocardium, representing a hallmark of maturing CMs (109). Stimulated constructs also had well-developed intercalated disks and gap junctions and elevated the levels of β-MHC, Connexin-43 (Cx-43), creatine kinase-MM and cardiac Troponin-I (cTn-I) (109).

4.3. The biofunctionalization approach

One of the basic ideas of TE concerns the use of a resorbable biocompatible material providing the initial temporary support to stimulate and orchestrate tissue repair, progressively disappearing and being replaced by newly developed tissue. Tailoring its structure, the scaffold would therefore, constitute a leading framework for stem cells attachment, differentiation and deposition of new ECM, gradually remodeled. The scaffold would then be degraded up to the final replacement with structured host tissue with all the mechanical and biological features of the native tissue. Another further step to promote stem cell differentiation within a three dimensional support and tissue regeneration concerns the association of a specific bioactive signaling to the scaffold.

The concept of fabricating a scaffold containing factors, able to induce stem cells differentiation and to exert important effects even once in *in vivo* settings, is relatively novel (3, 6, 7). The idea of designing a resorbable scaffold able to define an optimal microenvironment to induce tissue-oriented differentiation of stem lineages and at the same time to host cells in a structure resembling the natural ECM opens a new paradigm in TE. In this scenario, the biomaterial is intended as a differentiating system for stem cells seeded therein, and is capable to guide and orient the differentiation process within a 3D biomimetic structure.

Polymeric biomaterials have been engrafted with growth factors, cytokines and drugs, thus obtaining drug releasing systems capable of focused and localized delivery of molecules depending on the environment requirements and the actual milieu in which the scaffold is placed (6) (Table 1). Up to date, several methods to incorporate growth factors into synthetic scaffolds have been developed (110-115). Growth factors can be adsorbed to the scaffold (116). Adsorption efficiency is material related and concerns not only the chemical influence, but also physical properties of the scaffold itself, as the porosity and the nature of the pores. Moreover, it depends on the structure of the growth factors too, which can in turn affect release kinetics. As proteins are generally adsorbed through electrostatic attractions between anionic groups and the scaffold surface, growth factors presenting only a few of these reactive groups can display lower adsorption capacities on biomaterials, as elegantly showed by Ziegler and colleagues who combined ceramic materials with bFGF and VEGF (116). Adsorbing growth factors onto the

scaffold has the drawback of low loading efficiencies and rapid release, together with the degradation of growth factors such as rhVEGF, BMP-4 and bFGF during a very short release time (116).

It is also possible to blend growth factor-containing microspheres into the scaffold (117), without interfering with its macro and microstructure. Microspheres incorporation allows for a fine modulation of the release kinetics by adjusting the molecular weight and composition of the copolymer, eventually avoiding undesirable initial release bursts. Recently, Patil and colleagues developed a novel poly(lactide-co-glycolide) (PLGA) microsphere/polyvinyl alcohol (PVA) hydrogel composite drug delivery system, able to provide a zero-order release profile (118). However, loading growth factors into microspheres, even if eliciting a regulated sustained release, could be harnessed by a loss in bioactivity due to harsh solvents.

Alternatively, a direct mixing of growth factor containing protein powder into the scaffold during processing can be performed (119-121). Incorporating growth factors directly into the scaffold can potentially surmount the mentioned shortcomings. In particular, the use of a thermoplastic scaffold would be amenable to the mixing of growth factor during polymer processing providing an appropriately mild solvent or low processing temperature. Guan and colleagues have developed a biodegradable, elastomeric poly(ester urethane) urea (PEUU) scaffold capable of releasing bioactive bFGF over a period of several weeks (122).

Another approach to the controlled delivery issues regards the modification of the scaffold itself. Surface-modified PLGA scaffolds have been developed in order to enhance adhesion and function of rat hepatocytes, bovine chondrocytes, and bone marrow stem cells by surface immobilization of bioactive molecules such as galactose, cell adhesive peptides, and hyaluronic acid (123-126). Alternatively, exploitation of biological properties of some ECM components, as heparin, known to specifically bind various growth factors, has been described with the possibility to use natural bridging molecules for scaffold functionalization. (127-129). This technique allows both a sustained diffusion of growth factors and their protection against thermal denaturation and enzymatic digestion at physiological conditions (130).

With this in mind, a large variety of polymers has been functionalized with growth factors as drug delivery system, and also realized with structures closely simulating the ECM histoarchitecture. More interestingly, the potential of 3D ECM-mimicking scaffolds could be oriented in order to obtain a leading framework providing adequate signalling not only for engraftment and proliferation but also for differentiation of precommitted or stem cells towards different phenotypes.

Recently, an electrospun PLLA scaffold has been developed, that was functionalized with hydroxyapatite with the aim to recapitulate the native histoarchitecture and the molecular signalling of osteochondral tissue in order to facilitate cell differentiation towards chondrocyte. PLLA/hydroxyapatite nanocomposites induced differentiation of hMSCs in a chondrocyte-like phenotype with generation of a proteoglycan-based matrix (63). Moreover, data on scaffolds tailored for cardiovascular structures are available (8, 131). Heparin functionalized electrospun PLLA scaffolds have been shown to promote endothelial differentiation of mesenchymal stem cells (7). This data represents a proof of principle of the possibility to produce a scaffold suitable for stem cells seeding, containing the appropriate factors to induce a guided differentiation towards the desired phenotype. In these settings, differentiation would be realized within a three-dimensional ECM-like environment closely mimicking the tissue native architecture and allowing a harmonious ongoing cell growth and differentiation for tissue regeneration.

The intrinsic properties of the scaffolds could therefore provide correct sequences of signals to promote cell adhesion and matrix remodeling, creating a microenvironment able not only to assist and guide cell growth, differentiation and repopulation but also to mimic the mechanical properties of the native tissue, providing at the same time important signaling once in the *in vivo* settings.

To this extent, Authors recently developed a three-layered hybrid tubular scaffold with oriented drug-delivery capacity for the differentiation of hMSCs seeded therein, with the aim to recapitulate the native histoarchitecture of a vessel (132). In particular, the scaffold developed presents a middle pivotal collagen *lamina* between two differentially functionalized layers of poly(L-lactide), obtaining a compartmented architecture and two different controlled microenvironments, simultaneously enabling multiple differentiation processes for the single components of a tissue. Under differentially compartmented biological *stimuli*, a single stem cell type could find the appropriate signals to topographically differentiate into endothelium (inner side of the scaffold) and smooth muscle cells (outside), gradually leading to functional integration of the two layers in a totally regenerated structure (132).

Functionalization strategies of tissue engineered constructs could therefore overcome the current limitations of cardiac stem cell therapy. Currently, stem cell therapy by transfer of bone marrow (BM)-derived stem and precursor cells into the infarcted myocardium has been shown to improve left ventricular systolic function (133). Similar results were obtained by the application of granulocyte colony-stimulating factor (G-CSF), alone or in combination with stem cell factor (SCF), which is known to mobilize BM-derived cells (134-138). Interestingly, the beneficial effects of these therapies are claimed to be due to a significant degree of structural regeneration of the infarcted hearts by transdifferentiation of the immigrated BM-derived cells to CMs (139-143). However, this phenomenon of *in situ* cardiac transdifferentiation has not been conclusively demonstrated yet, and actual cell

viability and engrafting following cell administration have been found to be low, weakening the concept of a real tissue replacement or regeneration (3). Despite the mechanisms underlying their beneficial effect on cardiac performance, this non-functional integration of injected or endogenously mobilized cells might constitute an arrythmogenic load within the cardiac environment, increasing the risk for pro-arrhythmia. To this extent, recent reports have shown that the transfer of skeletal myoblasts (144) into decompensate hearts failed to electromechanically integrate and provoked ventricular tachycardia in patients (15). Moreover, certain types of CMs derived from *in vitro* differentiated embryonic stem cells exhibited prolonged action potential durations after depolarization, and a potential for arrhythmogenesis (145). The achievement of both a CM differentiation and a precise integration of the injected cells into the myocardial wall, in order to augment synchronized contractility and avoid potentially life-threatening alterations in the electrical conduction of the heart, still remains a major target to be pursued. With this in mind, the therapeutic potential of cell therapy alone needs to be carefully evaluated, especially in light of initial results using skeletal myoblasts that are considered to represent one of the greatest potential myogenic sources for cardiac cell therapy. Intramyocardial injection of these cells failed to consistently show transdifferentiation into CMs (57, 146) and, more importantly, electromechanical coupling with the host CMs (146-148).

Another major concern in myoblast transplantation, besides the caveat of arrhythmic events, it is the loss of a significant fraction of injected myoblasts upon engraftment.

TE approaches have been attempted with the aim to surmount these limitations. Siepe *et al.* recently used highly porous myoblast-seeded polyurethane scaffolds, demonstrating their ability to prevent post–myocardial infarction progression toward heart failure in a rat model, with no evidence of transdifferentiation of the seeded myoblasts towards cardiac cells or migration from the scaffold to the heart (149, 150). Then, in light of the importance of inducing neo-angiogenesis into the inner porosity of the scaffolds, incorporation of angiogenic growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) has been preformed to obtain polymers for controlled release and angiogenesis promotion (110, 151). Additionally, in consideration of the lack of functional electromechanical coupling between the majority of grafted myoblasts and CMs (147, 148), and the recently reported effect of G-CSF on angiogenesis and on the induction of expression of connexin 43 (152) – a cardiac-specific gap junction protein crucial for effective electromechanical association (153, 154) – an interesting TE approach has been recently reported (155). G-CSF has been shown to inhibit both apoptosis and remodeling in the failing heart following myocardial infarction through the receptor responsible for cardiac hypertrophy (137). Moreover, it activates the Wnt and Jak2 signals in CMs up-regulating Cx43, protecting from ventricular arrhythmia induced by myocardial infarction, and ameliorating survival in a rodent model of myocardial infarction (152). With this in mind, a PLLA electrospun scaffold functionalized with G-CSF has been developed and seeded with skeletal myoblasts. Authors demonstrated induction of a cardiac pre-commitment of skeletal myoblasts, demonstrated by the expression of cardiac specific connexin 43 and troponin-I. However, morphological and immunophenotypic changes achieved by myoblasts in this setting were not compatible with a complete differentiation in CM and cells did not acquire beating capabilities. In light of an *in vivo* application, a fully differentiated beating phenotype could paradoxically fail to integrate with the host because of asynchronous beating activity and potentially constitute an arrhythmogenic focus leading to life threatening arrhythmias. On the opposite, inducing a partial differentiation towards a cardiac pre-committed phenotype, expressing some of the key proteins of a mature CM, could represent an interesting strategy to provide a better integration within the cardiac environment. The pre-differentiated cells would receive signals from the new environment thus achieving a gradual ongoing complete differentiation (3).

Therefore, functionalization with growth factors could be an interesting alternative to ameliorate cardiac tissue engineered constructs. In our laboratory, we developed a set of techniques to produce 3D ECM-like matrices containing signals locally compartmented at different sides of the scaffold, to promote differential commitment of a single stem cell type towards the multiple phenotypes constituting an organ.

5. CONCLUSION

Considering the current limitations of both cell therapy and TE, the most amenable alternative could be represented by a combination of the two approaches. The idea of a bridge between stem cells plasticity and biomaterials that actively guide and provide the correct sequence of signals to allow ongoing lineage-specific differentiation of these pluripotent precursor cells may represent a promising answer to the problems related to cardiovascular healing.

3D systems could act as drug delivery systems, in a broad concept that includes gene delivery. These engineered DDSs are an alternative to apply localized doses of bioactive molecules over sustained periods of time. Therefore, undesired systemic effect can be minimized, defining a favourable environment for cell engraftment and proliferation, and improving the post-implantation management of the grafts.

7. REFERENCES

1. L Li, S Zhang, Y Zhang, B Yu, Y Xu and Z Guan: Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. *Mol Biol Rep*.36 (4),725-31 (2009)

2. W Dai, SL Hale and RA Kloner: Role of a paracrine action of mesenchymal stem cells in the improvement of

left ventricular function after coronary artery occlusion in rats. *Regen Med*.2 (1),63-8 (2007)

3. C Spadaccio, E Chachques, M Chello, E Covino, JC Chachques and J Genovese: Predifferentiated adult stem cells and matrices for cardiac cell therapy. *Asian Cardiovasc Thorac Ann*.18 (1),79-87 (2010)

4. A Prokop and MZ Rosenberg: Bioreactor for mammalian cell culture. *Adv Biochem Eng Biotechnol*.39,29-71 (1989)

5. MS Kim, HH Ahn, YN Shin, MH Cho, G Khang and HB Lee: An *in vivo* study of the host tissue response to subcutaneous implantation of PLGA- and/or porcine small intestinal submucosa-based scaffolds. *Biomaterials*.28 (34),5137-43 (2007)

6. C Spadaccio, M Chello, M Trombetta, A Rainer, Y Toyoda and JA Genovese: Drug releasing systems in cardiovascular tissue engineering. *J Cell Mol Med*.13 (3),422-39 (2009)

7. C Spadaccio, A Rainer, M Centola, M Trombetta, M Chello, M Lusini, E Covino, Y Toyoda and JA Genovese: Heparin-releasing scaffold for stem cells: a differentiating device for vascular aims. *Regen Med*.5 (4),645-57 (2010)

8. C Spadaccio, A Rainer, M Chello, E Covino, M Trombetta and J Genovese: Drug releasing hybrid scaffold: new avenue in cardiovascular tissue engineering. *Tissue Eng Part A*.14 (5),691-943 (2008)

9. EM Jolicoeur, CB Granger, JL Fakunding, SC Mockrin, SM Grant, SG Ellis, RD Weisel and MA Goodell: Bringing cardiovascular cell-based therapy to clinical application: perspectives based on a National Heart, Lung, and Blood Institute Cell Therapy Working Group meeting. *Am Heart J*.153 (5),732-42 (2007)

10. VF Segers and RT Lee: Stem-cell therapy for cardiac disease. *Nature*.451 (7181),937-42 (2008)

11. JC Chachques: Cellular cardiac regenerative therapy in which patients? *Expert Rev Cardiovasc Ther*.7 (8),911-9 (2009)

12. AC Assis, JL Carvalho, BA Jacoby, RL Ferreira, P Castanheira, SO Diniz, VN Cardoso, AM Goes and AJ Ferreira: Time-Dependent Migration of Systemically Delivered Bone Marrow Mesenchymal Stem Cells to the Infarcted Heart. *Cell Transplant* (2009)

13. H Zhang, P Song, Y Tang, XL Zhang, SH Zhao, YJ Wei and SS Hu: Injection of bone marrow mesenchymal stem cells in the borderline area of infarcted myocardium: heart status and cell distribution. *J Thorac Cardiovasc Surg*.134 (5),1234-40 (2007)

14. GA Ramos and JM Hare: Cardiac cell-based therapy: cell types and mechanisms of actions. *Cell Transplant*.16 (9),951-61 (2007)

15. P Menasche, AA Hagege, JT Vilquin, M Desnos, E Abergel, B Pouzet, A Bel, S Sarateanu, M Scorsin, K Schwartz, P Bruneval, M Benbunan, JP Marolleau and D Duboc: Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol*.41 (7),1078-83 (2003)

16. Y Iijima, T Nagai, M Mizukami, K Matsuura, T Ogura, H Wada, H Toko, H Akazawa, H Takano, H Nakaya and I Komuro: Beating is necessary for transdifferentiation of skeletal muscle-derived cells into cardiomyocytes. *FASEB J*.17 (10),1361-3 (2003)

17. C Badorff, RP Brandes, R Popp, S Rupp, C Urbich, A Aicher, I Fleming, R Busse, AM Zeiher and S Dimmeler: Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation*.107 (7),1024-32 (2003)

18. S Rangappa, JW Entwistle, AS Wechsler and JY Kresh: Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. *J Thorac Cardiovasc Surg*.126 (1),124-32 (2003)

19. S Rangappa, C Fen, EH Lee, A Bongso and EK Sim: Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg*.75 (3),775-9 (2003)

20. M Miura, Y Miura, HM Padilla-Nash, AA Molinolo, B Fu, V Patel, BM Seo, W Sonoyama, JJ Zheng, CC Baker, W Chen, T Ried and S Shi: Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. *Stem Cells*.24 (4),1095-103 (2006)

21. P Menasche: Stem cells for clinical use in cardiovascular medicine: current limitations and future perspectives. *Thromb Haemost*.94 (4),697-701 (2005)

22. JA Genovese, C Spadaccio, J Langer, J Habe, J Jackson and AN Patel: Electrostimulation induces cardiomyocyte predifferentiation of fibroblasts. *Biochem Biophys Res Commun*.370 (3),450-5 (2008)

23. JA Genovese, C Spadaccio, HG Rivello, Y Toyoda and AN Patel: Electrostimulated bone marrow human mesenchymal stem cells produce follistatin. *Cytotherapy*.11 (4),448-56 (2009)

24. A Shafy, T Lavergne, C Latremouille, M Cortes-Morichetti, A Carpentier and JC Chachques: Association of electrostimulation with cell transplantation in ischemic heart disease. *J Thorac Cardiovasc Surg*.138 (4),994-1001 (2009)

25. N Rodic, MS Rutenberg and N Terada: Cell fusion and reprogramming: resolving our transdifferences. *Trends Mol Med*.10 (3),93-6 (2004)

26. V Labovsky, EL Hofer, L Feldman, V Fernandez Vallone, H Garcia Rivello, A Bayes-Genis, A Hernando

Insua, MJ Levin and NA Chasseing: Cardiomyogenic differentiation of human bone marrow mesenchymal cells: Role of cardiac extract from neonatal rat cardiomyocytes. *Differentiation*.79 (2),93-101 (2010)

27. M Koyanagi, RP Brandes, J Haendeler, AM Zeiher and S Dimmeler: Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes? *Circ Res*.96 (10),1039-41 (2005)

28. JM Leferovich, K Bedelbaeva, S Samulewicz, XM Zhang, D Zwas, EB Lankford and E Heber-Katz: Heart regeneration in adult MRL mice. *Proc Natl Acad Sci U S A*.98 (17),9830-5 (2001)

29. S Itescu, MD Schuster and AA Kocher: New directions in strategies using cell therapy for heart disease. *J Mol Med*.81 (5),288-96 (2003)

30. NF Huang, J Yu, R Sievers, S Li and RJ Lee: Injectable biopolymers enhance angiogenesis after myocardial infarction. *Tissue Eng*.11 (11-12),1860-6 (2005)

31. D Philp, SS Chen, W Fitzgerald, J Orenstein, L Margolis and HK Kleinman: Complex extracellular matrices promote tissue-specific stem cell differentiation. *Stem Cells*.23 (2),288-96 (2005)

32. W Dai, LE Wold, JS Dow and RA Kloner: Thickening of the infarcted wall by collagen injection improves left ventricular function in rats: a novel approach to preserve cardiac function after myocardial infarction. *J Am Coll Cardiol*.46 (4),714-9 (2005)

33. O Schussler, C Coirault, M Louis-Tisserand, W Al-Chare, P Oliviero, C Menard, R Michelot, P Bochet, DR Salomon, JC Chachques, A Carpentier and Y Lecarpentier: Use of arginine-glycine-aspartic acid adhesion peptides coupled with a new collagen scaffold to engineer a myocardium-like tissue graft. *Nat Clin Pract Cardiovasc Med*.6 (3),240-9 (2009)

34. KL Christman, HH Fok, RE Sievers, Q Fang and RJ Lee: Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng*.10 (3-4),403-9 (2004)

35. A Remuzzi, S Mantero, M Colombo, M Morigi, E Binda, D Camozzi and B Imberti: Vascular smooth muscle cells on hyaluronic acid: culture and mechanical characterization of an engineered vascular construct. *Tissue Eng*.10 (5-6),699-710 (2004)

36. S Badylak, A Liang, R Record, R Tullius and J Hodde: Endothelial cell adherence to small intestinal submucosa: an acellular bioscaffold. *Biomaterials*.20 (23-24),2257-63 (1999)

37. PJ Schaner, ND Martin, TN Tulenko, IM Shapiro, NA Tarola, RF Leichter, RA Carabasi and PJ Dimuzio: Decellularized vein as a potential scaffold for vascular tissue engineering. *J Vasc Surg*.40 (1),146-53 (2004)

38. ME Davis, JP Motion, DA Narmoneva, T Takahashi, D Hakuno, RD Kamm, S Zhang and RT Lee: Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. *Circulation*.111 (4),442-50 (2005)

39. PC Hsieh, ME Davis, J Gannon, C MacGillivray and RT Lee: Controlled delivery of PDGF-BB for myocardial protection using injectable self-assembling peptide nanofibers. *J Clin Invest*.116 (1),237-48 (2006)

40. ME Davis, PC Hsieh, AJ Grodzinsky and RT Lee: Custom design of the cardiac microenvironment with biomaterials. *Circ Res*.97 (1),8-15 (2005)

41. ME Davis, PC Hsieh, T Takahashi, Q Song, S Zhang, RD Kamm, AJ Grodzinsky, P Anversa and RT Lee: 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*.103 (21),8155-60 (2006)

42. EJ Suuronen, JP Veinot, S Wong, V Kapila, J Price, M Griffith, TG Mesana and M Ruel: Tissue-engineered injectable collagen-based matrices for improved cell delivery and vascularization of ischemic tissue using CD133+ progenitors expanded from the peripheral blood. *Circulation*.114 (1 Suppl),I138-44 (2006)

43. P Zammaretti and M Jaconi: Cardiac tissue engineering: regeneration of the wounded heart. *Curr Opin Biotechnol*.15 (5),430-4 (2004)

44. WH Zimmermann, I Melnychenko and T Eschenhagen: Engineered heart tissue for regeneration of diseased hearts. *Biomaterials*.25 (9),1639-47 (2004)

45. QP Pham, U Sharma and AG Mikos: Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng*.12 (5),1197-211 (2006)

46. QP Pham, U Sharma and AG Mikos: Electrospun poly (epsilon-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules*.7 (10),2796-805 (2006)

47. T Courtney, MS Sacks, J Stankus, J Guan and WR Wagner: Design and analysis of tissue engineering scaffolds that mimic soft tissue mechanical anisotropy. *Biomaterials*.27 (19),3631-8 (2006)

48. O Ishii, M Shin, T Sueda and JP Vacanti: *In vitro* tissue engineering of a cardiac graft using a degradable scaffold with an extracellular matrix-like topography. *J Thorac Cardiovasc Surg*.130 (5),1358-63 (2005)

49. M Shin, O Ishii, T Sueda and JP Vacanti: Contractile cardiac grafts using a novel nanofibrous mesh. *Biomaterials*.25 (17),3717-23 (2004)

50. JJ Stankus, J Guan and WR Wagner: Fabrication of biodegradable elastomeric scaffolds with sub-micron morphologies. *J Biomed Mater Res A*.70 (4),603-14 (2004)

51. S Zhong, WE Teo, X Zhu, R Beuerman, S Ramakrishna and LY Yung: Formation of collagen-glycosaminoglycan blended nanofibrous scaffolds and their biological properties. *Biomacromolecules*.6 (6),2998-3004 (2005)

52. T Shimizu, M Yamato, Y Isoi, T Akutsu, T Setomaru, K Abe, A Kikuchi, M Umezu and T Okano: Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res*.90 (3),e40 (2002)

53. Q Ke, Y Yang, JS Rana, Y Chen, JP Morgan and YF Xiao: Embryonic stem cells cultured in biodegradable scaffold repair infarcted myocardium in mice. *Sheng Li Xue Bao*.57 (6),673-681 (2005)

54. WH Zimmermann and T Eschenhagen: Embryonic stem cells for cardiac muscle engineering. *Trends Cardiovasc Med*.17 (4),134-40 (2007)

55. WH Zimmermann, K Schneiderbanger, P Schubert, M Didie, F Munzel, JF Heubach, S Kostin, WL Neuhuber and T Eschenhagen: Tissue engineering of a differentiated cardiac muscle construct. *Circ Res*.90 (2),223-30 (2002)

56. M Cortes-Morichetti, G Frati, O Schussler, JP Van Huyen, E Lauret, JA Genovese, AF Carpentier and JC Chachques: Association Between a Cell-Seeded Collagen Matrix and Cellular Cardiomyoplasty for Myocardial Support and Regeneration. *Tissue Eng* (2007)

57. P Menasche: Skeletal myoblasts as a therapeutic agent. *Prog Cardiovasc Dis*.50 (1),7-17 (2007)

58. MA Laflamme, KY Chen, AV Naumova, V Muskheli, JA Fugate, SK Dupras, H Reinecke, C Xu, M Hassanipour, S Police, C O'Sullivan, L Collins, Y Chen, E Minami, EA Gill, S Ueno, C Yuan, J Gold and CE Murry: Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*.25 (9),1015-24 (2007)

59. Y Miyahara, N Nagaya, M Kataoka, B Yanagawa, K Tanaka, H Hao, K Ishino, H Ishida, T Shimizu, K Kangawa, S Sano, T Okano, S Kitamura and H Mori: Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med*.12 (4),459-65 (2006)

60. Y Zhang, X Cheng, J Wang, Y Wang, B Shi, C Huang, X Yang and T Liu: Novel chitosan/collagen scaffold containing transforming growth factor-beta1 DNA for periodontal tissue engineering. *Biochem Biophys Res Commun*.344 (1),362-9 (2006)

61. E Luong-Van, L Grondahl, KN Chua, KW Leong, V Nurcombe and SM Cool: Controlled release of heparin from poly (epsilon-caprolactone) electrospun fibers. *Biomaterials*.27 (9),2042-50 (2006)

62. G Zhang and LJ Suggs: Matrices and scaffolds for drug delivery in vascular tissue engineering. *Adv Drug Deliv Rev*.59 (4-5),360-73 (2007)

63. C Spadaccio, A Rainer, M Trombetta, G Vadala, M Chello, E Covino, V Denaro, Y Toyoda and JA Genovese: Poly-L-lactic acid/hydroxyapatite electrospun nanocomposites induce chondrogenic differentiation of human MSC. *Ann Biomed Eng*.37 (7),1376-89 (2009)

64. P Zilla, M Deutsch, J Meinhart, R Puschmann, T Eberl, E Minar, R Dudczak, H Lugmaier, P Schmidt, I Noszian and *et al.*: Clinical *in vitro* endothelialization of femoropopliteal bypass grafts: an actuarial follow-up over three years. *J Vasc Surg*.19 (3),540-8 (1994)

65. D Sheyn, O Mizrahi, S Benjamin, Z Gazit, G Pelled and D Gazit: Genetically modified cells in regenerative medicine and tissue engineering. *Adv Drug Deliv Rev*.62 (7-8),683-98 (2010)

66. Y Lei and T Segura: DNA delivery from matrix metalloproteinase degradable poly (ethylene glycol) hydrogels to mouse cloned mesenchymal stem cells. *Biomaterials*.30 (2),254-65 (2009)

67. SL Rong, YJ Wang, XL Wang, YX Lu, C Chang, FZ Wang, QY Liu, XY Liu, YZ Gao and SH Mi: Recombinant human growth hormone secreted from tissue-engineered bioartificial muscle improves left ventricular function in rat with acute myocardial infarction. *Chin Med J (Engl)*122 (19),2352-9 (2009)

68. E Ward, E Chan, K Gustafsson and SN Jayasinghe: Combining bio-electrospraying with gene therapy: a novel biotechnique for the delivery of genetic material via living cells. *Analyst*.135 (5),1042-9 (2010)

69. L De Laporte and LD Shea: Matrices and scaffolds for DNA delivery in tissue engineering. *Adv Drug Deliv Rev*.59 (4-5),292-307 (2007)

70. M Ou, TI Kim, JW Yockman, BA Borden, DA Bull and SW Kim: Polymer transfected primary myoblasts mediated efficient gene expression and angiogenic proliferation. *J Control Release*.142 (1),61-9 (2010)

71. SH Kim, JH Jeong, TI Kim, SW Kim and DA Bull: VEGF siRNA delivery system using arginine-grafted bioreducible poly (disulfide amine) *Mol Pharm*.6 (3),718-26 (2009)

72. C Spadaccio, A Rainer, M Trombetta, M Centola, M Lusini, M Chello, E Covino, F De Marco, R Coccia, Y Toyoda and JA Genovese: A G-CSF functionalized scaffold for stem cells seeding: a differentiating device for cardiac purposes. *J Cell Mol Med* (2010)

73. R Langer and DA Tirrell: Designing materials for biology and medicine. *Nature*.428 (6982),487-92 (2004)

74. MM Stevens and JH George: Exploring and engineering the cell surface interface. *Science*.310 (5751),1135-8 (2005)

75. Y Wan, Y Wang, Z Liu, X Qu, B Han, J Bei and S Wang: Adhesion and proliferation of OCT-1 osteoblast-like cells on micro- and nano-scale topography structured poly (L-lactide) *Biomaterials*.26 (21),4453-9 (2005)

76. EK Yim, RM Reano, SW Pang, AF Yee, CS Chen and KW Leong: Nanopattern-induced changes in morphology and motility of smooth muscle cells. *Biomaterials*.26 (26),5405-13 (2005)

77. W He, T Yong, ZW Ma, R Inai, WE Teo and S Ramakrishna: Biodegradable polymer nanofiber mesh to maintain functions of endothelial cells. *Tissue Eng*.12 (9),2457-66 (2006)

78. Z Ma, W He, T Yong and S Ramakrishna: Grafting of gelatin on electrospun poly (caprolactone) nanofibers to improve endothelial cell spreading and proliferation and to control cell Orientation. *Tissue Eng*.11 (7-8),1149-58 (2005)

79. HK Noh, SW Lee, JM Kim, JE Oh, KH Kim, CP Chung, SC Choi, WH Park and BM Min: Electrospinning of chitin nanofibers: degradation behavior and cellular response to normal human keratinocytes and fibroblasts. *Biomaterials*.27 (21),3934-44 (2006)

80. J Zeltinger, JK Sherwood, DA Graham, R Mueller and LG Griffith: Effect of pore size and void fraction on cellular adhesion, proliferation, and matrix deposition. *Tissue Eng*.7 (5),557-72 (2001)

81. MJ Moore, E Jabbari, EL Ritman, L Lu, BL Currier, AJ Windebank and MJ Yaszemski: Quantitative analysis of interconnectivity of porous biodegradable scaffolds with micro-computed tomography. *J Biomed Mater Res A*.71 (2),258-67 (2004)

82. SJ Hollister: Porous scaffold design for tissue engineering. *Nat Mater*.4 (7),518-24 (2005)

83. J Glowacki, S Mizuno and JS Greenberger: Perfusion enhances functions of bone marrow stromal cells in three-dimensional culture. *Cell Transplant*.7 (3),319-26 (1998)

84. TS Karande, JL Ong and CM Agrawal: Diffusion in musculoskeletal tissue engineering scaffolds: design issues related to porosity, permeability, architecture, and nutrient mixing. *Ann Biomed Eng*.32 (12),1728-43 (2004)

85. A Salerno, D Guarnieri, M Iannone, S Zeppetelli, E Di Maio, S Iannace and PA Netti: Engineered mu-bimodal poly (epsilon-caprolactone) porous scaffold for enhanced hMSC colonization and proliferation. *Acta Biomater*.5 (4),1082-93 (2009)

86. M Lovett, D Rockwood, A Baryshyan and DL Kaplan: Simple Modular Bioreactors for Tissue Engineering: A System for Characterization of Oxygen Gradients, Human Mesenchymal Stem Cell Differentiation, and Prevascularization. *Tissue Eng Part C Methods* (2010)

87. F Causa, PA Netti and L Ambrosio: A multi-functional scaffold for tissue regeneration: the need to engineer a tissue analogue. *Biomaterials*.28 (34),5093-9 (2007)

88. J Xu, X Liu, Y Jiang, L Chu, H Hao, Z Liua, C Verfaillie, J Zweier, K Gupta and Z Liu: MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell differentiation into endothelial cell. *J Cell Mol Med*.12 (6A),2395-406 (2008)

89. LS Nair, S Bhattacharyya and CT Laurencin: Development of novel tissue engineering scaffolds via electrospinning. *Expert Opin Biol Ther*.4 (5),659-68 (2004)

90. MS Khil, SR Bhattarai, HY Kim, SZ Kim and KH Lee: Novel fabricated matrix via electrospinning for tissue engineering. *J Biomed Mater Res B Appl Biomater*.72 (1),117-24 (2005)

91. GH Kim: Electrospun PCL nanofibers with anisotropic mechanical properties as a biomedical scaffold. *Biomed Mater*.3 (2),25010 (2008)

92. X Zong, H Bien, CY Chung, L Yin, D Fang, BS Hsiao, B Chu and E Entcheva: Electrospun fine-textured scaffolds for heart tissue constructs. *Biomaterials*.26 (26),5330-8 (2005)

93. JD Fromstein, PW Zandstra, C Alperin, D Rockwood, JF Rabolt and KA Woodhouse: Seeding bioreactor-produced embryonic stem cell-derived cardiomyocytes on different porous, degradable, polyurethane scaffolds reveals the effect of scaffold architecture on cell morphology. *Tissue Eng Part A*.14 (3),369-78 (2008)

94. MC McManus, ED Boland, HP Koo, CP Barnes, KJ Pawlowski, GE Wnek, DG Simpson and GL Bowlin: Mechanical properties of electrospun fibrinogen structures. *Acta Biomater*.2 (1),19-28 (2006)

95. Y Zhu, Y Cao, J Pan and Y Liu: Macro-alignment of electrospun fibers for vascular tissue engineering. *J Biomed Mater Res B Appl Biomater*.92 (2),508-16

96. MP Rubbens, A Driessen-Mol, RA Boerboom, MM Koppert, HC van Assen, BM TerHaar Romeny, FP Baaijens and CV Bouten: Quantification of the temporal evolution of collagen orientation in mechanically conditioned engineered cardiovascular tissues. *Ann Biomed Eng*.37 (7),1263-72 (2009)

97. T Barkhausen, M van Griensven, J Zeichen and U Bosch: Modulation of cell functions of human tendon fibroblasts by different repetitive cyclic mechanical stress patterns. *Exp Toxicol Pathol*.55 (2-3),153-8 (2003)

98. DW Hamilton, TM Maul and DA Vorp: Characterization of the response of bone marrow-derived progenitor cells to cyclic strain: implications for vascular

tissue-engineering applications. *Tissue Eng*.10 (3-4),361-9 (2004)

99. SP Hoerstrup, R Sodian, S Daebritz, J Wang, EA Bacha, DP Martin, AM Moran, KJ Guleserian, JS Sperling, S Kaushal, JP Vacanti, FJ Schoen and JE Mayer, Jr.: Functional living trileaflet heart valves grown *in vitro*. *Circulation*.102 (19 Suppl 3),III44-9 (2000)

100. MP Rubbens, A Mol, RA Boerboom, RA Bank, FP Baaijens and CV Bouten: Intermittent straining accelerates the development of tissue properties in engineered heart valve tissue. *Tissue Eng Part A*.15 (5),999-1008 (2009)

101. GC Engelmayr, Jr., E Rabkin, FW Sutherland, FJ Schoen, JE Mayer, Jr. and MS Sacks: The independent role of cyclic flexure in the early *in vitro* development of an engineered heart valve tissue. *Biomaterials*.26 (2),175-87 (2005)

102. RL Carrier, M Rupnick, R Langer, FJ Schoen, LE Freed and G Vunjak-Novakovic: Perfusion improves tissue architecture of engineered cardiac muscle. *Tissue Eng*.8 (2),175-88 (2002)

103. M Radisic, L Yang, J Boublik, RJ Cohen, R Langer, LE Freed and G Vunjak-Novakovic: Medium perfusion enables engineering of compact and contractile cardiac tissue. *Am J Physiol Heart Circ Physiol*.286 (2),H507-16 (2004)

104. M Radisic, H Park, F Chen, JE Salazar-Lazzaro, Y Wang, R Dennis, R Langer, LE Freed and G Vunjak-Novakovic: Biomimetic approach to cardiac tissue engineering: oxygen carriers and channeled scaffolds. *Tissue Eng*.12 (8),2077-91 (2006)

105. WH Zimmermann, M Didie, GH Wasmeier, U Nixdorff, A Hess, I Melnychenko, O Boy, WL Neuhuber, M Weyand and T Eschenhagen: Cardiac grafting of engineered heart tissue in syngenic rats. *Circulation*.106 (12 Suppl 1),I151-7 (2002)

106. M Radisic, H Park, H Shing, T Consi, FJ Schoen, R Langer, LE Freed and G Vunjak-Novakovic: Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. *Proc Natl Acad Sci U S A*.101 (52),18129-34 (2004)

107. Z Feng, T Matsumoto, Y Nomura and T Nakamura: An electro-tensile bioreactor for 3-D culturing of cardiomyocytes. A bioreactor system that simulates the myocardium's electrical and mechanical response *in vivo*. *IEEE Eng Med Biol Mag*.24 (4),73-9 (2005)

108. M Radisic, H Park, S Gerecht, C Cannizzaro, R Langer and G Vunjak-Novakovic: Biomimetic approach to cardiac tissue engineering. *Philos Trans R Soc Lond B Biol Sci*.362 (1484),1357-68 (2007)

109. N Tandon, C Cannizzaro, PH Chao, R Maidhof, A Marsano, HT Au, M Radisic and G Vunjak-Novakovic: Electrical stimulation systems for cardiac tissue engineering. *Nat Protoc*.4 (2),155-73 (2009)

110. A Perets, Y Baruch, F Weisbuch, G Shoshany, G Neufeld and S Cohen: Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. *J Biomed Mater Res A*.65 (4),489-97 (2003)

111. A Kedem, A Perets, I Gamlieli-Bonshtein, M Dvir-Ginzberg, S Mizrahi and S Cohen: Vascular endothelial growth factor-releasing scaffolds enhance vascularization and engraftment of hepatocytes transplanted on liver lobes. *Tissue Eng*.11 (5-6),715-22 (2005)

112. JL Cleland, ET Duenas, A Park, A Daugherty, J Kahn, J Kowalski and A Cuthbertson: Development of poly-(D,L-lactide--coglycolide) microsphere formulations containing recombinant human vascular endothelial growth factor to promote local angiogenesis. *J Control Release*.72 (1-3),13-24 (2001)

113. Y Tabata: Tissue regeneration based on tissue engineering technology. *Congenit Anom (Kyoto)*44 (3),111-24 (2004)

114. KY Lee, MC Peters and DJ Mooney: Comparison of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in SCID mice. *J Control Release*.87 (1-3),49-56 (2003)

115. AH Zisch, MP Lutolf and JA Hubbell: Biopolymeric delivery matrices for angiogenic growth factors. *Cardiovasc Pathol*.12 (6),295-310 (2003)

116. J Ziegler, U Mayr-Wohlfart, S Kessler, D Breitig and KP Gunther: Adsorption and release properties of growth factors from biodegradable implants. *J Biomed Mater Res*.59 (3),422-8 (2002)

117. G Wei, Q Jin, WV Giannobile and PX Ma: Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB. *J Control Release*.112 (1),103-10 (2006)

118. SD Patil, F Papadimitrakopoulos and DJ Burgess: Dexamethasone-loaded poly (lactic-co-glycolic) acid microspheres/poly (vinyl alcohol) hydrogel composite coatings for inflammation control. *Diabetes Technol Ther*.6 (6),887-97 (2004)

119. WL Murphy, MC Peters, DH Kohn and DJ Mooney: Sustained release of vascular endothelial growth factor from mineralized poly (lactide-co-glycolide) scaffolds for tissue engineering. *Biomaterials*.21 (24),2521-7 (2000)

120. DD Hile and MV Pishko: Solvent-free protein encapsulation within biodegradable polymer foams. *Drug Deliv*.11 (5),287-93 (2004)

121. H Lee, RA Cusick, F Browne, T Ho Kim, PX Ma, H Utsunomiya, R Langer and JP Vacanti: Local delivery of

basic fibroblast growth factor increases both angiogenesis and engraftment of hepatocytes in tissue-engineered polymer devices. *Transplantation*.73 (10),1589-93 (2002)

122. J Guan, JJ Stankus and WR Wagner: Biodegradable elastomeric scaffolds with basic fibroblast growth factor release. *J Control Release*.120 (1-2),70-8 (2007)

123. HS Yoo, EA Lee, JJ Yoon and TG Park: Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. *Biomaterials*.26 (14),1925-33 (2005)

124. JJ Yoon, SH Song, DS Lee and TG Park: Immobilization of cell adhesive RGD peptide onto the surface of highly porous biodegradable polymer scaffolds fabricated by a gas foaming/salt leaching method. *Biomaterials*.25 (25),5613-20 (2004)

125. JJ Yoon, YS Nam, JH Kim and TG Park: Surface immobilization of galactose onto aliphatic biodegradable polymers for hepatocyte culture. *Biotechnol Bioeng*.78 (1),1-10 (2002)

126. TG Park: Perfusion culture of hepatocytes within galactose-derivatized biodegradable poly (lactide-co-glycolide) scaffolds prepared by gas foaming of effervescent salts. *J Biomed Mater Res*.59 (1),127-35 (2002)

127. MJ Wissink, R Beernink, AA Poot, GH Engbers, T Beugeling, WG van Aken and J Feijen: Improved endothelialization of vascular grafts by local release of growth factor from heparinized collagen matrices. *J Control Release*.64 (1-3),103-14 (2000)

128. M Ishihara, M Sato, H Hattori, Y Saito, H Yura, K Ono, K Masuoka, M Kikuchi, K Fujikawa and A Kurita: Heparin-carrying polystyrene (HCPS)-bound collagen substratum to immobilize heparin-binding growth factors and to enhance cellular growth. *J Biomed Mater Res*.56 (4),536-44 (2001)

129. D Gospodarowicz and J Cheng: Heparin protects basic and acidic FGF from inactivation. *J Cell Physiol*.128 (3),475-84 (1986)

130. JJ Yoon, HJ Chung, HJ Lee and TG Park: Heparin-immobilized biodegradable scaffolds for local and sustained release of angiogenic growth factor. *J Biomed Mater Res A*.79 (4),934-42 (2006)

131. M Centola, A Rainer, C Spadaccio, S De Porcellinis, JA Genovese and M Trombetta: Combining electrospinning and fused deposition modeling for the fabrication of a hybrid vascular graft. *Biofabrication*.2 (1),014102 (2010)

132. A Rainer, M Centola, C Spadaccio, S De Porcellinis, F Abbruzzese, J Genovese and M Trombetta: A biomimetic three-layered compartmented scaffold for vascular tissue engineering. *Engineering in Medicine and Biology Society*.EMBC Annual International Conference of the IEEE (In press) (2010)

133. H Drexler, GP Meyer and KC Wollert: Bone-marrow-derived cell transfer after ST-elevation myocardial infarction: lessons from the BOOST trial. *Nat Clin Pract Cardiovasc Med*.3 Suppl 1,S65-8 (2006)

134. HJ Kang, HS Kim, BK Koo, YJ Kim, D Lee, DW Sohn, BH Oh and YB Park: Intracoronary infusion of the mobilized peripheral blood stem cell by G-CSF is better than mobilization alone by G-CSF for improvement of cardiac function and remodeling: 2-year follow-up results of the Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion (MAGIC Cell) 1 trial. *Am Heart J*.153 (2),237 e1-8 (2007)

135. HJ Kang, HS Kim, SY Zhang, KW Park, HJ Cho, BK Koo, YJ Kim, D Soo Lee, DW Sohn, KS Han, BH Oh, MM Lee and YB Park: Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet*.363 (9411),751-6 (2004)

136. HJ Kang, HY Lee, SH Na, SA Chang, KW Park, HK Kim, SY Kim, HJ Chang, W Lee, WJ Kang, BK Koo, YJ Kim, DS Lee, DW Sohn, KS Han, BH Oh, YB Park and HS Kim: Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. *Circulation*.114 (1 Suppl),I145-51 (2006)

137. M Harada, Y Qin, H Takano, T Minamino, Y Zou, H Toko, M Ohtsuka, K Matsuura, M Sano, J Nishi, K Iwanaga, H Akazawa, T Kunieda, W Zhu, H Hasegawa, K Kunisada, T Nagai, H Nakaya, K Yamauchi-Takihara and I Komuro: G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. *Nat Med*.11 (3),305-11 (2005)

138. Y Sugano, T Anzai, T Yoshikawa, Y Maekawa, T Kohno, K Mahara, K Naito and S Ogawa: Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction. *Cardiovasc Res*.65 (2),446-56 (2005)

139. D Orlic: Adult bone marrow stem cells regenerate myocardium in ischemic heart disease. *Ann N Y Acad Sci*.996,152-7 (2003)

140. D Orlic, J Kajstura, S Chimenti, DM Bodine, A Leri and P Anversa: Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci*.938,221-9; discussion 229-30 (2001)

141. D Orlic, J Kajstura, S Chimenti, DM Bodine, A Leri and P Anversa: Bone marrow stem cells regenerate infarcted myocardium. *Pediatr Transplant*.7 Suppl 3,86-8 (2003)

142. D Orlic, J Kajstura, S Chimenti, I Jakoniuk, SM Anderson, B Li, J Pickel, R McKay, B Nadal-Ginard, DM Bodine, A Leri and P Anversa: Bone marrow cells regenerate infarcted myocardium. *Nature*.410 (6829),701-5 (2001)

143. D Orlic, J Kajstura, S Chimenti, F Limana, I Jakoniuk, F Quaini, B Nadal-Ginard, DM Bodine, A Leri and P Anversa: Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A*.98 (18),10344-9 (2001)

144. JC Chachques, J Herreros, J Trainini, A Juffe, E Rendal, F Prosper and J Genovese: Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. *Int J Cardiol*.95 Suppl 1,S29-33 (2004)

145. R Gruber, F Karreth, F Frommlet, MB Fischer and G Watzek: Platelets are mitogenic for periosteum-derived cells. *J Orthop Res*.21 (5),941-8 (2003)

146. CE Murry, LJ Field and P Menasche: Cell-based cardiac repair: reflections at the 10-year point. *Circulation*.112 (20),3174-83 (2005)

147. M Rubart, MH Soonpaa, H Nakajima and LJ Field: Spontaneous and evoked intracellular calcium transients in donor-derived myocytes following intracardiac myoblast transplantation. *J Clin Invest*.114 (6),775-83 (2004)

148. B Leobon, I Garcin, P Menasche, JT Vilquin, E Audinat and S Charpak: Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A*.100 (13),7808-11 (2003)

149. M Siepe, MN Giraud, E Liljensten, U Nydegger, P Menasche, T Carrel and HT Tevaearai: Construction of skeletal myoblast-based polyurethane scaffolds for myocardial repair. *Artif Organs*.31 (6),425-33 (2007)

150. M Siepe, MN Giraud, M Pavlovic, C Receputo, F Beyersdorf, P Menasche, T Carrel and HT Tevaearai: Myoblast-seeded biodegradable scaffolds to prevent post-myocardial infarction evolution toward heart failure. *J Thorac Cardiovasc Surg*.132 (1),124-31 (2006)

151. DJ Mooney, PM Kaufmann, K Sano, KM McNamara, JP Vacanti and R Langer: Transplantation of hepatocytes using porous, biodegradable sponges. *Transplant Proc*.26 (6),3425-6 (1994)

152. M Kuwabara, Y Kakinuma, RG Katare, M Ando, F Yamasaki, Y Doi and T Sato: Granulocyte colony-stimulating factor activates Wnt signal to sustain gap junction function through recruitment of beta-catenin and cadherin. *FEBS Lett*.581 (25),4821-30 (2007)

153. DL Lerner, KA Yamada, RB Schuessler and JE Saffitz: Accelerated onset and increased incidence of ventricular arrhythmias induced by ischemia in Cx43-deficient mice. *Circulation*.101 (5),547-52 (2000)

154. HV van Rijen, D Eckardt, J Degen, M Theis, T Ott, K Willecke, HJ Jongsma, T Opthof and JM de Bakker: Slow conduction and enhanced anisotropy increase the propensity for ventricular tachyarrhythmias in adult mice with induced deletion of connexin43. *Circulation*.109 (8),1048-55 (2004)

155. AH Zisch, U Schenk, JC Schense, SE Sakiyama-Elbert and JA Hubbell: Covalently conjugated VEGF--fibrin matrices for endothelialization. *J Control Release*.72 (1-3),101-13 (2001)

156. SE Sakiyama-Elbert and JA Hubbell: Development of fibrin derivatives for controlled release of heparin-binding growth factors. *J Control Release*.65 (3),389-402 (2000)

157. S Koch, C Yao, G Grieb, P Prevel, EM Noah and GC Steffens: Enhancing angiogenesis in collagen matrices by covalent incorporation of VEGF. *J Mater Sci Mater Med*.17 (8),735-41 (2006)

158. P Lijnen, V Petrov and R Fagard: Transforming growth factor-beta 1-mediated collagen gel contraction by cardiac fibroblasts. *J Renin Angiotensin Aldosterone Syst*.4 (2),113-8 (2003)

159. AG Ravin, KC Olbrich, LS Levin, AL Usala and B Klitzman: Long- and short-term effects of biological hydrogels on capsule microvascular density around implants in rats. *J Biomed Mater Res*.58 (3),313-8 (2001)

160. JA Thompson, KD Anderson, JM DiPietro, JA Zwiebel, M Zametta, WF Anderson and T Maciag: Site-directed neovessel formation *in vivo*. *Science*.241 (4871),1349-52 (1988)

161. M Yamamoto, Y Ikada and Y Tabata: Controlled release of growth factors based on biodegradation of gelatin hydrogel. *J Biomater Sci Polym Ed*.12 (1),77-88 (2001)

162. M Ozeki, T Ishii, Y Hirano and Y Tabata: Controlled release of hepatocyte growth factor from gelatin hydrogels based on hydrogel degradation. *J Drug Target*.9 (6),461-71 (2001)

163. H Kobayashi, S Minatoguchi, S Yasuda, N Bao, I Kawamura, M Iwasa, T Yamaki, S Sumi, Y Misao, H Ushikoshi, K Nishigaki, G Takemura, T Fujiwara, Y Tabata and H Fujiwara: Post-infarct treatment with an erythropoietin-gelatin hydrogel drug delivery system for cardiac repair. *Cardiovasc Res*.79 (4),611-20 (2008)

164. KY Lee, MC Peters, KW Anderson and DJ Mooney: Controlled growth factor release from synthetic extracellular matrices. *Nature*.408 (6815),998-1000 (2000)

165. FM Andreopoulos and I Persaud: Delivery of basic fibroblast growth factor (bFGF) from photoresponsive hydrogel scaffolds. *Biomaterials*.27 (11),2468-76 (2006)

166. S Cai, Y Liu, X Zheng Shu and GD Prestwich: Injectable glycosaminoglycan hydrogels for controlled

release of human basic fibroblast growth factor. *Biomaterials*.26 (30),6054-67 (2005)

167. H Wang, X Zhang, Y Li, Y Ma, Y Zhang, Z Liu, J Zhou, Q Lin, Y Wang, C Duan and C Wang: Improved myocardial performance in infarcted rat heart by co-injection of basic fibroblast growth factor with temperature-responsive chitosan hydrogel. *J Heart Lung Transplant*.29 (8),881-7 (2010)

168. LS Liu, CK Ng, AY Thompson, JW Poser and RC Spiro: Hyaluronate-heparin conjugate gels for the delivery of basic fibroblast growth factor (FGF-2) *J Biomed Mater Res*.62 (1),128-35 (2002)

169. SK Hahn, JS Kim and T Shimobouji: Injectable hyaluronic acid microhydrogels for controlled release formulation of erythropoietin. *J Biomed Mater Res A*.80 (4),916-24 (2007)

170. JJ Yoon, JH Kim and TG Park: Dexamethasone-releasing biodegradable polymer scaffolds fabricated by a gas-foaming/salt-leaching method. *Biomaterials*.24 (13),2323-9 (2003)

171. Q Sun, RR Chen, Y Shen, DJ Mooney, S Rajagopalan and PM Grossman: Sustained vascular endothelial growth factor delivery enhances angiogenesis and perfusion in ischemic hind limb. *Pharm Res*.22 (7),1110-6 (2005)

172. DD Hile, ML Amirpour, A Akgerman and MV Pishko: Active growth factor delivery from poly (D,L-lactide-co-glycolide) foams prepared in supercritical CO (2) *J Control Release*.66 (2-3),177-85 (2000)

173. WL Murphy and DJ Mooney: Controlled delivery of inductive proteins, plasmid DNA and cells from tissue engineering matrices. *J Periodontal Res*.34 (7),413-9 (1999)

174. T Wang, XJ Jiang, T Lin, S Ren, XY Li, XZ Zhang and QZ Tang: The inhibition of postinfarct ventricle remodeling without polycythaemia following local sustained intramyocardial delivery of erythropoietin within a supramolecular hydrogel. *Biomaterials*.30 (25),4161-7 (2009)

175. HW Jun, LJ Taite and JL West: Nitric oxide-producing polyurethanes. *Biomacromolecules*.6 (2),838-44 (2005)

176. N Davies, S Dobner, D Bezuidenhout, C Schmidt, M Beck, AH Zisch and P Zilla: The dosage dependence of VEGF stimulation on scaffold neovascularisation. *Biomaterials*.29 (26),3531-8 (2008)

177. Y Yeo, W Geng, T Ito, DS Kohane, JA Burdick and M Radisic: Photocrosslinkable hydrogel for myocyte cell culture and injection. *J Biomed Mater Res B Appl Biomater*.81 (2),312-22 (2007)

178. G Zhang, Y Nakamura, X Wang, Q Hu, LJ Suggs and J Zhang: Controlled release of stromal cell-derived factor-1 alpha *in situ* increases c-kit+ cell homing to the infarcted heart. *Tissue Eng*.13 (8),2063-71 (2007)

Key Words: Tissue Engineering, Stem Cells, Cell Therapy, Scaffolds, Electrospinning, Review

Send correspondence to: Jorge A. Genovese, University Campus Bio-Medico of Rome, Department of Cardiovascular Science, Unit of Cardiovascular Surgery, Via Alvaro del Portillo 200, 00128 Rome, Italy, Tel: 3906225411140, Fax: 3906225411028, E-mail: genoveseja@yahoo.com