Handbook of Regenerative Medicine and Tissue Engineering

Shay Fisher

Volume II

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Edited by **Shay Fisher**

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Preface

Over the recent decade, advancements and applications have progressed exponentially. This has led to the increased interest in this field and projects are being conducted to enhance knowledge. The main objective of this book is to present some of the critical challenges and provide insights into possible solutions. This book will answer the varied questions that arise in the field and also provide an increased scope for furthering studies.

The basic concept of regenerative medicine and tissue engineering is intriguing for physicians and scientists as it involves healing tissues or organ defects that the present medical practice finds difficult or impossible to cure. Tissue engineering involves cells, materials methods and engineering supported by appropriate physiochemical and biological factors to enhance or replace biologic functions. Regenerative medicine is a new division of medicine which aims to change the course of chronic disease and regenerate failing organ systems lost due to damage, age, disease and congenital defects. This book reflects state-of-the-art of these two disciplines at this time, as well as their therapeutic application. It discusses various topics under scaffolds and matrices. This book provides as a reference for physicians, scientists and students and as an explanatory analysis for individuals in pharmaceuticals and biotech companies.

I hope that this book, with its visionary approach, will be a valuable addition and will promote interest among readers. Each of the authors has provided their extraordinary competence in their specific fields by providing different perspectives as they come from diverse nations and regions. I thank them for their contributions.

Editor

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Scaffolds and Matrices

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Chapter 1

Biomaterials for Cardiac Tissue Engineering

M. Arnal-Pastor, J. C. Chachques,
M. Monleón Pradas and A. Vallés-Lluch

Additional information is available at the end of the chapter

1. Introduction

1.1. Cardiovascular diseases

Cardiovascular diseases (CVD) are a leading death cause in developed countries (1 of every 3 deaths in the United States in 2008) [1]. Changes in diet and habits are causing CVD to become major mortality pathologies in developing countries too [2] (they are already responsible for a 30% of the world deaths). This group of diseases constitutes a great burden for the national health systems, consuming great percentages of the health systems budgets. In the particular case of the coronary heart diseases (CHD), 3,8 million men and 3,4 million women die a year worldwide because of them [3]. In the United States 1 of every 6 deaths in 2008 was caused by CHD [1].

The heart is a complex organ that pumps 7000 liters of blood to all the tissues in the body per day [4]. This pumping function precisely determines its anatomy. Heart tissue basically is formed by cardiac myocytes (contractile elements) [5], smooth muscle cells, fibroblasts, blood vessels, nerves and the extracellular matrix components (cardiac interstitium and collagen) [6] organized in a very particular way. Myocytes form muscular fibers with changing orientation across the ventricular wall up to 180º [7]. At the same time, muscular fibers are organized into myocardial laminas 4-6 myocytes thick separated from neighboring laminas by extracellular collagen [8]. The particular arrangement of the ventricular myocytes influence the mechanical and electrical function of the heart and small changes in it can lead to severe changes in these functions [9].

The extracellular matrix (ECM) connects the cells into a 3D architecture allowing the coupling of the forces produced by the myocytes. The anatomical model proposed by Torrent-Guasp [8], which considers the heart one muscle band plied in a double helical loop, explains how the

ventricles contract and get an efficient pumping in every heart beat, achieving an ejection fraction of the 60% when sarcomeres individually contract 15% only [10].

Myocytes are intimately connected, forming a functional syncytium [8]. Each myocardial cell is coupled in average to 9,1 ± 2,2 [11] myocytes, by 99 [12] gap junctions where the transfer of ionic currents takes place. Gap junctions are a specialized form of cell connection; they are formed by a cluster of ionic channels essential to the rapid propagation of the action potential. The action potential is the electrical impulse responsible for the contraction of the cells [13]. A proper electrical coupling of the cells is critical to avoid arrhythmias and reentries and essential for the contraction to spread as a wave front.

Acute myocardial infarction (AMI) occurs when a coronary artery is clogged, in 80% of the cases, by coronary atherosclerosis with superimposed luminal thrombus [14]. This occlusion leaves the downstream zone of the heart without blood supply, what means lack of oxygen, nutrients and metabolites wash for the affected zone. As a consequence, the aerobic metabolism changes to anaerobic glycolysis [14], leading to a decrease in the pH and reduction in the contractile function. Within 20 to 40 minutes without blood supply cells start to die and as times passes more myocardial tissue is compromised. There is also a zone of the heart affected by the infarction, where myocytes remain viable but lower their activity to reduce the metabolism and oxygen consumption to survive under hypoxic conditions; they can recover their contractibility after revascularization [15].

Clinical practices aim to limit the severity and extension of the AMI by rapidly restoring the blood flow (reperfusion), alleviating the oxygen demand [16] and reducing reperfusion injury. This can be done with different treatments or combinations of them. Pharmacological approaches involve the use of anticoagulant therapies and thrombolytic drugs to eliminate the clot. Vasodilatators like nitrates are also used to favor the dilation of the vessels, aspirin to avoid platelet aggregation, betabloqueants to reduce the heart pace, as well as morphine to reduce the pain are employed. Another group of therapies are the percutaneous coronary interventions; they physically reopen the vessel via catheterization. There are different techniques: the regular angioplasty uses a catheter with a balloon that is inflated in the place of the thrombus to reopen the lumen [17], or allows the permanent implantation of a stent in the vessel to keep it open. There is a wide variety of these devices depending on their composition, whether they release drugs or are biodegradable or not, etc [18, 19].

These therapies restore the blood flow to the infarcted zone; but reperfusion therapy is not exempt of risks: it is a complex process that can induce apoptosis by the microenvironmental changes that the recovery of the blood supply induces (formation of free radicals, calcium release, neutrophils, etc.) [20]. So it has to be done carefully and there is always a compromise between limiting the infarction extension due to the time without oxygen and the induced apoptosis due to the reperfusion. Reperfusion done soon after the onset of the ischemia is very advantageous, saving more tissue by restoring the blood flow than the tissue that will be lost because of the toxic substances released in the reperfusion. All the aforementioned treatments basically limit the damage of the acute episode but do not regenerate the damaged tissue and do not avoid the subsequent ventricular remodeling following an AMI.

In the infarcted area there is a great number of dead myocytes, and the host response to the injury consists in activating the inflammatory response and producing cytokines [21]. Thereupon neutrophils, monocytes and macrophages migrate into this area to remove the necrotic tissue [22]. Then, matrix metalloproteases (MMPs) are activated, which have a deleterious effect on the collagen matrix of the heart and in the surrounding coronary vasculature by degrading them [23]. The weakening of the collagen leads to wall thinning and ventricular dilation, as well as mural realignment of myocytes bundles [24]. After the inflammatory phase and the resorption of the necrotic tissue, there is an increase in the deposition of cross-linked collagen in the infarcted area that leads to scar tissue formation. During the remodelling process a change in the collagen composition occurs, the type I collagen fraction is reduced from 80% to 40% and the collagen III is increased [25].

Against what it was thought, this scar is a living tissue with a fibroblast-like cell population nourished by a neovasculature; these cells regulate the collagen turnover of the scar tissue [22]. The scar tissue has a reduced or absent contractility as compared with the original healthy myocardium [26], what leads to a reduction in the overall cardiac function [27].

The remodeling process initially is a compensatory mechanism to overcome the loss of contractile tissue. But with time this adaptative process of overload becomes maladaptative [15]. To compensate the additional effort, the remaining beating tissue hypertrophies trying to overcome the reduction in the cardiac function. This overload leads to myocyte slippage and fibrotic interstitial growth and to a degenerating process that may end in heart failure. The heart remodeling produces in the ventricles a set of anatomical and functional changes, including increased wall stress, slimming of the wall, chamber dilation, increase of the sphericity, and a significant loss of cardiac function.

The ventricular shape change from elliptical to spherical reduces its ejection fraction, because of a change in the apical loop fiber orientation [28]. Another problem caused by the shape change is that the papillary muscles are separated, what leads to regurgitation, contributing to the overload of the heart [24]. Besides, remodeled hearts are more prone to suffer arrhythmias as the membrane potential is altered and because of the interstitial fibrotic growth that may affect conductivity [15].

The end stage of the degeneration is the heart failure, when the heart is unable to pump enough blood to match the metabolic needs of the tissues. Current treatments aim to avoid reaching this point. Pharmacological treatments aspire to reduce the work load and to protect the cardiac tissues from the accumulated harmful substances [29]. Surgical therapy involves different techniques with different objectives: to restore a proper blood flow in areas that lack it (bypass surgery), to restore the normal elliptical geometry (Dor and Batista procedures), to restore the wall stress to normal (Dynamic Cardiomyoplasty), to limit the pathologic dilation, etc [10].

1.2. Cell therapy and cardiac tissue engineering

For many years, the heart has been considered a fully differentiated organ, with no myocyte regeneration after birth [30]. Recently it has been proved that myocytes have a limited regenerative capacity, around 1% of the cells per year at the age of 20 and it is reduced to 0,3%

at the age of 75 [31]. This regenerative capacity is achieved thanks to a small population of cardiac stem cells [32]. Nevertheless, their regenerative capacity is limited and in any case it is not enough to regenerate the heart if it suffers severe damage, like the one provoked by a myocardial infarction. New therapies under development like cell therapy or tissue engineering, aim to boost this limited regenerative potential of the native tissue by employing cells, drugs, factors or patches.

The aim of cardiac cell therapy is to heal the damaged infarcted tissue by the implantation of cells into or onto the pathologic myocardium by different techniques (figure 1 a). In tissue engineering strategies, different types of cells have been combined with materials and with bioactive molecules if necessary to again try to recover the injured tissue. The employed materials will support cells, provide them 3D organization, protect them, stimulate and guide its growth, maintain them in the site of interest, etc.; in sum, they will act as an artificial extracellular matrix during the regeneration process. But the use of materials either injectable, or *ex vivo* conformed (gels –patches- or scaffolds) (figure 1 b) has an additional and important effect: the implantation of a material in the scarred ventricular wall, increases its thickness and by Laplace's law, this increase leads to a reduction in the wall stress. This side-effect could be by itself very positive, even although regeneration did not arrive to happen, to limit ventricular remodeling and improve the quality of life of cardiac patients [29].

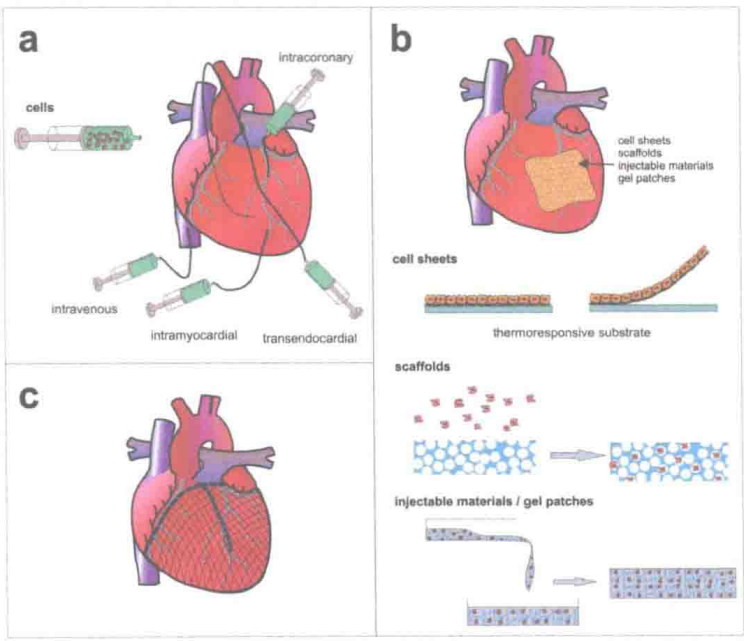

Figure 1. (a) Classical cell therapy in the heart (*freely inspired* in Strauer BE, Kornowski R, *Circulation* 2003; 107: 929-934). (b) Tissue engineering approaches with cell sheets, scaffolds or injectable materials (*freely inspired in* Masuda S *et al*, *Adv. Drug Del. Revs* 2008; 60(2): 277-85). (c) Ventricular restrain device.

2. Cardiomyoplasty

2.1. Need for cell cardiomyoplasty

Cardiomyoplasty has evolved from "dynamic" to "cellular cardiomyoplasty". The term dynamic cardiomyoplasty is referred to a surgical procedure developed in 1987 [33] to wrap the heart with the latissimus dorsi muscle, aiming to support the heart beating and limit the remodeling. Nevertheless, the obtained results were not as good as expected. With the advances in cell therapy, cellular cardiomyoplasty appeared as a promising therapeutical approach. This name encloses the therapies that use the injection of cells, from different origins, directly into the heart to try to obtain an improvement in the reduced heart function after an ischemic insult (figure 1 a).

The injected cells are envisaged to induce angiogenesis, inhibit apoptosis, help to recover hibernating myocardium, activate endogenous repair mechanisms, and create new contractile tissue that will replace the damaged one. Also they are expected to reverse the remodeling process that provoked ventricular dilation [34]. Many cells have been employed and the initial promising results obtained in animal models made this technique moved very fast to clinical trials, even if the mechanisms involved in the observed improvements were unknown. Unfortunately, the results obtained from the clinical trials were not as good as expected, and some were contradictory between them. One possible contributing cause to this discrepancy is that studies are carried out in young healthy animals, while patients susceptible to receive these treatments normally are aged people and in many cases with other co-morbidities [35].

Different ways to deliver cells into the damaged heart have been explored: intracoronary infusion (with the hope that cells will migrate through the vessels and be hosted in the infarcted area) or directly into the infarcted area either by intramyocardial or endocardial injection [36], as shown in figure 1 a. The advantage of injecting them directly into the infarcted area is that this will ensure that the cells are delivered in the site of interest.

2.2. Related problematic

Many different cell types have been employed in the numerous studies that have been done. Autologous cell sources are interesting because they do not require immunosuppression treatment of the patient and there is no risk of illness transmission. On the contrary, allogenic cells could be ready to use whenever a patient needs them, but would require immunosuppressive therapy after their implantation, and there is always a remaining risk of illness transmission. Another disadvantage is that prior to implantation cells need to be extracted and expanded. This whole process in some cases may take several weeks, limiting its application in the acute state. Besides, autologous cells coming from patients that suffer other conditions like diabetes or are simply aged, may have limited proliferation and attachment [37].

An important aspect of this technique is the low engraftment into the heart tissue of the supplied cells. The retention of the cells in the heart seems to be determined by the cell type and delivery route [38]. It has been estimated that in humans 50-75 min after intracoronary injection of bone marrow cells only 1,3-2,6% of the injected cells remain in the myocardium

[39]; after 2 hours less than 10% of the injected cells survive [32]. Many causes can be advanced: the heart beats, so cells can easily be pumped out of the heart; the solution in which cells are injected has a low viscosity, so cells can be washed away; the mechanical loss of the cells through the injection hole left by the needle, etc [40]. A different contributing cause to the low cell engraftment is that the injured heart is not a cell-friendly environment, type I collagen fibers have been substituted by type III, which has worse properties in terms of adhesion and promoting angiogenesis, what can induce anoikis [4]. Another problem is cell survival itself. The conditions in the infarcted myocardium are very hostile for the cells: hypoxic conditions (studies show that the survival of injected cells decreases towards the center of the scar), cytokines, inflammatory factors, etc., are present in the damaged myocardium, and can negatively affect the survival of the injected cells. Immunological rejection can be another cause reducing cell survival [41].

An interesting approach is to train cells prior to their implantation for them to resist the hostile conditions they will find in the implantation site. For instance, the resistance to hypoxic conditions is key and needs to be improved even for skeletal myoblasts (which are the cells that have better resistance to lack of oxygen). Privation of glutamine reduces the oxygen consumption rate, what has been proved to improve survival of myoblasts when implanted [42].

The fact that most of the cells did not graft into the host myocardium in the studies performed to date, that there is a very limited transdifferentiation of implanted cells into beating cardiomyocytes (the differentiation reported in animals may have been fusion events between native cardiomyocytes and injected cells [41]), and that a wide range of non-myogenic cells also induce an improvement of the ventricular function [36], suggests that the mechanism leading to this enhancement cannot be only myogenesis regenerating the myocardium. The pathways through which cell implantation induces improvements in cardiac function remain to be elucidated, but different events that can take place simultaneously have been proposed. The most remarkable are the induction of angiogenesis (formation of new vessels) and the improvement in the myocardial perfusion, the reduction of the wall stress because of the increase in cell mass [43] and the paracrine effect of the injected cells [32].

2.3. Cell types investigated

As previously said, many cell types from different origins have been employed: embryonic stem cells, mesenchymal stem cells, bone marrow cells, induced pluripotent stem cells, cardiac stem cells, skeletal myoblasts, umbilical cord blood cells and amniotic fluid stem cells, among others. In what follows the use of these cell types is discussed, with the advantages and disadvantages that each one presents for its application in heart regeneration.

Embryonic Stem Cells (ESC)

ESC can be obtained from the inner mass of an embryo in the blastocyst stage. These cells have the capacity of growing undifferentiated indefinitely, and when they differentiate they can form any cell from the three germ layers. But the use of ESC raises ethical issues, requires

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