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Cardiac tissue engineering: regeneration of the wounded heart

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New solutions are needed to regenerate hearts damaged by myocardial infarction, to overcome bad prognosis of patients with heart failure, and to address the shortage of heart donors. In the past few years, cardiac tissue engineering has emerged as a new and ambitious approach that combines knowledge from material chemistry with cell biology and medicine. In this short review, we present an overview on the most promising materials and cell-therapy strategies used in the past few years for the regeneration of the wounded heart.

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Abbreviations

ECM	extracellular matrix
PGA	polyglycolic acid
PIPAAm	poly(*N*-isopropylacrylamide)
PLGA	polylactic-*co*-glycolic acid

Introduction

Heart failure is the number one cause of death in industrialized countries. Myocardial infarction typically results in fibrotic scar formation and permanently impaired cardiac function because, after a massive cell loss due to ischemia, the myocardial tissue lacks intrinsic regenerative capability. Heart transplantation is the ultimate solution to end-stage heart failure. Owing to the lack of organ donors and complications associated with immune suppressive treatments, however, scientists and surgeons are continuously looking for new strategies to regenerate the injured heart.

Historically, all these strategies started with the development of the heart-lung machine, which enabled blood to be circulated while the patient's heart was surgically treated. This machine was first used by Miller and colleagues in Philadelphia [1]. Subsequently the surgeon's creativity resulted in the replacement of the mitral valve using either cadavers [2] or using the fascia lata to surgically recreate the valve [3]. The survival of the patients was very much dependent on the ability of the surgeon, but patients could survive for more than 20 years after operation using autologous tissue. The first artificial heart replacement arrived in 1969 from Kwan-Gett and co-workers [4]. The beginning of the 1970s saw the first engineering study of the heart, with the first geometrical modeling of the canine left ventricular myocardium.

Much later, studies in the mid-1990s started to apply gene delivery techniques followed by cell transplantation approaches to spark the failing heart [5–7]. Initially, it was thought that the myocardium could be treated by genetically engineered cells (e.g. transfected cells overexpressing the myogenic factor MyoD [8]). At that time, first studies appeared using synthetic biodegradable tissues and cells [9]. Since then, many studies have been published using different synthetic materials and different cells. In this review we give an overview on the most important works published in these last few years. The new trend is to use biodegradable tissues combined with different types of cells and stem cells.

Cells for cardiac cell therapy

Efforts to regenerate functional myocardial tissue are firstly being pursued through cell grafting by syringe injection directly in the ventricular wall or in the coronary vessels. The proof-of-principle experiments of cardiac cell implantation in the heart were first achieved nearly ten years ago using genetically selected embryonic stem cell derived cardiomyocytes [10–12]. Thereafter, several groups have enlarged our knowledge about the fate of implanted cells of various origin (embryonic, fetal or adult) in the myocardium of healthy and diseased hearts (reviewed in [13••,14,15]). In particular, over the past few years several teams have claimed that adult stem cells such as bone marrow stem cells can develop into a wide variety of tissues, including cardiomyocytes [16]. Most studies support the notion that cell engraftment in animal models of myocardial infarction can improve contractile function [17••]. The mechanism behind this functional improvement remains to be elucidated, however, and to date there has been no convincing demonstration of implanted haematopoietic or myoblastic adult stem cells taking on the cardiac phenotype [18–20]. Possibly, functional integration of non-cardiomyogenic cells might not be required to achieve a beneficial effect on cardiac function. In addition, the efficacy of cell engraftment is

Table 1

Cells used in the past three years for cardiac tissue engineering.

Cell type	References
Cardiomyocytes (embryonic or neonatal)	[32,37,38,44]
ESCs and ESC-derived cardiomyocytes	[45•,46,47]
Stem cells of different types	[48]
Skeletal muscle cells	[49,50•]
Monocytes	[49]
Bone marrow cells	[51–53]
Mesenchymal stem cells	[54]
Aortic valve interstitial cells	[55]
Endothelial progenitor cells	[56]
Umbilical cord cells	[57,58]
Vascular smooth muscle	[59]
Cloned cells by therapeutic cloning	[60,61•,62]

Besides embryonic and neonatal cardiomyocytes employed for the generation of cardiac tissue, other cell types derived from adult stem cells or adult tissue niches have also been employed for the regeneration of the heart. ESC, embryonic stem cell.

Table 2

Materials used in the past three years for cardiac tissue engineering.

Materials	References
Natural	
Gelatin scaffolds	[23]
Acellular porcine scaffolds	[63]
Porous alginate scaffolds	[34]
Alginate–gelatin–PEG[a] scaffolds	[64]
Collagen scaffolds	[25,26]
Fibrin glue	[50•]
Synthetic	
PLA–PGA	[35,43••]
Poly-L-lactide-gelatin–PGA	[36]
Electrically conducting membrane layers composed of PGA, gelatin, alginate and/or collagen	[40••,41,42]
Polyvinyl alcohol	[65]
PGA-*co*-polyhydrobutyrates	[56]
ε-Caprolactone-*co*-L-lactide	[59]
Polyurethanes	[66]
TMC-*co*-ε-caprolactone-*co*-D-L-lactide	[37,38]

A multitude of different polymers and copolymers of natural and synthetic origin have been used as scaffolds for the generation of cardiac tissues. Most of these materials were tested in animal trials with rats and some of them with dogs. Only a few were tested in humans [52]. PEG, polyethylene glycol ([a]synthetic material); PGA, polyglycolic acid; PLA, polylactic acid; TMC, 1,3-trimethylene carbonate.

very low as more than 90% of the cell suspension injected is lost and does not engraft. Nevertheless, there are presently several ongoing clinical studies in humans using adult stem cells (e.g. skeletal myoblasts and bone marrow stem cells) to investigate the safety and feasibility of such a cardiac cell therapy [21,22], despite concerns with timing of cell delivery and occurrence of arrhythmias (i.e. irregular myocardial contraction). The first clinical results are controversial and demonstrate the need to better understand stem cell biology and the way to successfully implant new cells in a diseased tissue. Therefore, much effort is now conveyed to the development of tissue-engineering strategies using biomatrices to successfully engraft new cells into the myocardium. Table 1 contains a list of scaffold-seeded cells that have been used so far.

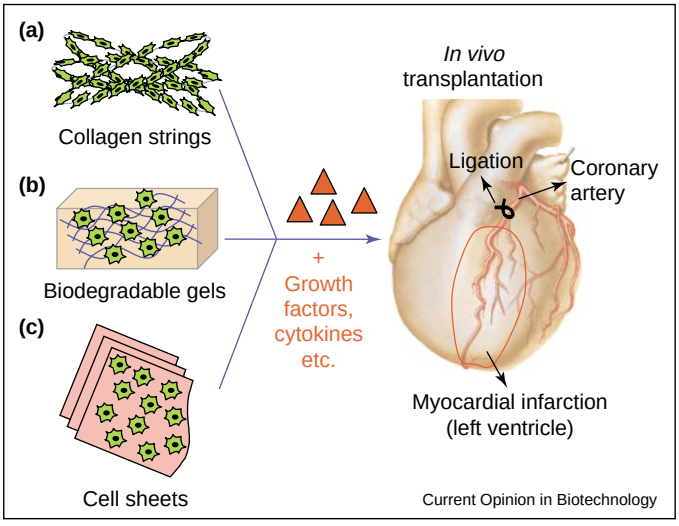
Materials as scaffolds for cell transplantation

One of the first materials used for tissue engineering of the heart was based on hydrolytically degradable biocompatible polymers composed of polylactic acid, polyglycolic acid (PGA) and their copolymer polylactic-*co*-glycolic acid (PLGA) [9]. Subsequently, researchers realized that the mechanical properties of the material used had to be adapted to the elastic properties of the heart tissue. Therefore, most research focused on the use of hydrogels made of different synthetic and/or natural polymers. Table 2 gives an overview on different materials used in the past few years for the regeneration of the heart. It has to be pointed out that only a few materials were tested in humans, while many animal studies were carried out in rats and dogs.

Several groups are currently working with scaffold materials composed of natural polymers such as collagen [23–28,29••,30–33], this latter being the major constituent of the cardiac extracellular matrix (ECM). Promising results in the development of collagen-based grafts or 'patches' containing beating cardiomyocytes were obtained in Canada [23] and Germany [24–28,29••,30–33]. These studies comprised the application of cardiomyocyte-seeded collagen strings that were cyclically stretched, thus providing patches with improved morphology and contractile function. Indeed, cyclical mechanical stress is essential to obtain optimal viable cell-seeded grafts [23,25]. Zimmermann *et al.* [32] demonstrated that these collagen patches could survive and beat for up to eight weeks after engraftment on the heart of immunosuppressed rats. Similar approaches and results were obtained using alginate-based scaffolds by Cohen *et al.* in Israel [34,35]. Alginate, a negatively charged polysaccharide from seaweed which forms hydrogels in the presence of calcium, offers the advantage of detecting ECM formation by cardiac cells to follow not only the proliferation and migration of the cells, but also the kinetics of ECM formation. After implantation into the infarcted rat myocardium, the alginate-biografts were shown to stimulate intense neovascularization and to attenuate left ventricular dilatation and failure, compared with control rat hearts [35].

Composites of natural and synthetic polymers were also developed; for example, sponges based on ε-caprolactone-*co*-L-lactide reinforced with knitted poly-L-lactide fabric (PCLA), gelatin or PGA. Using rat aortic smooth muscle cells, an increased colonization of the right ventricular outflow tract was obtained using gelatin or PCLA, but not with PGA-reinforced grafts [36].

Figure 1



Scheme of the present major strategies of cardiac tissue engineering using **(a)** collagen strings [32], **(b)** biodegradable gels [35] or **(c)** cardiac cell sheets [40••]. The incorporation of growth factors and/or cytokines (triangles) may have a crucial role to support cell differentiation, engraftment and survival, both within the scaffolds and *in vivo,* thus improving the overall cardiac function.

Another interesting synthetic material is based on 1,3-trimethylene carbonate and D,L-lactide copolymers [37,38], which have the ability to be readsorbed over a ten month period and to sustain the cyclic loading of the heart muscle under physiological conditions. However, as yet no animal studies have been carried out with this amorphous material.

To us, the most fascinating approach to the regeneration of heart has been proposed by Shimizu and co-workers [39,40••,41,42], who used materials to create electrically communicating three-dimensional cardiac tissue layers. In this case, cells were adhered on tissue-culture plates previously coated with poly(*N*-isopropylacrylamide) (PIPAAm), a temperature-sensitive polymer. At 37°C PIPAAm is hydrophobic, enabling cell adhesion and access to the binding sites offered on this modified surface; at a lower temperature such as 32°C, the surface becomes hydrophilic and inappropriate for cell adhesion due to the rapid hydration and swelling of PIPAAm. Using poly(vinylidene difluoride) (PVDF) membranes, which are hydrophobic, the detaching cell layers can be collected and handled, providing up to four conducting layers of synchronously beating cardiomyocytes. When these patches were implanted on rats with induced myocardial infarction, an improved myocardial contractility was observed, concomitant with the appearance of a vascular network within a few days after implantation. Figure 1 summarizes three of the major approaches to cardiac engineering described here, based on the use of collagen, hydrogel or multiple layers.

Conclusions

Recent data on the implantation of differentiated cardiac and non-cardiac cells as well as on adult stem cells of different origin has provided hope for the replacement of cells after the irreversible loss of viable cardiac cells that occurs during myocardial infarction. As compared with the direct injection of a cell suspension into the heart, cardiac tissue engineering strategies are now emerging as a better alternative to augment both cardiomyocyte number and contractile function of the failing heart. A critical step is the creation of suitable three-dimensional matrices composed of natural or synthetic scaffold materials that host the cells (defined as cardiac patches), to allow the maintenance of cellular viability and differentiation and favour cell integration. Clearly, the survival of these patches under the ischaemic conditions present in the injured heart is a major challenge that will have to be addressed. Therefore, the creation of engineered tissue that not only assembles cardiac cells but which also includes factors and/or cells favouring revascularization will be crucial. For an optimal achievement of cardiac patches, it might be necessary to cultivate three-dimensional cell constructs in bioreactors that reproduce the normal stress and flow experienced by the tissues.

Another important point is the possibility to deliver these patches by low-invasive techniques, such as the use of catheters, to avoid thoracotomy. Consequently, in the future, approaches considering these two points will be essential for the design and choice of cells that have to survive harsh ischaemic conditions. For example, after a three-dimensional culture period, cells might be

engrafted in a second step, as proposed by Leor and Cohen [43••]. Additionally, to envisage the regeneration of the human heart, 1 cm-thick patches of muscular tissue are necessary to achieve successful regeneration. This would enormously complicate the proposed elegant approaches involving cell sheets [40••], owing to hypoxic conditions. New solutions including the recruitment, *in vitro* proliferation and homing of the patients own stem cells, combined with biodegradable and biomimicking materials, will open new doors in the field of cardiac tissue engineering.

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