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Review

Stem cells used for cardiovascular tissue engineering☆

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Summary

Stem cell research and tissue engineering have become leading fields in basic research worldwide. Especially in cardiovascular medicine, initial reports on the potential of using stem cells to recover cardiac function and replace organ subunits such as heart valves seemed to offer the promise of widespread clinical use in the near future. However, the broad application of this new therapy failed due to safety and efficacy concerns. Due in part to the initial reports, major basic research efforts were undertaken to explore the specific cell types in greater detail and identify their mechanisms of supporting function, resulting in remarkable new findings in stem cell biology. For example, the notion of resident human cardiac stem cells has disproved the earlier supposition that the human heart is a finitely differentiated organ without the intrinsic potential for regeneration. Furthermore, new technologies emerged to produce pluripotent cells without the ethical and immunological drawbacks of embryonic stem cells (for instance by nuclear transfer). Other autologous cell sources are presently under investigation in myocardial tissue engineering. For tissue engineering of heart valves and small calibre vessels, the use of autologous endothelial (precursor) cells may be the optimal means of seeding a biological or artificial scaffold. It is important that ongoing basic and clinical research in cardiovascular surgery might explore the potential of different cell types either using tissue engineering constructs or in cell transplantation approaches.

Keywords: Tissue engineering; Cell transplantation; Stem cells; Endothelial precursor cells; Regeneration; Myocardium; Heart valves; Small calibre vessels

1. Introduction

Stem cells are defined by two main characteristics: the ability for self-renewal and the potential for differentiating into mature cell types. We distinguish the various stem cell types in general by their age and potency. Developmentally early and pluripotent cells (e.g. embryonic stem cells) can give rise to almost every mature cell type, while adult stem cells are classified as restricted to differentiation into only few types of mature cells. For example, mesenchymal stem as an adult stem cell type can give rise to muscle, bone, cartilage, and adipogenic tissue. In contrast, cell types that can only differentiate to one specific mature cell type, are referred to as precursor cells.

Parallel to the high differentiation potency of immature stem cells such as embryonic stem cells (ESC), these cells are suspected of incorporating an inherent tumourigenic risk once the wrong differentiation pathway has been taken.

First clinical applications of stem cells for cardiac regeneration comprised cell transplantation trials. For the most part, these trials were less successful than promising preclinical studies. However, these efforts initiated intense research activities providing new insight into the mechanisms of tissue growth and differentiation. New hope is now based on the further development of stem cell application in the mode of tissue engineering constructs.

Cardiac tissue engineering is focused on three different organ subunits: the myocardium, valves, and vessels. These three compounds of the heart can already be replaced by artificial or biological transplant constructs with their respective limitations (assist devices, commercial heart valves, autologous coronary bypasses, etc.). However, approaches to engineering these tissues must compete with the durability, efficiency and safety of existing substitutes and be affordable at the same time.

By definition, tissue engineering is the 'development of biological substitutes that restore, maintain, or improve tissue function or a whole organ' [1]. In most circumstances tissue engineering includes an in vitro step and at least two main compounds: a matrix and cells. We review herein the most relevant cell sources currently under investigation for use in cardiac tissue engineering constructs. The matrix selection will be discussed separately in a different review.

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2. Cells used for myocardial tissue engineering

Tissue engineering of myocardial tissue requires stem cells with the potency to differentiate into functional cardiomyocytes (Fig. 1). Mesenchymal stem cells (MSC) and endothelial precursor cells (EPC) have not been demonstrated conclusively to possess this potency to the degree needed for a beating construct. However, there is conflicting evidence concerning their ability to give rise to cardiomyocytes in general. In vitro and without the use of supporting cells, MSC can be differentiated in the cardiomyogenic lineage (e.g. with the use of Azacytidine enrichment). However, only cardiogenic surface marker expression (but no beating cells) was achieved [2,3]. In co-culture with cardiomyocytes, and after injection in the heart as well, both types of cells might undergo cardiogenic transdifferentiation to a certain degree. The in vivo transdifferentiation of bone marrow-derived MSC to cardiomyocytes has been the subject of intense conflict between researchers. While Anversa's groups found evidence for this transdifferentiation [4], other groups found evidence to exclude the same [5,6]. The debate has been recently influenced by more robust findings of MSC transdifferentiation in a myocardial infarction model [7]. However, no one has yet demonstrated an effective and feasible way of promoting the transdifferentiation of these adult cell types to cardiomyocytes in vitro [8]. Moreover, skeletal myoblasts are unlikely to play a role in future approaches to generate myocardial tissue engineering constructs because of lacking electrical coupling to the host myocardium after differentiation in myotubes [9]. However, the therapeutic potential of a skeletal myoblast-based scaffold might be due to a paracrine support of the failing myocardium, regardless of the presence of differentiated contractile elements [10].

Embryonic stem cells are derived from the inner cell mass of preimplanted blastocysts. These cells are regarded as an advantageous cell source due to their auto-regenerative capacity, high proliferation capability and pluripotency. The immunogenicity of these cells and their offspring cell types may not be as irrelevant as initially suggested. It is currently accepted that, along with the progress of cell differentiation, immunological incompatibilities potentate, a process that questions the full range of future options of clinical application of allogeneic ESC-derived cells [11].

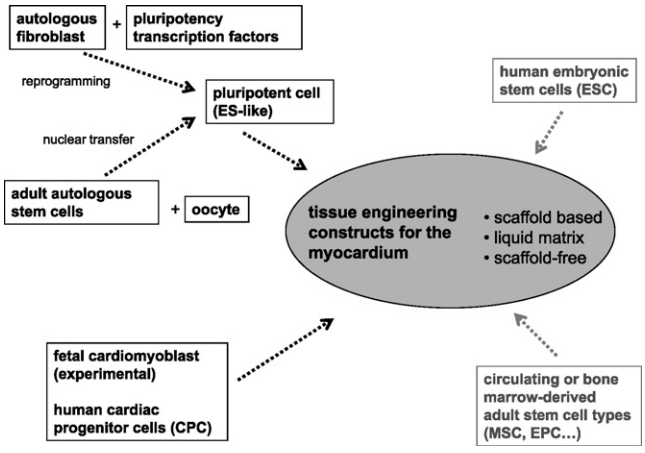

Fig. 1. The figure illustrates likely (black) and less likely (grey) sources for future tissue engineering approaches replacing the myocardium.

Human embryonic stem cells can be differentiated into functional cardiomyocytes under certain conditions. The pluripotent nature of these cells may be particularly important in cardiac tissue engineering when considering the role of non-cardiomyocytes in the development of beating myocardial constructs. Therefore, several myocardial tissue engineering approaches are based on ESC use. Its application in a clinical setting might be complicated by their allogeneic origin and the fear of tumourigenicity. The risk of neoplastic transformation seems to be dose-dependent, and is lower in the diseased heart due to the inflammatory environment [12]. Cardiopoietic programming might be a suitable strategy to ensure the correct differentiation pathway. The risk of malignancies and the hazard of transmission of pathogens derived from animal-derived components during in vitro culture must first be eliminated. Finally, strategies to avoid an adverse immune response, like antigen matching and immune tolerance induction, need to be developed to address immunological issues. However, the safety issues associated with the tumourigenicity and immunogenicity of ESC remain unsolved [13].

A more recent strategy to overcome these limitations is using autologous adult stem cells and incorporating immaturity genome into them (e.g. from oocytes). This concept is called nuclear transfer and may result in autologous pluripotent cell lines. This cellular-cloning strategy has been described in rodents. Mitalipov's group reported recently that they were able to reprogram adult mammalian skin cells by nuclear transfer, obtaining a pluripotent cell line [14]. At the same time, researchers from Kyoto, Japan used a similar approach. They transduced adult human dermal fibroblasts with four defined transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) to generate induced pluripotent cells [15]. These had all properties of embryonic stem cells. These approaches might have value for future research on myocardial tissue engineering.

Traditionally, murine fetal cardiomyoblasts have been the optimal cell source for experimental purposes. The leading groups in myocardial tissue engineering used cardiomyoblasts in their constructs.

Okano and co-workers used a cell sheet system to engineer a three-dimensionally contracting graft [16]. The trick to obtain a stable cell sheet was the use of temperature-responsive culture surfaces [17]. Thereby, a stable cell sheet of single-cell thickness can be harvested without losing the intercellular connection and then be transferred to a second and third cell sheet. These beating grafts were successfully transplanted to injured hearts, subsequently integrated into the host, and succeeded in regenerating myocardial function [16]. The cell sheet can also be placed around the abdominal aorta to assist cardiac function. It has been shown, that these functional myocardial tubes have the potential for circulatory support in a rat model [18].

Zimmermann et al. also used fetal cardiomyoblasts in their approach to engineer contractile cardiac tissue. Originally, these beating rings were meant to act as an in vitro drug-testing device. By mixing murine cardiomyoblasts with liquid collagen type I, matrigel, and serum-containing culture medium, they were able to design beating rings and

called them 'engineered heart tissue' (EHT) [19,20]. The further development of these constructs to large, force-generating grafts of multiple rings led to a remarkable improvement in ventricular function once transplanted on an infarcted heart [21].

However, immunological considerations prohibit the application of the above-mentioned constructs in humans. New hope of engineering cardiac tissue from human cells was raised by the demonstration of resident cardiac progenitor cells that have the capacity to differentiate into functional cardiomyocytes and repair the heart. The human heart used to be considered as a terminally differentiated organ. A decade ago, researchers became aware of resident cardiac stem cells in the adult murine heart [22—25]. There have been several reports recently on cardiac progenitor cells organised in niches in the adult human heart that could be clonally expanded in the absence of a co-culture with primary heart cells [4,26,27]. When injected into infarcted hearts, these cells can generate new viable myocardium and support heart function [28]. Various surface markers have been proposed for identifying these cardiac progenitor cells, such as c-kit, sca-1, isl-1 and others; most likely describing different cellular entities. A more homogenous view of these endogenous cardiac stem cells with different markers awaits clarification.

These exciting findings may open the door not only to an autologous cell therapy approach but to the engineering of autologous cardiac tissues as well. Such tissues would be ideal for cardiac repair, being most likely free of tumourigenic and immunological problems. However, beside their pure existence, a realistic means to make use of these cells yet needs to be shown.

3. Cells used for heart valve tissue engineering

Heart valve tissue engineering has already become a clinical reality, although the number of patients with a bioartificial valve remains very low. Most of the active scientific groups have put cell seeding into their heart valve engineering practice independently of the synthetic or biological origin of the employed valve scaffold. For obvious reasons, the ideal condition of a mature bioartificial heart valve includes full coverage of the scaffold surface area by endothelial cells (EC), which is complete endothelialisation (Fig. 2). What precisely the optimal cell source is remains unclear. Assuming that only autologous cells are of clinical interest, there are three options: primary vascular endothelial cells, endothelial cells derived from patient's existing progenitor or stem cells, or theoretically, endothelial cells may be differentiated from developmentally early phase stem cells that would have to be generated from the patient's genetic material, e.g. via therapeutical cloning.

The first group of cells includes endothelial cells from other vessels. Venous endothelial cells are generally preferred, due to unproblematic access and harvesting of peripheral venous segments with no or minor morbidity for the patient. Further, the venous system is less prone to degenerative events such as atherosclerosis and calcification, factors that may significantly reduce the efficacy of cell isolation [29,30]. Arterial cells can be isolated from the wall

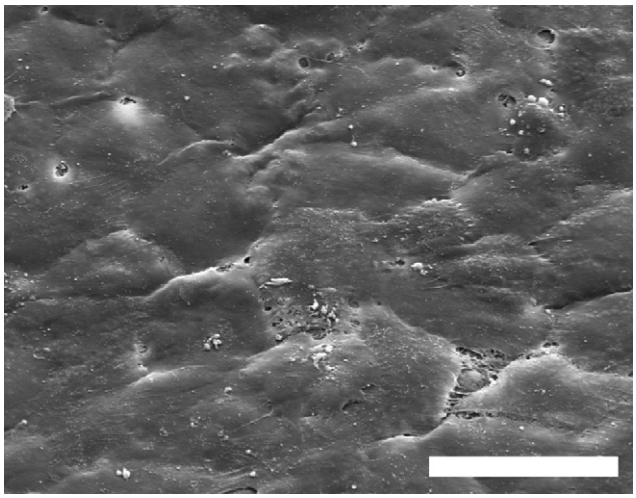

Fig. 2. Complete re-endothelialisation of PV reseeded with jugular vein EC and exposed to pulsatile circulation (2.0 l/min flow) in a bioreactor system. SEM of the cusp. Magnification 1000, bar = 10 mm.

of a few arteries at body sites possessing arterial supply by two vessels, e.g. from the radial artery. These cells express a suitable phenotype for the tissue engineering of valvular conduits, and have been successfully used in a mixture with myofibroblasts for bioengineering of functional cardiovascular structures in animals [31]. Their native adaptation to high flow patterns has been suggested as one reason for the superior performance of arterial EC. However, Schnell et al. demonstrated that saphenous vein myofibroblasts cultured on biodegradable scaffolds showed excellent in vitro tissue generation. Collagen formation and mechanical properties were superior to aortic tissue derived constructs [32].

In 1997, Asahara et al. identified a small population of CD34+-circulating mononuclear haematopoietic progenitor cells in adult human peripheral blood that revealed endothelial characteristics in culture. These cells are normally isolated from mononuclear cell pools of peripheral blood and are selected based on their adherence to fibronectin-coated surfaces and growth in the presence of endothelial growth factors [33]. Endothelial progenitor cells (EPC) represent a rare cell population capable of replacing vascular EC and promoting angiogenesis at sites of injury or inflammation. The option of a very convenient access via peripheral blood with virtually no trauma or morbidity for the donor, as well as the enhanced plasticity in culture, are major advantages of working with EPC. Main drawbacks lie in their very low frequency in peripheral blood with a steady decline in number with ageing of the tested individual. In contrast, when isolated from cord blood [34] or other perinatal sources (e.g. amniotic fluid) high numbers of EPC have been reported [35]. Consistently throughout existing reports, EPC have proven to be highly suitable for endothelialisation of cardiovascular tissue engineered implants [36,37]. Most remarkably, this has been documented by a small clinical paediatric series involving tissue engineering heart valves based on EPC seeded on decellularised human pulmonary valves [36]. In that series, evidence of tissue growth was documented for the first time by echocardiographic follow-up observations of increasing diameter of functional, competent tissue engineered pulmonary valves.

MSC-derived EC represents another option for heart valve tissue engineering. They may be isolated from a bone marrow biopsy for adult heart valve recipients. By seeding MSC on a biodegradable scaffold and subsequent culture for two weeks in a pulsatile duplicator, markers for mesenchymal stem cells, as well as specific markers of smooth muscle cell lineage including alpha smooth muscle actin, desmin, and calponin have been demonstrated. Remarkably, the mechanical properties of the resulting tissue engineered valve resembled those observed in native heart valves. It has thus been concluded that bone marrow may be a potential cell source for the tissue engineering of trileaflet heart valves, particularly in children with congenital heart disease [38]. Moreover, Hoerstrup et al. investigated the feasibility of human mesenchymal stem cells for tissue engineering finding that the cells demonstrated characteristics of myofibroblast differentiation after dynamic culture [39].

Similar potency regarding the frequency of MSC and EPC and their cell proliferation and differentiation has been reported for cord blood [40]. Schmidt et al. reported on the construction of living patches engineered from human umbilical cord-derived fibroblasts and endothelial progenitor cells. EPC of endothelial phenotype was obtained and confirmed by Ac-Dil-LDL, CD31, von Willebrand factor, and eNOS staining before cell seeding. The resulting newly formed tissue fragments expressed myofibroblast markers such as desmin and alpha-smooth muscle actin. Also, at the same time constant endothelial phenotype markers (CD31, vWF) identified persisting EPC, while significant amounts of major components of the extracellular matrix were detected such as collagen and proteoglycans. After mechanical testing, the authors reported on comparable features of native tissues. Other reports underline the capacity of EPC, a novel cell source that may make the tissue engineering of versatile, living, autologous organ fragments possible for congenital cardiac interventions [35].

Although in vitro experiments have revealed exciting properties of these cells of embryonic stem cells and their endothelial descendants, many questions regarding their biological safety and efficacy upon implantation remain unanswered. However, serious ethical concerns from the public, and a multitude of unanswered experimental questions leave little room for further attention to this cell source [13], and there may be no need for another ideal endothelial cell source. Numerous practical cell sources with established handling protocols and ethical acceptance have already made the endothelial cell the most convenient representative of the cells employed for heart valve tissue engineering.

4. Cells used for vessel tissue engineering

Every year there is a large and growing number of surgical procedures, in which arterial grafts are needed. For large-calibre vessels, artificial grafts fabricated from Dacron or polytetrafluoroethylene (PTFE) have proven excellent short-term and long-term function. In contrast, to date artificial grafts for small-calibre vessel replacement have failed mainly due to thrombogenicity and underlying disease progression [41]. That is why surgeons prefer autologous vessels for bypass grafting. In particular, autologous vessels account for almost 100% of all grafts in coronary bypass procedures since the risk of early occlusion of artificial vessels is too high. The preferred grafts are saphenous veins, the internal mammary, and radial arteries. Sometimes, the cardiovascular surgeon faces a shortness of autologous vessels and is forced to progress with less or poorly suited grafts. Furthermore, the process of procuring autologous grafts is an additional risk for the patient, occasionally prolonging the operation substantially. Tissue engineering of small-calibre vessels would thus represent an attractive means of avoiding such shortcomings.

The necessary prerequisites for a perfect tissue engineered graft are sophisticated: failing thrombogenicity, mechanical properties able to withstand arterial pressure over years, together with a certain degree of compliance and elasticity, and the correct degree of biocompatibility to heal without inflammation and encapsulation. Other desirable properties include physiological properties of vasoconstriction and relaxation on demand, permeability for solids and fluids and saturability of the grafts' ends. These challenging demands have led to a widely accepted principle of tissue engineered, small-diameter vessels having two main components: (1) a confluent and non-activated endothelial layer as the inner layer to prevent early and late thrombosis, and (2) a scaffold to support the tensile strength as mechanical support, and an elastic component to prevent ecstatic growth.

The cells to implement the endothelial layer (1) are almost exclusively endothelial (precursor) cells. This type of cell is discussed in the above chapter on heart valve engineering in detail. In small vessel engineering, most groups use an in vitro step with a dynamic bioreactor to seed the endothelial cells onto the scaffold's inner surface. An important tool to ensure a stable and functioning endothelium seems to be the intense simulation of the physiological blood stream [42].

There are currently four principles for the preparation of the matrix containing the endothelium: (1) synthetic (polymeric) scaffolds, (2) biopolymeric scaffold of hydrogel, (3) decellularised tissues, and (4) cell based engineered constructs. Since this review is focused on cells used for cardiovascular tissue engineering, we do not discuss (1)—(3) and concentrate on cell based strategies.

One group in particular, focused on a completely biological design of small-calibre vessel tissue engineering. The approach of Auger and colleagues is presented in greater detail as an example of cell based studies [43]. They engineered the three components of a vessel separately from each other. First, a cell sheet composed of human neonatal smooth muscle cells was designed to construct the media. This sheet was wrapped around a porous mandrel to allow nutrition by medium diffusion through the pores. After an incubation period, a second sheet composed of fibroblasts was imposed representing the adventitia. Finally, the intimal layer was again formed by endothelial cells, which were applied in a rotating bioreactor manner.

This construct has demonstrated good microscopic and physical properties as well as promising physiological behaviour [44]. However, the vessels' compliance may be inferior to that of native small-calibre vessels and might therefore aid and abet anastomosis problems [45].

5. Conclusion

Initial clinical successes using stem cells and tissue engineering constructs have initiated intense basic research activities in stem cell biology. The optimisation of existing strategies to replace subunits of the heart, and the generation of new approaches may eventually enjoy widespread clinical use.

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