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Future trends: cell engineering for cardiac repair

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Abstract

Stem cells can be defined as the cells capable of unlimited self-renewal with an ability to give rise to multiple tissue types. Not all stem cells have the capability for unrestricted differentiation towards all tissues of the body. Only primary embryonal stem cells can be considered as totipotent stem cells, since they may give rise to all embryonic and extra-embryonic tissues. Both embryonic, as well as some somatic stem cells, can be defined as pluripotent (three germ layers), while multipotent somatic cells may differentiate into the tissues of the only one germ leave (eg. ectoderm). Some stem cells of the tissue reservoir can be even more limited in their plasticity, therefore they are often implanted into post-infarction myocardium in ongoing clinical trials. Both circulating autologous bone marrow cells (BMC), as well as their specific subsets, are applied with a certain degree of success. Progenitor cells of tissue reservoir (MDSC – muscle-derived stem cells) have also been tried for myocardial repair. The optimisation strategy of stem cell delivery, including novel cell subsets (CPC – cardiac progenitor cells), as well as possible genetic modifications of currently used stem cells, are also discussed.

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Introduction

The development of congestive heart failure (as a consequence of myocardial infarction) is related to myocardial cell loss in an area supplied by the infarct-related artery and the subsequent formation of a scar. Important strategies during the acute phase of MI, primary angioplasty and fibrinolysis, are aimed at restoration of the blood flow to minimise local necrosis. Nevertheless, often a billion or more myocytes can be lost as the result of myocardial infarction (MI). Ischaemia also kills vascular cells, fibroblasts and nerves in the tissue. Late revascularisation procedures may enable recovery of contractility, but only in areas of hibernated myocardium (in a bordering zone) that usually contain a relatively low number of viable, reversibly injured, myocytes. In patients with large myocardial necrotic areas resulting from acute MIs, and especially when collateral vessels supplying the infarcted region are weakly developed, the loss of cardiomyocytes results in left ventricular remodelling, aneurysm formation, and progression of congestive heart failure.

Limited availability of donor organs for heart transplantation, along with the poor results of current pharmacological therapies, prompted investigation into alternative methods of treatment. Various experimental studies provided evidence that the infusion or injection of stem or progenitor cells may lead to a process whereby multiple damaged cell types are replaced to restore the previous histoarchitecture and function of the damaged tissue[1].

Stem cells are defined as those cells capable of unlimited self-renewal, with the ability of giving rise to multiple tissue types. Generally, stem cells may by classified into two main groups according to the source of their origin: (1) embryonal stem cells, and (2) adult somatic stem cells. The second group is comprised of somatic multipotent stem cells (mostly originating from the bone marrow, but also identified in cordal blood), and somatic progenitor cells of the tissue reservoir (eg, satellite cells, neural stem cells).

Many organs have tissue specific stem cells, but these populations differ in their proliferative capacity. Some organs regenerate poorly after injury, yet evidence is mounting that they harbour cells capable of rebuilding their tissues. Haematopoietic and epithelial tissues exhibit high cell turnover, while brain and heart muscle[2] contain low numbers of stem cells with limited ability for self-renewal. Strategies for regenerating these latter tissues thus rely on overcoming the fibrotic response and engrafting the lesions with regenerating cells that may replace or rescue the dying cells.

Stem cell classification and plasticity

A criterion which distinguishes different populations of stem cells is their potential to generate multiple types of specialised cells, i.e. whether the stem cell is totipotent, pluripotent, multipotent, bipotent or unipotent. Embryonic stem cells are totipotent, as they are capable of generating all the cells of the body including embryonic as well as extra-embryonic tissues. Adult stem cells (somatic) which can give rise to all three of the primary germ layers – ectoderm, mesoderm and endoderm – can be pluripotent as well as the embryonic cells derived from the inner cell mass. The term multipotent refers to those cells with the potential to generate all cell types of a particular germ layer (eg. mesoderm). A cell with a more limited potential is considered a bipotent stem cell; for example, lymphoid progenitor, which can give a rise to both T and B lymphocytes. A representative of a unipotent stem cell is skeletal myoblast. Recent reports suggest that tissue-derived stem cells could have a broader potential than suspected, and may even show quite a range of plasticity allowing them to transdifferentiate across tissue borders when the environment is changed[3]. Somatic stem cells (specifically those residing in specific niches of tissue reservoir) are relatively quiescent in adults and may undergo self-renewal into perpetuity, while progenitor cells divide very rapidly and generate very large numbers of off-springs (myoblasts, CPC's).

Reports on stem cell transdifferentiation have been recently questioned in a variety of ways. It has been proposed that tissue-derived stem cells appear to undergo a fusion with other cell types rather than transdifferentiation which remains, at this stage, an *in vitro* phenomenon[4]. However, it is very likely that the cell fusion and differentiation are not mutually exclusive, for example, skeletal muscle development involves both cell differentiation and fusion. Controversial, and not fully convincing, data points to a low rate of transdifferentiation that can be observed in rodents[5].

Stem cells and their application

Human embryonal stem cells – potential therapeutic significance

Embryonal stem cells (ESCs) originate from the inner mass of the blastocyst and can be propagated *in vitro* for a virtually unlimited time at the stage of their pluripotent ability, raising the possibility that they may be of use in the regeneration of every tissue and organ in the human body[6].

Human embryonic stem cells are advantageous due to their pluripotency and minimal immunoreactivity (reduced expression of immune-related cell-surface proteins), but clinical application of embryonic stem cells is not very likely in the next few years because of ethical considerations and problems with differentiation control (eg. teratoma formation). Thus, in clinical studies, the use of a patient's own cells is, at this moment, preferred.

Adult stem cells and tissue self-renewal

The mature heart belongs to the group of organs with extremely low "turn-over". Although, human cardiomyocytes are reported to proliferate and contribute to the increase in a muscle mass of the myocardium[7], the existence of adult heart-derived cardiac progenitor cells (CPC) was also documented[8]. Still, the human heart has a very limited regenerative potential. Cellular cardiomyoplasty, which is the replacement of cardiomyocytes through cell transplantation, has been therefore undertaken. Such procedure is based on the principle of augmentation of insufficient intrinsic repair mechanisms within the diseased heart. Several sources of stem cells for cardiac muscle regeneration were reported as a potential therapeutic option, including bone marrow cells (BMC) and skeletal-derived stem cells[9]. The aim to deliver the stem cells to the site of cardiac injury was to restore a blood flow and contractility to dysfunctional heart muscle.

Pluripotent tissue stem cells and their role in cardiac muscle repair

The best established source for adult stem cells is bone marrow. It contains different cell types that have been broadly categorised

into haematopoietic and mesenchymal lineages. These cells are further divided into subpopulations on the basis of their surface markers. One can distinguish haematopoietic stem cells (HSC), endothelial progenitor cells (EPC), mononuclear cells (MNC), mesenchymal stem cells (MSC) and their subpopulation, multipotent adult progenitor cells (MAPCs)[10].

Currently, in many centres worldwide investigations are proceeding into the transplantation of bone marrow-derived cells as an adjuvant therapy in acute MI. Bone marrow stem cells have a relatively high plasticity, so they can differentiate - depending on the environmental influence - into different cellular lineages. Therefore, at least theoretically, transplantation of these cells may also lead to the full regeneration of myocardial tissue, including both the myocardium itself as well as its coronary vasculature. Transplantation of undifferentiated pluripotent stem cells into recipient tissue is based on the hypothesis of milieu-dependent differentiation. These stem cells may differentiate into cardiomyocytes and endothelial cells if they are transplanted into the area of the myocardium, which contains cardiomyocytes and vessels, thus resulting in improvement of myocardial function and perfusion. But when they are injected into the area of post-infarction scar, they can differentiate into fibroblasts. Differentiation into tissues other than cardiomyocytes (within the heart) may lead to areas of arrhythmogenicity. This effect was shown, not only in animal models, but also in the initial human studies. Beneficial effects of BMC cell transplantation were observed after intracoronary cell delivery in acute infarction, as well as in patients with ischaemic myocardium. In both situations, cell transplantation led to the improvement of myocardial function and perfusion. Intracoronary, autologous bone marrow-derived $CD34^+$ stem cells delivery (during acute phase of myocardial infarction) resulted in improvement of myocardial function and perfusion. This effect was shown both in preclinical animal studies and in initial human trials[11]. Since pluripotent stem cells after transplantation may differentiate into more mature (differentiated) cell types depending on the information provided by the surrounding micro-environment, the effect of BMC transplantation in post-infarction heart regeneration may be related to the time of the cell delivery after the onset of infarction. Experimental data suggest that administration of BMCs very early after infarction may not increase myocardial contractile performance, and it may be speculated that the cell transplantation during the inflammatory phase of myocardial healing might result in the involvement of BMCs in the inflammatory reaction itself. On the other hand, very late BMC transplantation into a fibrous post-infarction scar may result in their differentiation into fibroblasts. Despite these observations, a clinical study was conducted involving the administration of bone marrow cell suspensions during CABG several months after MI which suggested a beneficial effect[12]. This was next followed by a report of Perin et al[13] in which percutaneous intramyocardial injections of autologous BMCs with the NOGA system in patients with heart failure, weeks to months after acute MI, were performed. The increased perfusion was detected by using a single photon emission computed tomography and increased ejection fraction was observed during echocardiography. Unfortunately further experiments showed that in mouse model of myocardial infarction, bone marrow-derived cells underwent very low levels of transdifferentiation into cardiomyocytes, and that the fate of most of these cells was to continue differentiation along haematopoietic cell lineage[14,15].

Finally, today we have arrived at a moment where we have performed 20 clinical studies with bone-marrow-derived cells involving 535 patients[16]. The ad hoc analysis may indicate that there is no clear difference in efficacy of bone-marrow-derived mononuclear cells or more closely defined BMC subsets ($CD133^+$, $CD34^+$) which questions the "stemness" of the cells included in heterogeneous BMC suspensions. Furthermore, some trials indicate no significant benefit in terms of heart haemodynamic parameters or contraction ability[17,18] indicating, at best, a temporary phenomenon of improvement. In some studies, a high rate of in-stent restenosis was observed[19] or accelerated atherosclerosis[20,21]. We must advocate caution in the conduct of more randomised double-blind studies, with comprehensive monitoring which keeps in mind that most bone marrow cells are aimed to periodically replace our haematopoietic potential and their endpoints are the cells of leukocyte lineage.

Mobilisation of bone marrow stem cells for organ regeneration is a highly controversial subject. At least half the attempts until now to boost bone marrow stem cells by G-CSF during acute myocardial infarction did not bring about any improvement[22]. Original studies initiated by Orlic and colleagues in 2001 were never successfully repeated in primates[23], negating common regeneration mechanisms in mammals.

Muscle-derived stem cells in myocardium repair

The satellite cells, located on the surface of the myofiber, and beneath the basement membrane, appear to be muscle precursor cells of the tissue reservoir. They are normally quiescent cells in mature skeletal muscle, and become activated only in response to the growth stimuli or the muscle injury. Beside the satellite cells, another population of adult stem cells resides in the adult skeletal muscle. They are the previously mentioned muscle-derived stem cells (MDSC). MDSCs exhibit the capacity to reconstitute the entire haematopoietic repertoire after intravenous injection into lethally irradiated mice[24,25]. It was shown that MDSCs could reconstitute the adipogenic, endothelial and myogenic cell types[26].

Recently, a population of myogenic-endothelial progenitor cells has been also identified that are derived from skeletal muscle and are believed to reside in the interstitial spaces of the tissue[26]. Distinct from the satellite cells, these cells are $CD34^+$ and $CD45^-$. The $CD34^+/45^-$ cells can fully differentiate into vascular, endothelial cells and form skeletal muscle fibres *in vivo* after transplantation. They are distinct from the satellite cells, as they express Bcrp1/ABCG2 gene[27]. These findings confirm that myo-endothelial progenitors reside in the interstitial spaces of mammalian skeletal muscles, and that they can potentially contribute to postnatal skeletal muscle growth and repair.

For heart regeneration, satellite cells can be isolated and propagated *in vitro*. They have a relatively good capacity to proliferate in routine cell culture procedures. Skeletal myoblasts (satellite cells) are natural, progenitor cells located at the basal lamina of the adult skeletal muscle, where they are skeletal myocyte precursors maintaining the self regenerative properties of the muscle. Experimental

studies have shown that foetal cardiomyocytes and skeletal myoblasts when implanted into a post-infarction scar have similar efficacy at improving the left ventricle haemodynamic parameters, including contractile function[28,29]. The possible use of autologous skeletal myoblasts in a clinical setting is attractive because these cells are readily accessible, can be easily multiplied *in vitro* to an appropriate number and avoid immunosuppression. Additionally, they do not form tumours, and display much higher levels of ischaemic tolerance; graft survival is much better then when compared to other cell types. Skeletal myoblasts do not raise ethical controversies. Numerous human trials indicate that autologous skeletal muscle-derived stem cell transplantation into the region of the post-infarction heart have resulted in an increase of segmental contractility which is related to natural properties of these cells[30].

Up to this date, approximately 12 clinical trials using human myoblasts have been conducted[31], of which the vast majority were performed using the intramyocardial approach (adjunct to CABG), with only a few trials using the myoblasts alone. Several side effects have to be underlined such as arrhythmia, specifically when the positive effect of myoblasts is seen to be correlated with their large quantities (as in the prematurely terminated MAGIC trial). He second setback in myoblast properties is their lack of 'gap junctions' with neighbouring cardiomyocytes, therefore no electro-mechanical coupling is formed. Transplanted myoblasts may, however, contract synchronously by direct transmembrane channelling of electric current, or may fuse with cardiomyocytes to form chimeric cells. There is no experimental data for their transdifferentiation, instead they retain morphological and electrophysiological characteristics of skeletal muscle cells[30].

The other possible indirect effects of myoblasts on the improvement of cardiac function may involve: a) prevention of post-infarction remodelling; b) paracrine effects of released vascular endothelial growth factor and insulin growth factor; c) attenuation of matrix metalloproteinase–2 and –9.

Future perspectives for cell engineering

A variety of stem cells sources have been tried in our attempts to regenerate and/or rejuvenate damaged organs and tissues. We are far from understanding the optimal strategy to achieve such ambitious goals. However, international trials, primarily focused on heart regeneration (generally speaking, on organs with low "turn-over") have already been initiated with the aim of testing safety and feasibility, and some studies have already reached the level of being phase II clinical trials. The first optimistic reports have, unfortunately, faded in the light of randomised double-blind studies[11,16]. Nonetheless, intensive research on stem cells and their application has already begun, and undoubtedly has opened a new era of gene and cell therapy.

Several additional ideas and approaches can be envisaged, not only to optimise our use of the already explored stem cell types, but also in discovering: a) novel cell candidates (CPC, ES – cell derived progenitor cells); b) stem cells (currently tried) with over-expressed deficient genes; c) various combinations of stem cells as vehicles for variety of transfected genes (pro-angiogenic, antioxidants, Ca^{2+} channels). Current clinical trials have spotlighted the hypothesis of the indirect (paracrine) effect of applied stem cells instead of their transdifferentiation and/or their integration into recipient tissue, prompting another hypothesis on the stimulating effect of these cells towards resident, progenitor cells sitting in tissue reservoir.

Another primary target in the repair of damaged hearts is therefore cardiac progenitor cells[32], pre-clinical trials in animal models have already started. The road ahead would include those heart progenitors that could give rise to several basic cell types such as cardiac muscle cells, cardiac conduction cells and endothelial cells. A problem among CPC's is to choose the appropriate markers in order to secure the complete regeneration of the particular heart region. Secondly, is the pressing question of whether sufficient amounts of CPC's can be generated *in vitro* for the entire organ, or at least the post-infarction region.

Another goal would include ES-cell derived progenitor cells of every type. This approach should omit the controversial cloning step (or acquiring cells from early human embryos). Current attempts are focused on nuclear re-programming of DNA in differentiated cells, that is de-differentiation and then re-differentiation along another tissue lineage. Several approaches have already been attempted, using fusion between somatic (differentiated) cells and human embryonic stem cells, also using pluripotent cell extracts and *in vitro* cell explantation[33,34].

The third line of research arises from some functional failures of the applied stem cells used until now, and their interaction with recipient cells of the pathological organ. This observation, combined with transcriptome analysis of implanted cells, indicates several candidate genes of which over-expression would benefit the applied cells. Some observations indicated positive results of over-expression of connexin 43 in skeletal myoblasts on gap junction proteins[35,36], however until now there have been no clinical trials with genetically modified myoblasts or muscle-derived stem cells.

Finally, stem cells integrated into the recipient's tissue (pathological organ) could potentially serve as vehicles for genes that have been previously administered alone for induction of local angiogenesis, cardioprotection etc. Intensive work in this area is ongoing. The interesting cycle of experiments performed by Rosenthal group should be cited, where insulin-growth factor was transfected to muscle cells showing their anti-apoptotic and anti-ageing properties in respect to muscle mass and function[37,38]. Recently, during the 5th International Ascona Workshop, this same group reported that transgenic supplementation of a locally acting insulin-like growth factor 1 isoform (mIGF-1) promoted efficient tissue repair of damaged skeletal and cardiac muscle without scar formation and prevented muscle atrophy in heart failure[39]. It is an open question whether experiments in rodents warrant us to move on to clinical trials, this time with genetically modified stem cells. But this is only a question of time.

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