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Cell survival and redistribution after transplantation into damaged myocardium

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Abstract

Cell transplantation has become an attractive option for cardiac regenerative therapy. However, poor cell survival and extensive redistribution throughout the body can drastically affect the outcome and safety of cell therapy. Although various approaches have been attempted to support the survival and engraftment of implanted cells, we need to apply a new comprehensive strategy by melding the *in vitro* and *in vivo* approaches to recondition the cells and infarcted myocardium. Here we summarize our understanding of cell survival and migration after transplantation into the damaged heart.

Keywords: myocardial infarction • cell transplantation • preconditioning • cell survival

Introduction

Myocardial infarction (MI) is one of the leading causes of morbidity and mortality throughout the world. In China, a newly industrialized country, it is estimated that 3 million patients have suffered from MI and an additional 0.5 million patients experience acute MI yearly [1]. Substantial progress has been made in myocardial reperfusion strategies, revascularization procedures and novel pharmacological approaches in the past 30 years. However adult cardiomyocytes have a very limited capacity to regenerate themselves, and therapies for rescuing dead cardiomyocytes remain limited [2]. Cell transplantation (CTx) has become an attractive option for cardiac regenerative therapy. Basic research in this area has progressed rapidly since the early 1990s, and furthermore, the evidence from preclinical studies has also been encouraging. The first clinical trial of CTx for MI was performed by the group of Dr. Menasche in 2002 and since then hundreds of clinical safety and efficacy studies have been performed worldwide [3]. Bone marrow (BM)-derived cells have been the most popular cell source in current studies. Recently, a meta-analysis was performed to review the outcome of 18 studies involving 999 patients who received BM-derived CTx for cardiac repair [4]. Although the administration of BM cells was safe, it only improved heart function by 3% increase in ejection fraction and a 5% reduction in infarct scar size. From a clinical point of view, this moderate improvement is far from satisfactory. In addition to a search for a new more robust cell source, some other potential hurdles must be considered. In the end there is the possibility that cell survival and redistribution after transplantation might drastically improve the outcome and safety of cell therapy.

Poor cell survival limits the efficacy of cell transplantation

Poor survival of engrafted cells has adversely influenced the therapeutic efficacy of CTx for MI. After intracoronary infusion of BM-mononuclear cells, only 5% of transplanted cells could be

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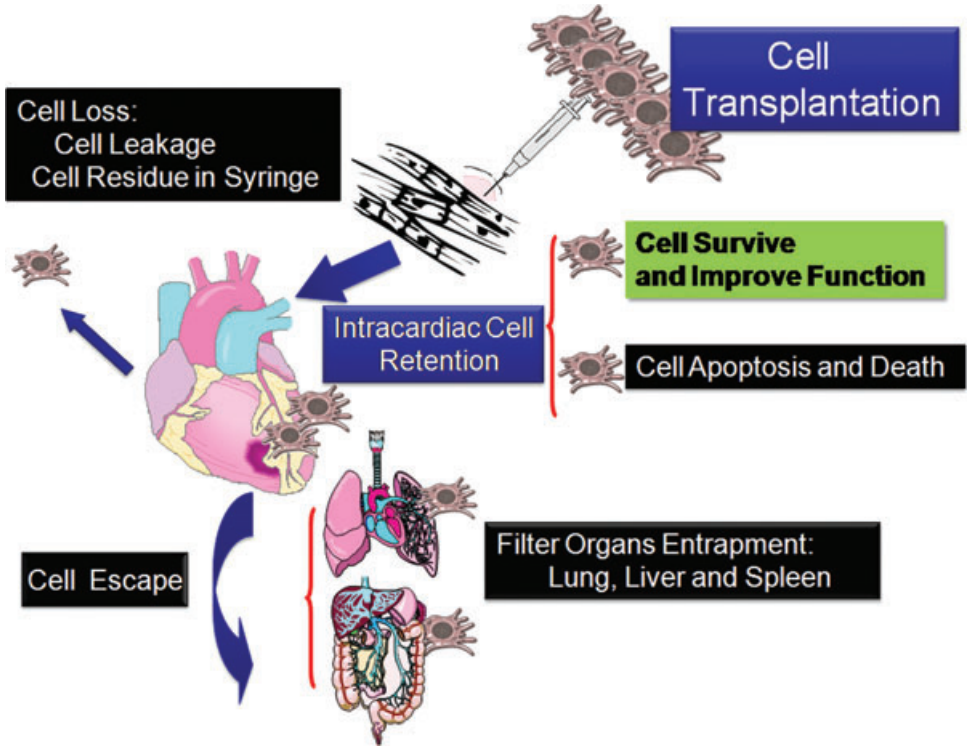

Fig. 1 Illustration of the reasons for cell loss, escape and redistribution.

detected in the myocardium within 2 hrs after infusion; furthermore, only 1% of infused cells resided in the heart 18 hrs after transplantation [5]. Although intramyocardial injection was considered to be the most effective route for precise cell delivery to target areas, the intramyocardially injected cells decreased rapidly from 34–80% at 0 hr to 0.3–3.5% at 6 weeks [6]. Besides the delivery route and cell types, several reasons may account for the poor cell survival rate, as illustrated in Fig. 1. Thus, the transplanted cells face the serious challenge of an unfriendly environment in which they may not survive. The deprivation of oxygen and nutrient supply, the mechanical tension from myocardial motion and various cytotoxic factors in the ischemic myocardium can result in large-scale cell apoptosis and death. The *in vivo* cell proliferation and differentiation of transplanted cells usually occurs weeks after transplantation [7]. The first week is the crucial time for cell survival and can determine the final therapeutic efficacy. In our previous *in vitro* study, we demonstrated that serum deprivation, not hypoxia, was the main reason for the apoptotic cell death of BM-derived ischemic mesenchymal stem cells (MSCs) [8]. Mitochondrial dysfunction leading to increased caspase-3 activation could be a major contributor to this process. However, the choice of different oxygen tensions, serum concentrations and various cell types makes it difficult to elucidate the precise mechanisms underlying apoptotic cell death [9]. Clearly, with the increasing understanding of the signalling pathways involved in cell survival, dedicated strategies should be capable of creating a friendly milieu of host infarcted myocardium to improve long-term cell viability *in vivo*.

Extracardiac cell redistribution and consequent safety issues

In a non-human primate model, culture-expanded MSCs were infused by an intravenous route. The cells were distributed to a wide range of tissues including gastrointestinal tissues, lung, skin, thymus, etc. [10]. Our previous study also revealed extensive extracardiac cell distribution after intramyocardial injection into the border zone of an acute MI area [11].

In another preliminary study, we injected male MSCs into a sex-mismatched rat chronic MI model (Fig. 2). One hour after CTx, 56% of injected cells were untraceable and 8% of cells were harboured by filter organs. Interestingly, we found 3% and 4% of cells in venous and arterial blood, respectively. These findings indicate that cell migration is initiated in an ultra-early stage after transplantation and that blood flow is the main 'highway' for cell escape. Thus venous blood could collect escaped cells from the right and left side of the heart and transport them into the lung. Similarly, cells washed from the left side of the heart could be pumped into the systemic circulation *via* the left ventricular space and then captured by reticuloendothelial systems, mainly located in liver and spleen. Two weeks later, no evidence for cell viability in peripheral blood was found. We presume that the survived cells had been integrated into the microenvironment at a late stage after CTx. Therefore, the myocardial contractile force would not squeeze more retained cells into the blood at a late stage. However, the total amount of escaped cells accounted for 37% of total traceable cells at 2 weeks after transplantation.

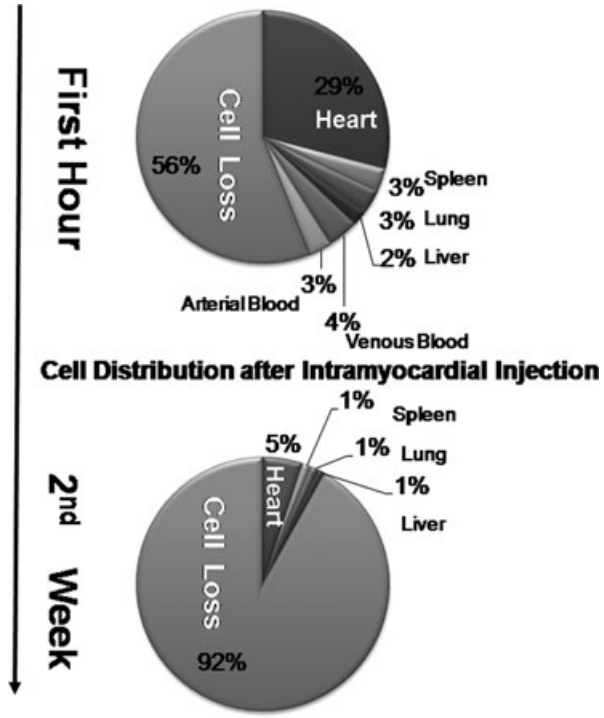

Fig. 2 Quantification of implanted male MSCs in female rats by real-time polymerase chain reaction. The data are expressed as the average percentage, relative to the initial number of injected cells.

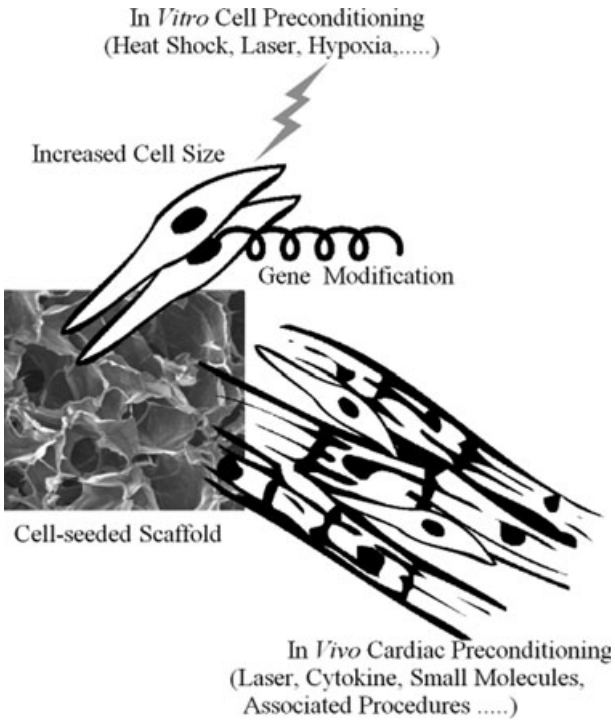

Fig. 3 Illustration of the current preconditioning approaches to enhance cell survival.

At this time, little is known about the impact of escaped progenitor cells on extracardiac organs. These migrated progenitor cells could participate in ongoing local cellular turnover and replacement. A previous study has shown that transplanted BM cells could be a double-edged sword with an unexpected negative impact on the liver [12]. In the clinical experience of angiogenic gene therapy, unwanted angiogenesis, including liver haemangioma and retinopathy, was observed. Therefore, a control of the expressed angiogenic factors to the local ischemic myocardium was advocated [13].

Recently, more robust cell types, including induced pluripotent stem cells (iPS) and cardiac stem cells were introduced into the field of heart cell therapy [14, 15]. Until now, the purity of iPS or embryonic stem cells derived cardiomyocytes has been unsatisfactory. Undoubtedly, transplanted iPS or embryonic stem cells without a cardiomyocyte phenotype hold more potential for differentiation and teratoma formation. Once these cells escape from the heart, they could have a more serious impact on the extracardiac organs.

A hybrid approach to support cell survival and decrease cell escape

Various approaches have attempted to support the survival and engraftment of implanted cells (summarized in Fig. 3). *In vitro* gene modification could be very labour intensive and carry inherent risks for future clinical translation. Therefore, some more convenient approaches such as hypoxia, heat shock and low-level laser irradiation (LLLI) have also been applied for *in vitro* cell manipulation [16]. These approaches could induce faster cell proliferation, augment cytokine release and increase preconditioning or anti-hypoxia ability. Another easy way to enhance cell retention is to increase the size of the injected cells with microencapsulation [17]. The size of freshly isolated progenitor cells is usually 10–20 μm, so the cells could be packed into microcapsules (150–250 μm). With the increased size, the cells would not be drained to the myocardial vein, collateral channels or ventricular lumen and thus reside in the injection sites. Fabrication of cell-seeded biodegradable scaffolds is a promising tissue-engineering approach to nourish grafted cells and maintain neo-cardiac tissue geometry and structure [18]. However, many issues remain unsolved, including the optimal scaffold biomaterial, cell adhesion and diffusion of nutrients. Besides direct pre-treatment with cytokines, recently some small molecules have been identified as novel regulators that are capable of enhancing cell survival rates. Lysophosphatidic acid, as an endogenous lipid mediator, could prevent cell apoptotic death and increase cell survival 2-fold within 1 week after cell injection [19]. Recently, it was demonstrated that microRNAs participate in the ischemic preconditioning. The increased expression of miR-210 promoted transplanted cell survival in an acute MI model [20]. Tannic acid is a natural plant polyphenol. Local injection of

tannic acid could cross-link fibrous collagen and inhibit matrix metalloproteinase activity [21]. The consequent stabilization of the extracellular matrix would reduce myocardial mechanical tension and thereby promote cell survival. These small molecules could be injected simultaneously into the damaged myocardium during CTx.

Some associated procedures could be combined with direct cell injection during open-chest revascularization surgery for patients with MI. We initially applied non-invasive LLLI to precondition the infarcted myocardium. We found that LLLI augments the expression of growth factors and superoxide dismutase in the myocardium, and hence markedly enhances early survival of transplanted MSCs [22]. Beginning in 2009, we initiated a clinical study at our centre to evaluate the efficacy of coronary bypass surgery, combined with pedicled omentum-wrapped heart tissue patch implantation [23]. The omentopexy could significantly stimulate angiogenesis in the locally wrapped area and hence improve cell survival [24].

Poor cell survival and extensive cell migration have caused clinicians and scientists to re-evaluate the safety and efficacy of heart cell therapy. To achieve satisfactory cell survival, we need to apply a comprehensive strategy to join *in vitro* and *in vivo* approaches that precondition both the cells and the infarcted myocardium. Although there are clearly many obstacles to overcome, new understanding and technologies will hopefully move cell-based regenerative therapy closer to the clinic.

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Conflict of interest

The authors confirm that there are no conflicts of interests.

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