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Heart Regeneration with Embryonic Cardiac Progenitor Cells and Cardiac Tissue Engineering

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Abstract

Myocardial infarction (MI) is the leading cause of death worldwide. Recent advances in stem cell research hold great potential for heart tissue regeneration through stem cell-based therapy. While multiple cell types have been transplanted into MI heart in preclinical studies or clinical trials, reduction of scar tissue and restoration of cardiac function have been modest. Several challenges hamper the development and application of stem cell-based therapy for heart regeneration. Application of cardiac progenitor cells (CPCs) and cardiac tissue engineering for cell therapy has shown great promise to repair damaged heart tissue. This review presents an overview of the current applications of embryonic CPCs and the development of cardiac tissue engineering in regeneration of functional cardiac tissue and reduction of side effects for heart regeneration. We aim to highlight the benefits of the cell therapy by application of CPCs and cardiac tissue engineering during heart regeneration.

Keywords: Heart regeneration; Myocardial infarction; Cell therapy; Cardiac progenitor cells; Cardiac tissue engineering; Biomaterials

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the world. According to WHO 17.3 million people died from CVD in 2008 and the number is estimated to reach 23.3 million by 2030 [1]. In the United States alone, the medical cost of CVD is expected triple from $273 billion in 2008 to $818 billion in 2030, constituting a heavy economic burden [2]. Myocardial infarction (MI) is the most common type of CVD with high morbidity and mortality. Approximately 1 million people suffer from MI annually in the US [3]. MI frequently progresses to heart failure accompanied by ventricle remodeling with the permanent loss of up to 1 billion cardiomyocytes that are replaced by myofibroblasts to form scar tissue [4]. In contrast to amphibians, reptiles, and zebrafish, human cannot sufficiently regenerate the injured heart after MI. The current therapeutic approaches, such as medication, intervention and surgical bypass, can limit the disease developments, but they are ineffective in completely restoring reduced ventricular function and reversing scar formation. While whole heart transplantation is one of the most effective option to treat patients with severe MI, it is limited by the shortage of donor hearts and immune rejection complications [5].

Over the past decade, great breakthroughs in stem cell biology have offered several potential strategies for heart regeneration, such as cell therapy and cell reprogramming [6]. Cell therapy is considered to be a promising option for patients afflicted with heart disease. A variety of candidate cell types, including embryonic stem cells, induced pluripotent stem cells, cardiac progenitor cells (CPCs), cardiomyocytes, mesenchymal stem cells, skeletal myoblasts and others, have been explored to repair the injured hearts in animal models by vasculogenesis, cardiomyogenesis and paracrine effects (Figure 1). Several approaches have moved into clinical trials and applications, providing evidence of the cardiac regenerative possibility by cell therapy. The transplanted cells have been shown to take place of the fibrotic scar tissue, form vascular structure and generate new cardiomyocytes. However, it remains difficult to replace the entire infarcted area with newly generated cardiac tissue by the transplanted cells. Several challenges involving cell survival, cell retention, immune rejection, and vascular blood supply need to be technically and practically overcome before the promise of stem cell therapy is fulfilled.

Appropriate cell types and delivery methods are being considered to address these challenges. CPCs, which can give rise to cardiomyocytes, smooth muscle cells and endothelial cells, have been recently reported to significantly improve cardiac functions. Thus, CPCs are believed to be an optimal cell source to address current challenges facing cell therapy.

Cardiac tissue engineering is a vital strategy aimed at improving cell therapy for heart regeneration. It involves application of a series biomaterials designed for facilitating cell delivery and supporting cell functions after transplantation, thus enhancing the regenerative capacity. Moreover, seeding cardiac cells into biomaterials can be used to fabricate engineered vascular and myocardial grafts following transplantation.

In this review, we aim at highlighting the recent advances and major issues in cardiac cell therapy. We especially focus on the advantage and development of CPCs and cardiac tissue engineering during cardiac cell therapy. Furthermore, we attempt to shed light on optimized application of CPCs and biomaterials for heart regeneration after MI.

Current Challenges of Cell Therapy for Heart Regeneration

Since the first application of stem cells in human in 2001, a number of studies have been performed to prove the efficacy and safety of stem cell therapy for MI using multiple cell types. Some studies have advanced from laboratory into clinical trials. However, none of the current cell therapy strategies have been shown to effectively regenerate the injured heart after MI with most clinical

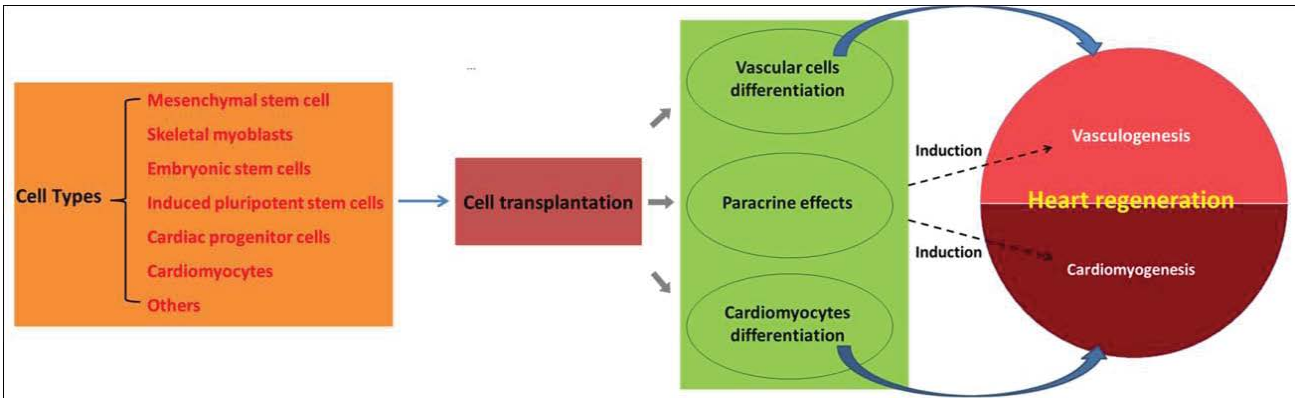

Figure 1: Diagram of cardiac cell therapy. Multiple cell types have been applied to investigate therapeutic potential after transplantation into MI heart. The transplanted cells aim to produce new vascular cells, cardiomyocytes and paracrine effects, leading to vasculogenesis and cardiomyogenesis.

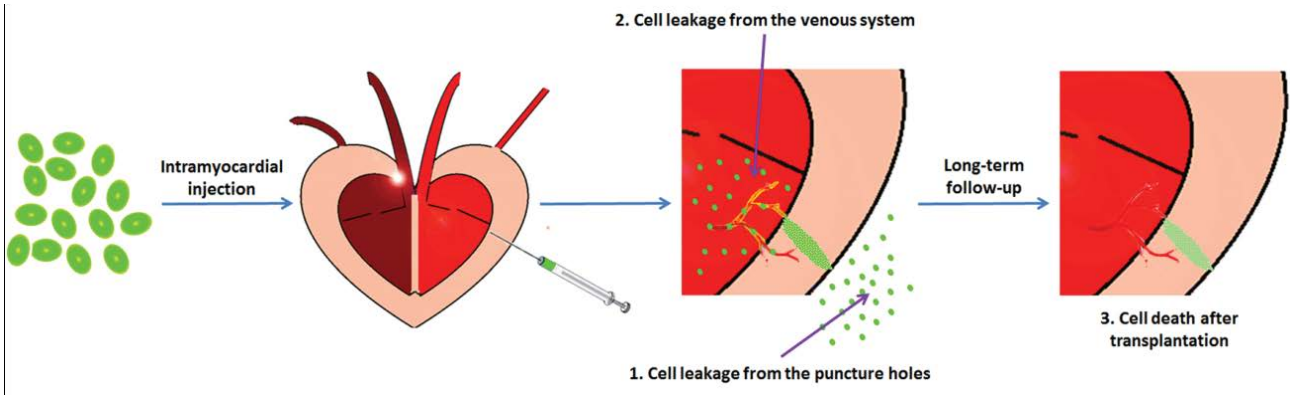

Figure 2: Possible reasons for low cell retention and survival after transplantation. Currently, intramyocardial injection is the most widely used method for cell transplantation. However, most of the transplanted cells are lost for three possible reasons: 1) the cells rapidly leak from the puncture holes after injection; 2) the injected cells leak through the transepicardial/transendocardial puncture holes and then they are washed out from the venous system; and 3) cell death occurs after injection into the infarct region due to the induction of hypoxia, inflammation, cytotoxic cytokines and apoptosis after MI.

trials reporting limited improvement of cardiac function. There are still a number of unsolved problems for cell-based heart regeneration that need to be addressed.

Cell retention and survival

MI typically results in cardiac cell loss, requiring a large number of cells to replace scar tissue and regenerate functional cardiac tissue. However, implanted cells show poor survival and retention after transplantation. Commonly used methods for cell transplantation involve intravenous injection, intracoronary injection and intramyocardial injection. By intravenous and intracoronary injection methods, cells are promptly removed by the circulation with very low numbers homing to the target sites. Intramyocardial injection, which can directly deliver cells into the infarcted region, is more efficient and currently more widely used [7]. But even by intramyocardial injection, more than 90% of injected cells are lost within 2 h [8,9]. Over 75% of retained cells in the first 2 h are lost after 24 h [10]. Possible reasons of the low cell retention and survival after transplantation are shown in Figure 2. -Immediate leakage of injected cells from the puncture holes is one of the major reasons. Blockage of the puncture holes may increase the retained cells by up to 60% 1 h after injection [8,11]. Another route for leakage of cells is through the transepicardial/transendocardial puncture holes and subsequent removal through the venous system. 2 h after injection, up to 30% of lost cells can be found in the lung [12]. Even though a small portion of cells can be retained after injection, numerous cells will also die in the challenging environments of the infarction region characterized by low oxygen, inflammation, cytotoxic cytokines and apoptosis induced by MI. As a result less than 5% of the injected cells can be observed after 4 weeks [7,10].

Regeneration of the functional cardiac tissue

In order to restore the cardiac function after MI, the well-organized cardiac structure should be constructed to replace the infarcted tissue. An ideal cell therapy should develop new vascular network, provide new contractile muscle tissue and synchronize with the contraction of the host heart. Although cardiomyocytes, smooth muscle cells and endothelial cells can be generated from pluripotent stem cells, no cell transplantation has successfully regenerated a fully organized and functional cardiac tissue due to the failed alignment and integration of transplanted cells with host cells.

After MI, the infarction region is replaced by cardiac fibroblasts. Cardiac fibroblasts excrete collagen to form new extracellular matrix (ECM). Deposition of collagen will create scar fibrosis, leading to a distortion of cardiomyocytes alignment [13-16]. Therefore, the transplanted cells are hampered from aligning properly due to the presence of collagen. Furthermore, the transplanted cells need to be mechanically and electrically integrated with the host myocardium where Connexin43 serves as a key protein for cell-cell coupling and electrical signals transduction [17]. Overexpression of Connexin43

in transplanted cells can improve intercellular mechanical and electrical coupling and reduce post-infarct arrhythmia [18,19], highlighting the importance of reestablishment of gap junction coupling. However, cardiac fibroblasts build up solid gap junction coupling after MI by increased expression of Connexin43 [20], impeding the integration of transplanted cells with host cells.

Side Effects: Tumorigenesis, Arrhythmia, and Immune Rejection

Several safety issues with regard to application of cell therapy for heart regeneration have been raised, including tumorigenesis, immune rejection and arrhythmia. Concern of tumorigenesis and immune rejection exists in all kinds of stem cell therapy and should also be considered in heart regeneration.

Tumorigenesis: Pluripotent stem cells can differentiate into multiple cell types as well as tumor cells. The migration of pluripotent stem cells has been found to result in off-target effects. If the undifferentiated pluripotent stem cells migrate from heart into other organs, it is possible to induce tumor formation [21], although, up to now no tumor formation has been found in animal and human studies using differentiated cells.

Immune rejection: Non-autologous cells are recognized as foreign cells by the immune system of the patients. The activated immune response will eliminate the transplanted cells and reduce the therapeutic efficacy. The transplanted cells may also modulate and influence the patient immune system [22]. Immunosuppressive drugs can inhibit the immune rejection but induce immunodeficiency as an adverse consequence. The risk of immune rejection should be carefully considered in order to maximize the benefits of cell therapy.

Arrhythmia: Ventricular arrhythmia after cell transplantation for heart regeneration has been observed in animal experiments and clinical trials. The first reported occurrence of arrhythmia was reported following transplantation of skeletal myoblasts in a clinical study [23]. Arrhythmia also occurred after injection of human ES cells-derived cardiomyocytes into injured monkey hearts [24]. Although arrhythmia was detected shortly after cell transplantation into heart, the arrhythmia symptoms disappeared after long-term follow-up in both studies [25]. The potential mechanism of arrhythmia is proposed by the different action potential and lack of electrical coupling between transplanted cells and host cells [26,27].

To address these challenges, a variety of cell types and several delivery approaches have been proposed. Among those, CPCs and tissue engineering have shown promising benefits of cell therapy to regenerate the heart tissue.

Embryonic Cardiac Progenitor Cells (Ecpcs) For Heart Regeneration

Endogenous heart regeneration

Heart is able to activate endogenous regenerative mechanism after injury. Zebrafish can fully regenerate the heart after resection of the apex and maintain the regenerative capacity during the whole life [28]. Neonatal mice also exhibit regenerative potential, although the extent of neonatal mice regeneration may need further investigation [29-31]. Recent studies show that proliferation of cardiomyocytes is the major mechanism for endogenous heart regeneration [32,33]. Adult mammalian heart retains the potential of cardiomyocytes proliferation after injury [34]. However, the capacity of endogenous adult cardiomyocytes proliferation is very limited thus providing an inadequate source to fully regenerate the injured heart.

Origin of eCPCs during heart development

CPCs have been identified and characterized during heart development (Figure 3). Cardiac precursors are initiated from

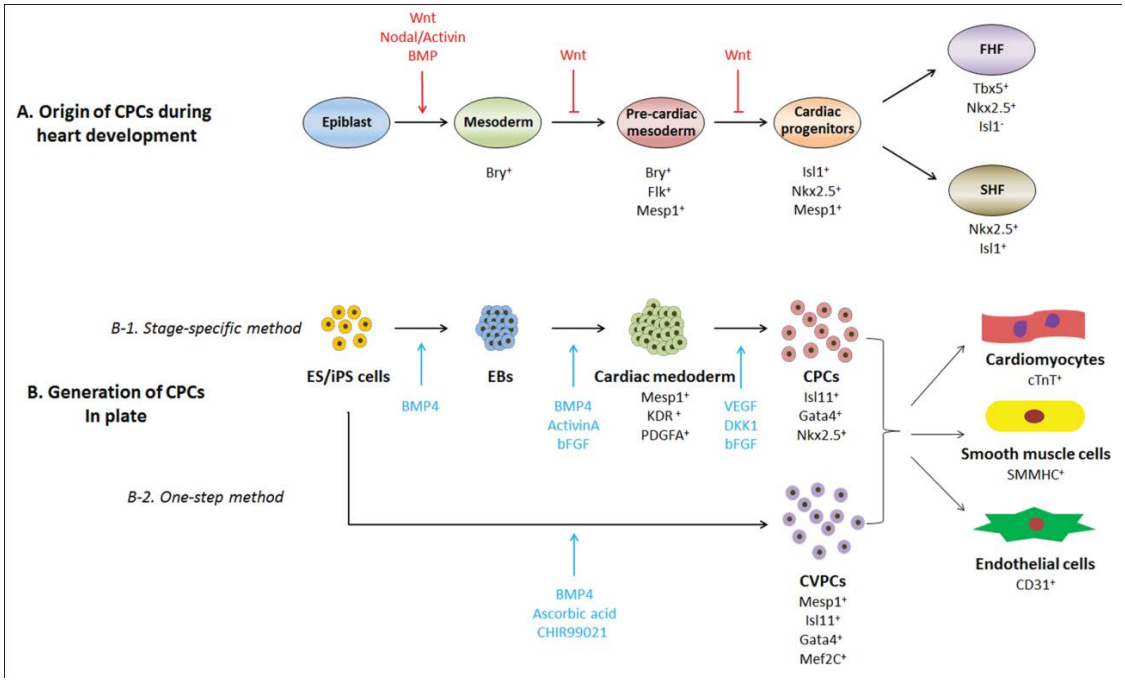

Figure 3: Origin and in vitro generation of eCPCs. (A) Lineage tracing method has been applied to describe specific stages and the origin of CPCs during heart development. Specific marker genes are used to define different stages. In this process, Wnt signaling pathway plays important roles. (B) Stage specific differentiation protocol of CPCs from ES/iPS cells was generated according to the Wnt signaling pathway. An optimized one-step differentiation protocol was developed to generate CPCs in plate. Both methods derived CPCs could further differentiate into cardiomyocytes, smooth muscle cells and endothelial cells.

lateral plate mesoderm, which arises from the primitive streak during gastrulation. Mesp1, a key transcription factor, is identified as the earliest marker of cardiac precursors. The expression of Mesp1 occurs transiently in the primitive streak stage before being extinguished as the cardiac precursors migrate away from the primitive streak. In addition to cardiac lineages, $Mesp1^+$ cells are also involved in formation of paraxial mesoderm and skeletal muscle of the head and neck [35]. $Mesp1^+$ cells develop into cardiac crescent, when the cardiac precursor cells become CPCs by expression of marker genes, such as Nkx2.5, Gata4 and Isl1 [36]. CPCs irreversibly commit to cardiac lineages, contributing to cardiomyocytes, endothelial cells, and vascular smooth muscle cells [37,38]. The cardiac progenitors are further divided into first heart field (FHF) and second heart field (SHF) [39]. By lineage tracing studies, the FHF, which is marked by the expression of TBX5, gives rise to left ventricle and some part of atrium [40,41]; while SHF, which specifically express Isl1, gives rise to right ventricle, the outflow tract, and some part of atrium[42,43]. With the rapid proliferation and differentiation of CPCs, the heart will undergo heart tube formation, looping and four chambers formation, and finally develop into the functional heart.

Embryonic stem (ES) cells/induced pluripotent stem (iPS) cells- derived eCPCs

Generation of ES/ iPS cells-derived CPCs: ES cells are pluripotent cells that can give rise to all the cell types found in the adult organism. However, due to the ethical issues, ES cells cannot be directly used for cell therapy. In 2006, adult mice skin fibroblasts were reprogrammed into ESC-like cells by retrovirus-mediated expression of four transcription factors: Oct3/4, Sox2, Klf4, and c-Myc [44]. This technique was soon reproduced in human cells to generate human iPS cells using human fibroblasts [45,46]. Recently, iPS cells were generated by defined chemicals alone without application of virus transfection, providing a more promising method that would not introduce mutation and might be safer for clinical use. iPS cells could be maintained in the ES cell like state in culture medium and have been successfully used to generate various somatic cells for regenerative medicine.

ES/iPS cells can be used to generate cardiac cells for cell therapy (Figure 2). The specification and differentiation of cardiac lineages during in vitro differentiation are modulated by several signaling pathway, including BMP, GSK3, TGF-β/activin/nodal, WNT/β-catenin, and FGF pathway [47,48]. In recent years, multiple differentiation protocols have been developed to direct ES/iPS cells into cardiac lineages and eCPCs can arise during the differentiation process.

The most commonly used method to generate eCPCs was the stage specific differentiation through embryonic body (EB) formation [49] to mimic the embryos development. ES/iPS cells are digested into single cells and suspended in differentiation medium on low-adherence plates to generate EBs, which can differentiate into all 3 germ layers [50]. Then eCPCs are induced by stepwise addition of growth factors in defined medium. By addition of BMP4, ActinvinA and bFGF, EBs are induced into cardiac mesoderm cells marked by Mesp1. $Mesp1^+$ cells specifically differentiate into eCPCs in the presence of VEGF, DKK1 and bFGF. eCPCs can be identified by the expression of surface markers, such as KDR/Flk-1 and PDGFR-α, or by the expression of transcription factors, such as Isl1, Gata4 and Nkx2.5. The derived eCPCs can efficiently differentiate into cardiomyocytes and vascular cells [51,52].

Another method has been reported to directly induce cultured human ES/iPS cells into cardiovascular progenitor cells (CVPCs) by application of BMP4, GSK3 inhibitor CHIR99021 and ascorbic acid [53]. The CVPCs can be characterized by expression of multipotent cardiovascular progenitor cell markers of Mesp1, Isl1, Gata4 and Mef2c. The CVPCs generated by this method can be maintained long-term and expanded by a combination of GSK3 inhibitor, BMP inhibitor and Activin/Nodal inhibitor. The derived CVPCs exhibited expected potential to generate cardiomyocytes, smooth muscle cells and endothelial cells using specific differentiation medium. Further research should be performed to describe whether the CVPCs are subpopulation of eCPCs, which are specifically commited to cardiac lineage and can be used for cell therapy.

Application of human iPS cells-derived eCPCs for heart regeneration: ES/iPS cells-derived eCPCs have shown great advantage for heart regeneration. Heart regeneration requires replacement of injured heart with new cardiac tissue accompanied with myocardiogenesis and angiogenesis. eCPCs have the potential to realize this goal by simultaneously differentiation into three major cardiac lineages. The specific commitment to cardiac lineages results in low potential of teratoma formation after cell transplantation. Furthermore, the autologous cells derived iPS cells could reduce the immune rejection for cell therapy. The therapeutic potential of eCPCs has been well investigated after transplantation into MI heart in multiple animal models, including mouse, rat and monkey.

Ali Nsair [54] reported that the combination of $Flt1^+/Flt4^+$ facilitated enrichment for mouse iPS cell-derived $Isl1^+/Nkx2.5^+$ CPCs with ability to differentiate into all three cardiac lineages. After transplantation into mouse heart, the transplanted cells were demonstrated to robustly engraft into host tissue and differentiate into mature cardiomyocytes. To verify the therapeutic effects of eCPCs, mouse ES cell-derived CPCs, labeled with EGFP driven by CPC marker gene Nkx2.5, were injected into the mouse heart after MI [55]. Injected CPCs significantly reduced scar area and improved cardiac function within two weeks. The injected cells differentiated into three major cardiac cell types with formation of mechanical and electrical coupling to host cells. No teratomas or arrhythmias were observed after transplantation.

Therapies using human iPS cell-derived eCPCs have been investigated. A modified 6-day generation protocol was established using human iPS cells as a monolayer and the derived CPCs were identified by expression of KDR and PDGFR-α [56]. After transplantation into the MI/reperfusion rats, the CPCs differentiated into cardiomyocytes and smooth muscle cells and persisted for at least 10 weeks, leading to a non-significant trend to protect declined cardiac function after MI. In another study, eCPCs were derived from Rhesus ES cells by treatment of BMP2 and characterized by expression of Oct4, SSEA-1 and Mesp1. eCPCs were purified by cell sorting using an anti–SSEA-1 antibody [57]. After transplantation into the MI Rhesus heart, $SSEA\text{-}1^+$ CPCs differentiated into cardiomyocytes and reconstituted 20% of the scar tissue. The $SSEA\text{-}1^+$ CPCs did not develop teratomas, whereas the non-purified CPCs, containing $SSEA\text{-}1^-$ cells could induce teratomas.

Application of eCPCs derivatives for heart regeneration

ES/iPS cell-derived eCPCs can further differentiate into cardiac cells, which have also been used for heart regeneration after MI. The effects of human pluripotent stem cell- derived cardiomyocytes for regenerating injured heart have been extensively investigated. After injection of human ES cell-derived cardiomyocytes into infarcted murine heart, the cells could engraft and electrically couple with host cells, but significant improvement of cardiac remodeling and function was not shown [58-60]. Significantly, a recent study has shown great promise for cell therapy against MI by

injection of human ES cell-derived cardiomyocytes into non-human primate heart. After cell transplantation, obvious improvement of infarcted heart was shown. 40% of the infarcted region was replaced by injected cardiomyocytes and the host vessels could extend into the graft to form new vascular network [24]. Besides cardiomyocytes, the therapeutic efficacy of ES cell-derived vascular cells has been investigated. After injection of mouse ES cell-derived endothelial cells into MI mice, the cardiac function was improved by formation of capillaries and venules in the infarct region. Using a single cell type appears inefficient to regenerate the injured heart. To simultaneously regenerate myocardium and vasculature, a recent study was performed where a mixture of human iPS cell-derived cardiomyocytes, endothelial cells, and smooth muscle cells (1:1:1) was transplanted using pig MI model [61]. The survived cardiomyocytes could integrate into the host cells and endothelial and smooth muscle cells involved in vasculature formation, contributing to cardiac function improvement. This study supports potential advantage of application of eCPCs for heart regeneration since eCPCs can automatically differentiate into cardiomyocytes, smooth muscle cells and endothelial cells with optimal efficiency under regulation of in vivo signaling pathways.

Adult CPCs

Identification and isolation of adult CPCs: Adult CPCs have been identified in the adult heart and characterized by the expression of stem cell surface markers, such as c-Kit, Sca-1 and SSEA-1. After MI, adult CPCs can be activated and, they are able to home in on the infarction region [62] but cannot produce sufficient numbers of cardiac cells to efficiently regenerate the injured heart [63]. Nevertheless, adult CPC is considered as an ideal cell source for cell therapy (Figure 2). Adult CPCs have been successfully isolated from cardiac biopsies of mice [64], rat [65], sheep [66], pig [67] and human [68]. Despite small numbers of adult CPCs that can be derived from the biopsies, they can be propagated for long periods of time to obtain sufficient cells for transplantation [65,69]. Notably, this kind of autologous cells may not induce immune rejection. Furthermore, the adult CPCs are committed to the cardiac lineages, indicating that the cells present the possibility to generate new cardiac tissue without tumorigenesis.

Application of adult CPCs for heart regeneration: Several types of adult CPCs, including c-Kit$^+$, Scar-1$^+$, SSEA-1$^+$, "cardiosphere" progenitor cells and side population CPCs, have been applied in vivo studies after MI and have demonstrated the therapeutic potential. Transplantation of c-Kit$^+$ CPCs reduced infarct size and improved cardiac function after MI by vascular system restoration, especially by formation of large coronary arteries [70,71]. Even in a 30-day infarction rat model, intracoronary administration of c-Kit$^+$ CPCs could alleviate the cardiac dysfunction [72]. By 2011, a phase 1 clinical trial was completed by transplantation of c-Kit$^+$ CPCs into MI patients. The results were very encouraging with regard to autologous c-Kit$^+$ CPCs ability to effectively improve LV systolic function and reduce infarct size in MI patients. Similar therapeutic potential was confirmed with Sca-1 and SSEA-1 CPCs. Sca-1$^+$ CPCs have been isolated from adult heart with expression of early cardiac markers of Gata4 and Mef2C, lacking c-Kit [73], and SSEA-1$^+$ CPCs obtained from adult heart have been characterized by expression of SSEA-1 and Oct3/4 without c-Kit and Sca-1[74]. Transplantation of Sca-1$^+$ or SSEA-1$^+$ CPCs showed improvement of cardiac function and attenuation of cardiac remodeling. "Cardiosphere" progenitor cells and side population CPCs have also been isolated from the adult heart and exhibited therapeutic potential for MI. However, they were identified with a mixture of CPCs, vascular progenitor cells and mesenchymal progenitors, which limited their clinical application [75,76].

Although improvement of cardiac function after MI has been described after transplantation of adult CPCs, their utility for clinical application remains controversial due to the origin and differentiation issues. The c-Kit and Sca-1 are the most used surface markers to isolate adult CPCs. They were initially used to isolate hematopoietic stem cells from bone marrow. The mesenchymal markers are found co-expressed in several subpopulations of c-Kit$^+$ and Sca-1$^+$ CPCs [77,78]. It is presumed that the adult CPCs are a different subset of mesenchymal stem cells or a mixture of CPCs and mesenchymal stem cells. Besides of the origin of adult CPCs, the differentiation potential of adult CPCs is also under debated. The CPCs are defined by their ability to differentiate into three types of cardiac cells. Cardiomyocytes are particularly the most important cells to restore the cardiac function. c-Kit$^+$ and Sca-1$^+$ CPCs have been shown to have a minimal potential to differentiate into cardiomyocytes. In vitro, Sca-1$^+$ CPCs were shown to differentiate into cardiomyocytes with very low efficiency, while addition of specific chemicals and growth factors could induce adult CPCs differentiation into cardiomyocytes [73,79,80]. c-Kit$^+$ CPCs were characterized by differentiation into cardiomyocytes in vitro[81], but less than 0.8% of adult c-Kit$^+$ CPCs contributed to cardiomyocytes by lineage tracing studies in vivo, even after injury [82,83]. However, some recent studies showed that after injection of c-Kit$^+$ or Sca-1$^+$ CPCs into infarcted heart, improvement of cardiac function could be observed by formation of vasculature and cardiomyocytes [71,72,84,85], although the efficiency of cardiomyocytes formation of transplanted cells should be further investigated. In addition, a single c-Kit$^+$ progenitor cell isolation and culture method was developed, providing a potential to generate real cardiac specific c-Kit$^+$ CPCs for cell therapy [86].

Cardiac Tissue Engineering

The previous cell transplantation methods for heart regeneration include intravenous injection, intracoronary injection and intramyocardial injection. These methods have severe cell retention and cell survival issues, leading to limited cell therapy potential. The emergence of tissue engineering offers an encouraging strategy to improve cell delivery. Over the past two decades, various engineered tissues were constructed in vitro, such as bone, skin, nerve, liver, blood vessel and myocardium [87]. Cardiac tissue engineering strategies, in which biomaterials play important roles in facilitating cell transplantation, have been developed to repair injured heart.

Currently used biomaterials for heart regeneration

Currently, hydrogel, decellularized ECMs and synthetic matrices are the main reported biomaterials applied for heart regeneration (Table 1) [88]. There are at least several general principles of biomaterials construction for heart regeneration: (1) the materials should be biocompatible and degradable; (2) the materials should not influence transplanted cells' proliferation and differentiation; (3) the materials should facilitate transplanted cells to establish mechanical and electrical couplings with host cells; and (4) the quality of the materials should be controllable to realize commercialization.

Hydrogel from natural polymers, such as collagen, fibrin, matrigel, gelatin and alginate, has been extensively investigated for cell-based tissue engineering approaches for heart regeneration. Particularly, the collagen matrix was the pioneered biomaterial to generate engineered cardiac tissue [89]. The natural materials are derived from native tissues thus exhibiting beneficial properties of being bioactive and biocompatible [88]. Additionally, they proivde ECM-like features of natural environment and structure for cell adhesion, proliferation and differentiation. Application of natural

Biomaterials	Hydrogel	Decellularized ECMs	Synthetic matrices
Components or Origin	Collagen Fibrin Matrigel Gelatin Alginate Others	SIS Pericardium Valves Whole pig heart	PU PGS PLLA PLGA PCLA Others
Advantage	Bioactive Biocompatible Natural environment	Organized tissue Provide donor heart	Mechanical strength Consistent qualities Biocompatible Biodegradable
Disadvantage	Less mechanical strength Pathogen risks potential Variable qualities	Pathogen risks potential Variable qualities	Integration to host Foreign responses

Table 1: Biomaterials applied for cardiac tissue engineering.

biomaterials can form better cell-cell interaction, but it leads to similar mechanical strength to native tissue, which is a very soft tissue, resulting in the difficulties for transplantation. The weakness of mechanical strength can be partly solved by combination of multiple scaffolds [90].

Decellularized ECMs are prepared by removing the cells from tissue, leaving ECMs, cell matrix interactions and blood vessels intact. It provides native ECM as biological scaffolds, following recellularization to fabricate the cardiac tissue. Decellularized ECMs have been generated from blood vessel [91], small intestine submucosa [92,93], pericardium [94,95] and valves [96]. Even the whole heart has been constructed by the decellularized ECMs in mouse and rat [97,98]. The decellularized ECMs cannot be derived from human heart, but pig heart, with many similarities to human heart in histology and physiology, can be used as an alternative to develop decellularized whole heart ECMs, providing a promising candidate for heart transplantation to treat MI [99,100].

Although several advantages have been shown in hydrogel and natural ECMs, the mechanical issues, pathogen risks and variable qualities between batches hamper their wide application [101]. Much effort has been devoted to exploring synthetic matrices due to their the advantages of being biocompatible and biodegradable [102]. Synthetic matrices, which mimic the natural ECMs and in vivo environments, are made with predictable and consistent mechanical, chemical and physical properties [103]. Their properties can be tuned to match specific applications, and they can realize commercialization with precise quality control. Multiple synthetic matrices have been reported for stem cell based heart regeneration, such as polyurethane (PU), polyglicerolsebacate (PGS), poly-L-lactic acid (PLLA), poly-lactic-co-glycolic acid (PLGA) and poly-chitosan-g-lactic acid (PCLA) [104]. Some shortcomings of synthetic materials such as difficulties with the host tissue integration and inability to conduct appropriate signals have been described [105]. These problems can be addressed by combination of regulatory molecules, growth factors and proteins [106,107].

Numerous biomaterials have been used to deliver stem cells and construct cardiac tissue. Some publications have shown the enhanced outcome of cell therapy. According to their application, these biomaterials can be classified into injectable biomaterials and engineered cardiac grafts. Both natural and synthetic materials have been extensively explored as injectable biomaterials and cardiac tissue grafts. The injectable biomaterials aim to directly deliver cells into the injured region with increased cell survival, while the engineered cardiac grafts are designed to generate a functional tissue to replace the infarcted ventricle.

Injectable biomaterials for cardiac cell delivery

Injectable biomaterials, which allow the injected cells to be directly delivered into the target site of the infarct region, are being developed to improve cell retention and survival during cell injection. After direct cell injection, the single cells are rapidly lost due to leakage from the puncture holes. The lack of cell-cell and cell-ECM interactions limit cell survival. The injectable materials offer a temporary ECM as a support structure for cell adherence thereby preventing cell ejection and increasing cell survival after injection [108]. The injectable biomaterials can also be used as a controlled release system to package drugs and growth factors during cell transplantation to further improve cell survival, proliferation, differentiation and cell coupling. In addition, injectable biomaterials can deliver cells with minimal invasive procedure by combination of catheter mediated cell delivery approaches [108,109].

Hydrogel and nanoparticles are the most used injectable biomaterials for heart regeneration. The improved cell retention accompanied by restored cardiac function and reduced scar tissue have been demonstrated by co-injection of injectable materials and various cell types [9,110-113]. Some advanced injectable materials have been designed to improve therapeutic effects. Thermosensitive hydrogel system, which underwent a sol-gel reversible transition upon heating or cooling at ≈32°C, was designed to increase injected cells retention compared with injection of dissociated cells [114]. With this biomaterial, the cardiac function significantly improved in rats and pigs with MI. Thermosensitive biomaterials were also able to suppress oxidative stress damage to cells so that the cell survival could be effectively improved [115]. Magnetic targeting method was combined with nanoparticles to prevent washing out of the injected cells. Magnetic targeting with ferumoxytol nanoparticles delivered cardiosphere-derived stem cells into MI region, leading to augmented cell engraftment and therapeutic benefit without any toxicity [116].

Engineered cardiac grafts

Engineered cardiac grafts are fabricated by seeding cells into biomaterials. Some of them are cultured in a bioreactor for a certain time. An ideal engineered cardiac grafts should exhibit contractility forces, extensive vascularization and electrical transduction. Different types of cell sheets and 3-D scaffolds have been developed by natural or synthetic biomaterials to generate cardiac grafts [117]. Despite some positive therapeutic potential observed from cardiac grafts, constructing a well-organized and functional cardiac tissue remains a significant challenge. Several strategies present promising advantage to improve the engineered cardiac grafts.

Contractility forces formation is a big challenge for generation of cardiac graft in vitro due to the cell alignment and mechanical

coupling issues, further leading to limited electrical coupling. Cardiomyocytes are striated for the parallel arrangement of actin and myosin filaments. To address this problem, some biomaterials were designed into parallel channels with certain width so that the seeded cells were aligned in a highly organized parallel manner [118-120]. The laser-patterned array was also reported to provide guidance to direct cells alignment [121,122]. The well-aligned cells revealed higher expression of Connexin43, suggesting improved cell-cell interaction and mechanical coupling. The electrical coupling was also improved in the well-organized engineered grafts. Some natural biomaterials, such as epicardial mimetics and decellularized ECMs, were applied to fabricate recellularized heart tissue with the presence of alignment and electrical coupling [123,124]. Even the acellular biomaterials themselves could attenuate remodeling and improve cardiac function after MI [125]. Mechanical and electrical stimulation approaches were broadly investigated in 2D and 3D engineered cardiac tissue. The stimulation appeared to generate functional cardiac tissue in vitro by mechanical and electrical coupling and improve cardiomyocytes maturation as well [126-130].

Although therapeutic effects of engineered cardiac grafts have been widely reported after MI, the cell survival issue remains unresolved [131-133]. The low survival rate is believed to be due to the low oxygen supply caused by graft thickness and poor revascularization. In a recent study, cardiac tissue slice was used as a model to investigate the maximum thickness of grafts. The results indicated that the cell death was higher at the edge of the grafts and <400 μm was the optimal thickness [134]. The revascularization issue can be addressed by optimization of cellular composition of the grafts. Co-culture of endothelial cells and fibroblasts was shown to revascularize the grafts and improve cardiac function [135,136]. Revascularization of transplanted grafts was also achieved by addition of growth factors [137].

CPCs Based Cardiac Tissue Engineering For Heart Regeneration

There are two potential strategies to regenerate injured heart by application of CPCs and cardiac tissue engineering (Figure 4). One is biomaterial-mediated CPCs injection into infarcted region, allowing CPCs to replace scar tissue with newly formed cardiac tissue; the other is fabrication of cardiac tissue by cultivation of CPCs with biomaterials in vitro, followed by transplantation into ventricle wall of infarct region.

The application of injectable biomaterials has shown the improvement of retention and survival of CPCs. Matrix-enriched hydrogel capsules were used to deliver human biopsy-derived resident CPCs into mice heart with MI [138]. The cell retention and survival were significantly improved and restored cardiac function was observed. The transplanted CPCs could differentiate into both cardiomyocytes and vascular cells, suggesting the potential to regenerate new cardiac tissue. When naturally derived cardiac ECM was explored to deliver CPCs [139], strong adhesion of CPCs to the cardiac ECM was demonstrated. CPCs exhibited enhanced proliferation and increased tolerance to apoptosis on the cardiac ECMs. Modified biomaterials have been explored to further improve cell survival or for certain other application. For example, an oxygen-releasing system, which consisted of hydrogen peroxide-releasing microspheres, catalase and an injectable, thermosensitive hydrogel, was demonstrated to augment CPCs survival and differentiation under hypoxic condition [140].

Several CPC-based engineered cardiac grafts have been generated in vitro. Gelatin and collagen scaffolds seeded with human adult CPCs were used to construct cardiac grafts [141]. The cells were viable and normally proliferating in the scaffold. CPCs were selectively committed towards cardiomyocytes lineage to generate the myocardium grafts. Printing technology was applied to generate 3-D cardiac grafts by combination of CPCs and alginate scaffold [142]. The CPCs could survive, proliferate and differentiate into cardiac lineages during culture. The printed cells were able to migrate from the matrix to form tubular-like structures. A defined organized cardiac graft was constructed by this method. The engineered heart was successfully constructed in vitro by seeding human iPS cells-derived CPCs into decellularized mouse heart [98]. The existence of natural ECMs directed CPCs into well-organized alignment and generation of functional myocardium. The engineered heart exhibited similar physiological response to normal heart. Repopulation of decellularized heart provided a promising strategy to generate donor heart for transplantation.

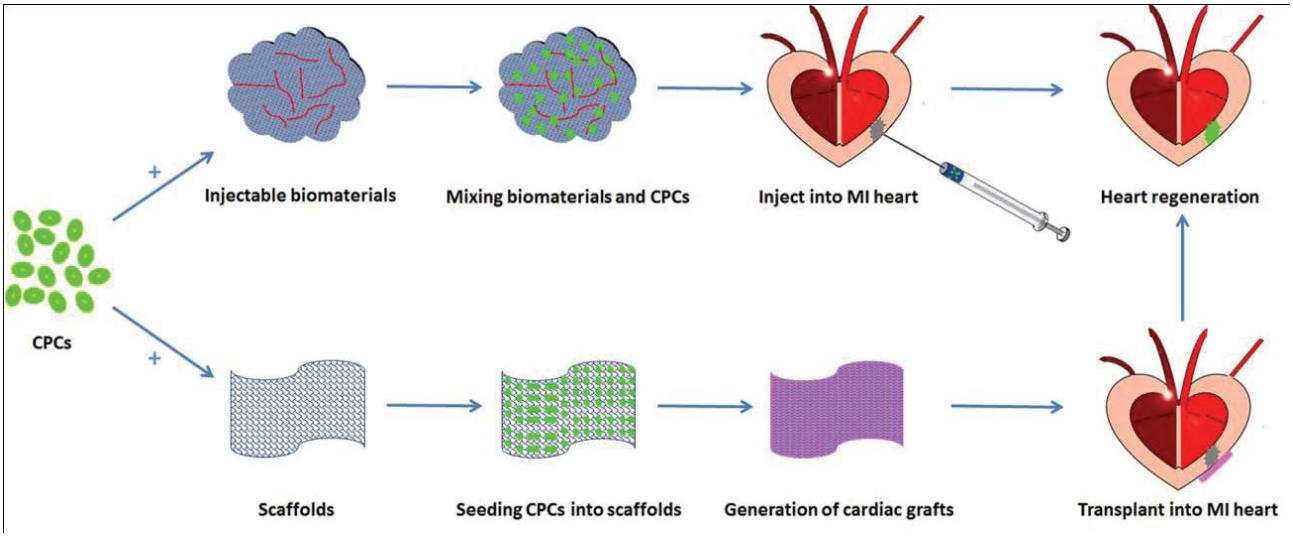

Figure 4: Potential strategies of CPCs based cardiac tissue engineering for heart regeneration. One strategy is to mix CPCs and injectable biomaterials and then directly inject into the infarcted region, allowing CPCs to replace scar tissue with newly formed cardiac tissue; the other involves fabrication of cardiac tissue by cultivation of CPCs with biomaterials in vitro and then transplantation of the tissue engineered cardiac grafts to the surface of the infarct region to achieve heart regeneration.

Since most of the engineered cardiac grafts by application of CPCs and biomaterials are constructed in vitro, their therapeutic effects should be further investigated in the injured heart *in vivo*.

Conclusions

Collectively, the major challenges for current cell-based heart regeneration include cell retention and survival, functional cardiac tissue formation and side effects. Application of CPCs and cardiac tissue engineering has the potential to address these key issues. The CPCs are committed to cardiac lineages, differentiating into cardiomyocytes, smooth muscle cells and endothelial cells, suggesting the potential to generate a cardiac tissue without tumorigenesis. The autologous derived CPCs can reduce immune rejection after transplantation. Meanwhile, tissue engineering technique has shown advantages in improving cell retention and survival, cell alignment, cell coupling and integration into the host tissue. However, there are still various limitations of application of CPCs and tissue engineering. In order to optimize the application of CPCs, future studies are required to illustrate the mechanism of cardiac remodeling after MI and the mechanisms of CPCs in heart regeneration. Furthermore, novel biomaterials and delivery systems are required to further advance the tissue engineering for heart regeneration.

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