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Review

Insight on stem cell preconditioning and instructive biomaterials to enhance cell adhesion, retention, and engraftment for tissue repair

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ABSTRACT

Stem cells are a promising solution for the treatment of a variety of diseases. However, the limited survival and engraftment of transplanted cells due to a hostile ischemic environment is a bottleneck for effective utilization and commercialization. Within this environment, the majority of transplanted cells undergo apoptosis prior to participating in lineage differentiation and cellular integration. Therefore, in order to maximize the clinical utility of stem/progenitor cells, strategies must be employed to increase their adhesion, retention, and engraftment *in vivo*. Here, we reviewed key strategies that are being adopted to enhance the survival, retention, and engraftment of transplanted stem cells through the manipulation of both the stem cells and the surrounding environment. We describe how preconditioning of cells or cell manipulations strategies can enhance stem cell survival and engraftment after transplantation. We also discuss how biomaterials can enhance the function of stem cells for effective tissue regeneration. Biomaterials can incorporate or mimic extracellular function (ECM) function and enhance survival or differentiation of transplanted cells *in vivo*. Biomaterials can also promote angiogenesis, enhance engraftment and differentiation, and accelerate electromechanical integration of transplanted stem cells. Insight gained from this review may direct the development of future investigations and clinical trials.

1. Introduction

Cell-based therapies show promise for providing cures to a multitude of debilitating diseases as well as repairing tissue damages. A variety of cell populations capable of repairing and rebuilding tissues or organs have been identified [1,2]. Among cell populations, stem cells are appealing therapeutic agents due to their rapid and extensive proliferation, self-renewal, and multipotency. Adult stem cells hold great potential to regenerate damaged tissues, and thus can be utilized as a treatment to accelerate healing and regenerate ischemic or damaged tissues. Many clinicians currently prefer to utilize autologous stem cells (i.e. cardiac stem cells, CSCs for myocardial infarction (MI) and chronic heart failure (CHF)) due to the relative low cost and safety compared to embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Additionally, adult stem cells are not blocked by various religious, ethical, legal, and immune rejection barriers that inevitably arise with the use of ESCs, or the high cost associated with the use of iPSCs as indicated below. Parallelly, ESCs and iPSCs have shown considerable benefits for regenerative medicine and tissue engineering applications [3–5]. ESCs fulfill all of the requirements of stem cells including self-renewal, clonality, and multi-potency. These cells can differentiate into any cell type present in the adult organism and have the potential to regenerate ischemic tissues [6,7]. Despite their ability to differentiate into all three germ layers (ectoderm, mesoderm, and endoderm), ESCs have limitations that hinder progress and clinical translation of their use in therapies: 1) ethical concerns because ESCs are isolated from the inner cell mass of the human embryo, 2) immune rejection problems because these cells are isolated from an allogenic source, and 3) religious concerns because an embryo may be considered by some as human life. Similarly, iPSCs are promising cells for transplantation and these cells are one step closer to the "ideal" stem cell because they have the differentiation potential of ESCs but potentially have a lower risk of teratogenicity and their

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use raises fewer ethical issues. The discovery of iPSCs led to many more studies, including those performed for developing "disease-in-a-dish" models for drug-screening platforms, generating disease-specific iPSC lines to study the pathophysiology of disease, and creating personalized therapies for autologous stem cell transplantation. However, iPSCs could be biased toward differentiation into certain lineages because of epigenetic memory [8].

Moreover, there are still key limitations that continue to complicate the clinical translation of pluripotent stem cells (PSC) sources (defined as derivatives of both ESCs and iPSCs). Preclinical challenges that must be addressed include the inherent tumorigenic potential of PSC due to their properties of self-renewal and pluripotency and the problems arising from PSCs differentiating into heterogeneous mature cell types, as well as issues with immunogenicity, engraftment, and survival [9]. One of the most significant challenges in using PSCs is the formation of teratomas [10]. Because stem cell-based products may consist of a heterogeneous population of cells, it is crucial to avoid neoplasms. This heterogeneity may arise from products being contaminated with undifferentiated cells or the use of a differentiation process that yields cells of multiple lineages [11]. Moreover, cell product may dedifferentiate into cells capable of forming neoplasms. Poor stem cell survival and engraftment after transplantation is partly due to the rejection of cells by the host's immune response. The lack of an effective method to induce immune tolerance to maintain cell survival is a bottleneck for cell therapy. While ESCs might be considered immune privileged, differentiated derivatives of ESCs can trigger an immune response [12].

When using PSCs for commercialization, investigators need to seek approval from regulatory agencies such as the European Medicines Agency in Europe and the Food and Drug Administration (FDA) in the US. Readers are referred to valuable reviews for the regulatory considerations of stem cells and stem cell-based products [13–17]. We discuss key points for these regulatory considerations. With PSCs, the use of defined culture conditions should be implemented. It is preferable to avoid using chemically undefined media or materials of animal origin, including fetal bovine serum and mouse embryonic fibroblasts, because they carry a risk of transmitting xenopathogens. For preclinical animal studies, it is crucial that stem cell-based products are manufactured using processes comparable to those intended for the final Good Manufacturing Conditions (GMP) product. This is an important step because these studies may be used to support future investigational new drug (IND) applications filed with the FDA. Preclinical studies are intended to assess product safety, off-target effects, and the potential for teratoma formation from undifferentiated cells within the transplanted cell product. Recent histopathological techniques for preclinical studies cannot pinpoint the underlying mechanisms of stem cell biodistribution, engraftment, and migration in real time. Therefore, the risk of ectopic engraftment is unclear. Recent advances in positron emission tomography (PET), magnetic resonance imaging (MRI), fluorescence imaging (FLI), bioluminescence imaging (BLI), and other techniques may need to be utilized for analysis on understanding the behavior of transplanted cells in tissues of interest, enabling the spatiotemporal mapping of transplanted cells for both long-term and short-term safety studies required by the FDA [18]. In addition, the FDA requires that all stem cell clinical products undergo safety/clinical studies before obtaining IND approval [13]. Moreover, cell products must be tested rigorously for acute infusion toxicity that might result in damage to the site of implantation or collateral damage to adjacent tissues originating from an immune response against the cell product. For these studies, the FDA requires investigators to look for organ toxicity and measure blood counts of animals after cell transplantation.

1.1. Stem cell transplantation

Cells are introduced into the body via injection, through systematic circulation, or directly into the tissue of interest (Fig. 1). A variety of cell types have been transplanted in these ways including bone marrow mesenchymal stem cells (BM-MSCs), adipose tissue-derived stem cells (ADSCs), cardiac progenitor cell (CPCs), cardiomyocytes (CMCs), cardiosphere-derived cell (CDCs), cardiac stem cells (CSCs), neural progenitor cells (NPCs), neural stem cells (NSCs), iPSCs, and ESCs [19,20]. These studies have shown the potential of these therapeutic approaches as the transplanted stem cells participated in the repair of damaged tissues either directly or via paracrine mechanisms [21,22]. For example, animal studies have revealed differentiation of transplanted hematopoietic stem cells (HSCs) into CMCs in infarcted myocardium (IM), but at an exceptionally low rate [23]. Despite these encouraging outcomes, the success of cell therapy has been limited due to marginal cell survival and retention after transplantation as well as limited engraftment (typically < 3% cells engraft) in the hostile ischemic environment [24]. A major cause of this low success rate may be due to cellular apoptosis, which is triggered by the disruption of cell-cell and cell-extracellular matrix (ECM) interactions during cell harvesting for transplantation [24].

There are several barriers that limit the therapeutic efficacy of transplanted stem cells in IM, including exposure of cells to ischemia and inflammation, mechanical washout of cells by the constantly beating myocardium, flushing of cells by the coronary vasculature, leakage of the cell suspension from the injection site, anoikis, and fibrosis. This hostile microenvironment might reduce the success of exogenous cell therapies. Critically, this occurs within a few days of transplantation. The reported rate of cell retention in animal hearts, where cells were transplanted intramyocardially as a simple suspension in saline or media, varies with the cell type and number; however, cell retention is very low [24]. For example, 11% of MSCs remained in infarcted rat or porcine hearts 90 min after transplantation [25]. This value decreased to 0.6% after 24 h. In addition, the retention of cells immediately after delivery is highly dependent on the delivery strategy. For example, if cells are injected intramyocardially, many cells are lost through the vasculature, and only a few cells infused into the coronary arteries engraft [6,26]. Thus, insufficient cell numbers and inadequate cellular interactions are adverse factors with respect to obtaining therapeutic effects [24]. Similarly, many studies have shown low retention and engraftment of NPCs and NSCs transplanted into the brains of model animals [27]. This is likely due to oxidative stress, necrosis, mechanical damage, immunological rejection, the infiltration of inflammatory microglia, or the lack of trophic factors, thus limiting the effectiveness of these stem cell therapies [28]. The number of cells surviving the initial days in the host tissue is limited, which may reduce the therapeutic effects of cell transplantation [27]. In addition, some research reports demonstrate that therapeutic efficacy of transplanted cells may be closely related to the successful engraftment at early stages or *in situ* survival of cells implanted in hostile environment of hypoxia, inflammation, and scarring [29,30]. Therefore, strategies focusing at making cells less vulnerable to these effects and thereby improving cell therapy hold great promise, which can be accomplished by providing cells with a nurturing and protective microenvironment before and after transplantation.

1.2. Enhancing transplantation outcomes by creating a permissive microenvironment

Tissue engineering is one avenue to enhance the impact of transplanted cells [31]. Two approaches are the focus of active