Chapter 14

Extracellular Matrix Enhances Therapeutic Effects of Stem Cells in Regenerative Medicine

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Abstract

Stem cell therapy is a promising option for regenerative of injured or diseased tissues. However, the extremely low survival and engraftment of transplanted cells and the obviously inadequate recruitment and activation of the endogenous resident stem cells are the major challenges for stem cell therapy. Fortunately, recent progresses show that extracellular matrix (ECM) could not only act as a spatial and mechanical scaffold to enhance cell viability but also provide a supportive niche for engraftment or accelerating stem cell differentiation. These findings provide a new approach for increasing the efficiency of stem cell therapy and may lead to substantial changes in cell administration. In order to take a giant stride forward in stem cell therapy, we need to know much more about how the ECM affects cell behaviours. In this chapter, we provide an overview of the influence of ECM on regulating stem cell maintenance and differentiation. Moreover, the enhancement of supportive microenvironment function of natural or synthetic ECMs in stem cell therapy is discussed.

Keywords: extracellular matrix (ECM), stem cell therapy, microenvironment, growth factor, regenerative medicine

1. Introduction

Stem cells reside within a specific extracellular microenvironment, which consists of a complex mixture of soluble and insoluble, short- and long-range signals [1]. Extracellular matrix (ECM) to which stem cells adheres is one of the microenvironmental parameters regulated stem-cell fates [2–4]. Once moving outside of their niche, stem cells will quickly lose their developmental potential, which limits the application of stem cell therapy [5]. Besides, mounting evidence on stem cells and their niche indicates that transplanted stem cells are unable to survive and

adapt at the site of administration where there is lack of functional vascular network to transport blood, supply oxygen and nutrients, and remove metabolites [6].

Through enhancing cell retention and engraftment after transplantation, modulating stem cell fate, and promoting functional vasculature formation, co-transplantation stem cells with natural or synthetic ECM that mimic natural extracellular milieu could be a potentially powerful tool to break the current bottleneck and maximize the effectiveness of stem cell therapy [7–9]. These strategies provide considerable hope for the development of stem cell therapy in degenerative diseases. This chapter will provide the insights into the interaction between stem cells and ECM, as well as current knowledge and involvement of stem cell therapy. Moreover, we will discuss the strategy of co-transplantation stem cells and ECM for tissue regeneration with enhanced therapeutic efficacy.

2. Why extracellular matrix is necessary for stem cell therapy

With the capacity of self-renewal and differentiation, stem cells have shown promising potential in regenerative medicine and tissue engineering. So far, stem cell transplantation have been proposed as future therapies for degenerative diseases or injury, including Alzheimer's disease [10], type 1 diabetes [11], Parkinson's disease [12], cardiac disease [13], muscle damage [14] and many others [15–17]. However, some studies showed that stem cell therapy only had modest improvement, which could be attributed to the fact that transplanted cells were unable to survive and adapt in the diseased area. For instance, low cell retention and engraftment and remarkably cell death after transplantation have been observed by using bioluminescence imaging (BLI) [18].

Though it is not clear what signals and underlying pathways cause the acute donor cell death following transplantation, increasing evidence suggests that that a supportive microenvironment is of crucial importance for stem cell survival, proliferation and differentiation [5,19]. For this reason, the strategy to seed stem cells on biomaterials that mimic the biochemical and biophysical properties of native niche could be a viable solution to the above mentioned problems [20] and optimize functional recovery of injured tissue (**Figure 1**). For instance, Matrigel, a product derived from the Engelbroth-Holm-Swarm (EHS) mouse sarcoma, is one of the most commonly used plate-coating materials for stem cell culture in vitro and effectively applied vehicles for transplanted stem cells [21]. Mounting evidence has demonstrated that Matrigel could affect cell fate in a variety of dimensions [21,22]. However, Matrigel is a complex with unknown variable matrices and numerous mixed growth factors, which makes it impossible for us to get further insights into the interplay of stem cells and ECM. Besides, another reason for safety concern is that Matrigel has been reported contaminated with Lactate Dehydrogenase Elevating Virus [23]. To avoid these problems, artificially synthetic ECM with both high purity and defined components in qualitative and quantitative measures for safe application is strongly demanded [24,25]. Recently, developments in engineered ECM-based microenvironments have gradually exhibited their ability for directing stem cell behaviours, such as adhesion, proliferation, and differentiation [26].

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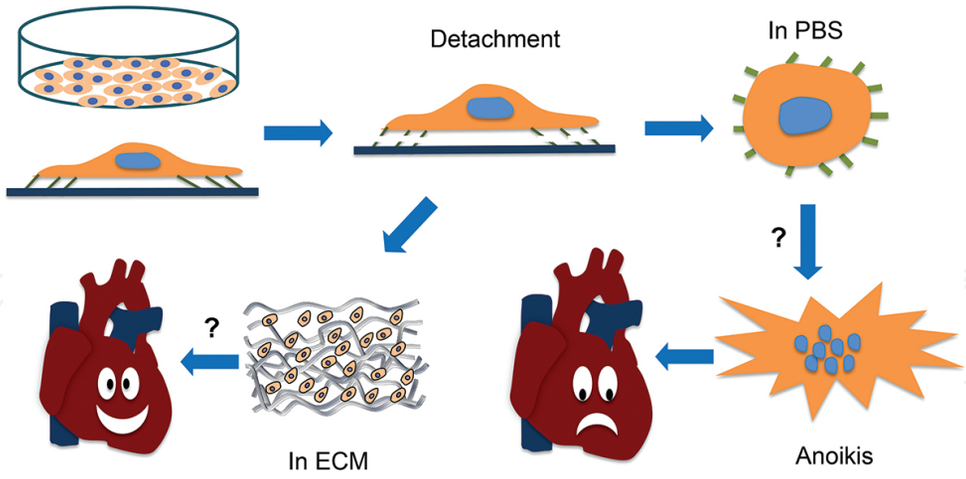

Figure 1. Extracellular matrix(ECM) is necessary for stem cell therapy. A form of apoptosis, called "anoikis", will be initiated once interactions between stem cells and ECM are cut off. Re-establish the connection between ECM and detached cells could increase cell viability and promote function recovery of injured tissue [4]. Reprinted by permission of the publisher.

3. Influence of the extracellular matrix on stem cell behaviour

Extracellular matrix (ECM), acting in conjunction with the biophysical properties and biochemical extracellular stimuli, is critical to regulate stem cell maintenance and differentiation [27,28]. It has been reported that a form of apoptosis, called "anoikis", would be initiated when interactions between stem cells and ECM were cut off [19]. Great effort has been made in an attempt to detail the mechanisms, which provides some key information for cell–ECM interplay. For example, recent study investigated changes of genes' expression after cell detachment by using PCR Array [2]. In that study, researchers found that adhesion molecules expression had no significant difference between cultured human embryonic stem cell-derived

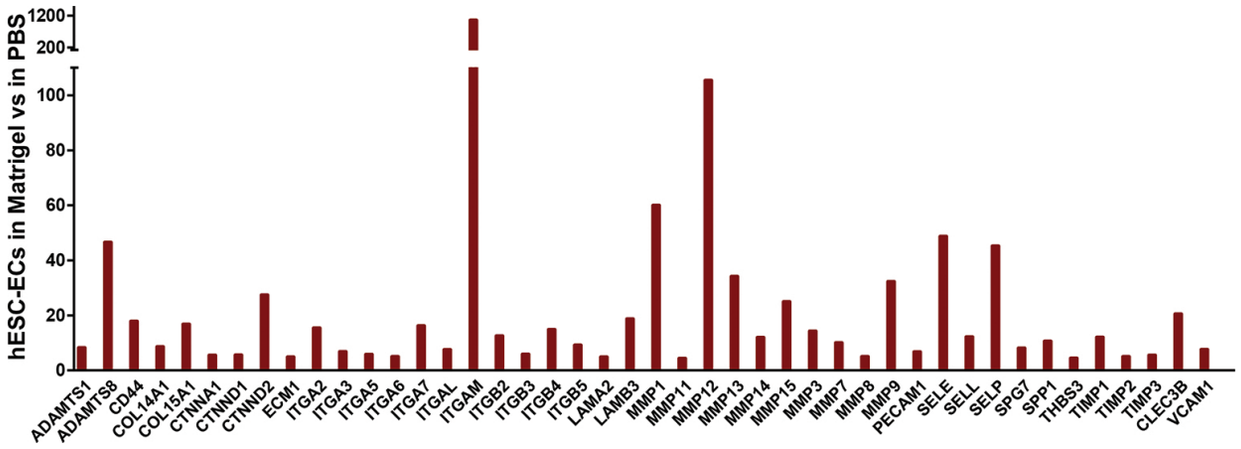

Figure 2. ECM and cell adhesion related gene-expression patterns of hESC-EC at different conditions. Expression of more than 4-fold changes genes of hESC-ECs in Matrigel compared with hESC-ECs in PBS [4]. Reprinted by permission of the publisher.

endothelial cells (hESC-ECs) and enzymatically dispersed hESC-ECs suspended in Matrigel. However, a series of ECM and adhesion molecules-specific genes was considerably down-regulated in hESC-EC suspended in PBS (**Figure 2**). These gene-expression data indicated that adding ECM to detached cells could reverse genes down-regulation of ECM pathway, cell adhesion molecules pathway, ECM and adhesion signalling.

3.1. Biochemical stimulus

The assignment of cell fate results from a response to sophisticated extracellular signals [29,30]. There is mounting evidence suggesting that ECM could deliver numerous soluble and immobilized factors that play vital roles in making the fate choice between self-renewal and lineage commitment [31]. Further insights and exquisite control of signals transported by ECM could provide opportunities for enhancing the regenerative efficacy in both in vitro and in vivo and further accelerating the translation of basic science to the clinical setting.

3.1.1. Release of soluble factors

The propagation of soluble signalling molecules controls a great variety of cellular responses, including proliferation [32], polarity [33], migration [34], and differentiation [35]. It has been

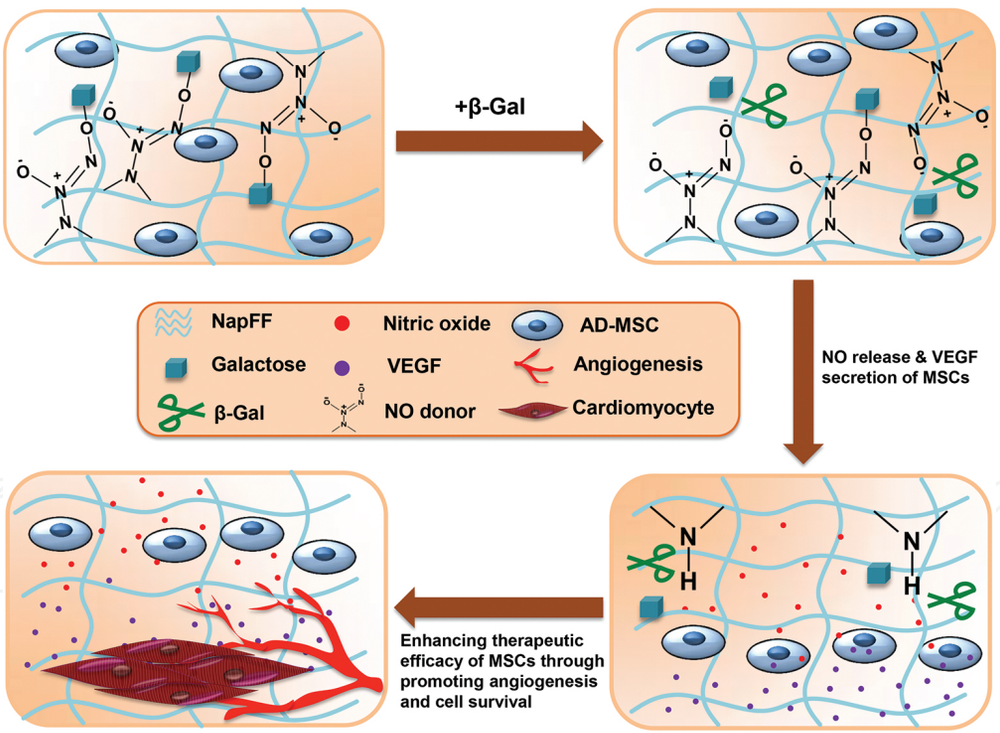

Figure 3. Putative model outlining the controlled nitric oxide (NO)-releasing hydrogel enhances the therapeutic effect of adipose derived-mesenchymal stem cells (ADSCs) for myocardial infarction. Encapsulation of ADSCs by NO-releasing hydrogel prevented transplanted cells effusing from injection positions. NO molecule released from the hydrogel catalyzed by β-galactosidase can facilitate angiogenic cytokines secretion of ADSCs, resulting in promoting angiogenesis, ADSCs survival and cardiac function. β-gal, β-galactosidase [9]. Reprinted by permission of the publisher.

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demonstrated that a specific interaction between cells and ECM is required for the ultimate biological response of soluble molecules [29,36,37]. Interactions with ECM can affect the responses of cell toward signalling messengers. For instance, insulin-triggered activation of insulin receptor substrate (IRS) was intensely enhanced in cells cultured on basement membrane than on collagen I, whereas higher levels of tyrosine phosphorylation of the EGF receptor and Erk was triggered by EGF in cell adhesion to collagen I [36].

In living systems, the coordinated effort among cells, growth factors and ECM is required for the successful tissue regeneration. The ability of manipulating biological signals transduced by ECM in a controlled and spatiotemporal manner that mimic the natural regenerative process could provide specific control over the stem cell-based regenerative therapy [38]. The potential therapeutic effect of a peptide-based ECM with the capacity of controlled release nitric oxide (NO), NapFF-NO, was tested in a mouse model of myocardial infarction [9]. The therapeutic effect of adipose-derived-mesenchymal stem cells (ADSCs) was elevated through co-transplantation with NapFF-NO and on-demand NO release (**Figure 3**). Additionally, the administration of growth factors within the context of the ECM niche could accentuate their therapeutic effects for tissue repair [39]. It was reported that a recombinant fragment of fibronectin (FN) could significantly enhance the regenerative effects of growth factors in models of chronic wounds and bone defects [40].

3.1.2. Immobilized factors

Sustained release and improved local retention of regenerative factors, such as growth factors and extracellular substances, are required during tissue regeneration [41]. However, these molecules are suffering from rapid degradation, and therefore they will quickly lose their functionality and clinical efficacy [42]. Additionally, there is evidence that cellular processes are also affected by the interactions between cells and non-soluble constituents of the ECM [43]. For these reasons, immobilization of signalling molecules or functional components to ECM could be suitable for stabilizing these highly reactive molecules, increasing local concentration of biochemical stimuli, and increasing the bioactivity of engineered ECM.

A growing number of studies have utilized short synthetic peptides to mimic the biological properties of full-length growth factors and to substitute parent proteins [44,45]. For example, insulin-like growth factor 1 (IGF-1) is considered as an essential biochemical stimulus in tissue regeneration. The C domain of IGF-1 (IGF-1C), a 12 amino acids sequence, had already been proved as the active region of IGF-1 [46]. IGF-1C has been used as substitute for IGF-1 and applied into hydrogel biomaterials as biomimetic material for tissue engineering and regenerative medicine. The proliferation, apoptosis resistance, and paracrine effects of ADSCs were significantly enhanced after they were seeded on chitosan (CS) hydrogel with immobilization of IGF-1C [47]. When co-transplanted ADSCs with CS-IGF-1C hydrogel into ischemic organ, this biomimetic matrix could create a favourable microenvironment for the survival and adaptation of transplanted cells and further promote functional and structural recovery of injured organ (**Figure 4**).

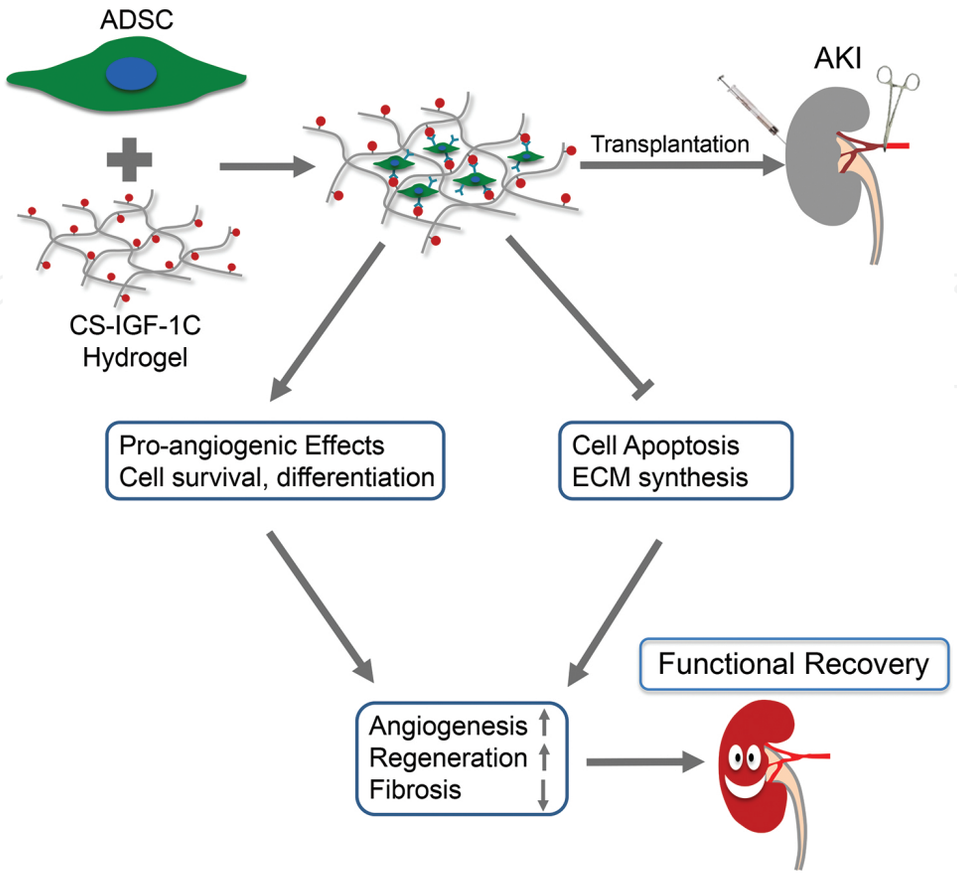

Figure 4. Schema of renoprotective effects of ADSCs and synthetic ECM (CS-IGF-1C hydrogel). When co-transplanted into AKI model, CS-IGF-1C hydrogel could protect delivered ADSCs, facilitated their paracrine and anti-inflammatory effects, and inhibit ECM synthesis in kidney, which result in enhanced angiogenesis, regeneration and alleviated fibrosis after kidney injuries. Consequently, CS-IGF-1C hydrogel therapy leads to improved functional and structural recovery of kidney [47]. Reprinted by permission of the publisher.

3.2. Physical interaction

Although it has commonly acknowledged that signals transduced by ECM could direct stem cell fate, there is increasing evidence that physical properties of ECM could also make a great impact on cell behaviours [48–50]. Some of these factors are proven to be of great influence, but we still have a long way to go and a lot of work to do to establish a complete theory. For example, in response to injury, the accumulation of ECM is excess and abnormal, which would cause significant changes to the stiffness of ECM and ultimately lead to tissue fibrosis [51,52].

3.2.1. Stiffness and elasticity

To test the effect of different stiffness (EY) on cell behaviors, substrates with EY ranging from <1 kPa to 30 kPa were synthesized [53]. The results showed that ECM stiffness has influence on cell proliferation as well as cell differentiation. For instance, neural stem/progenitor cells (NSPCs) could proliferated on substrates with EY <10 kPa. On soft substrates (<1 kPa), neuronal differentiation was promoted; whereas, on relatively stiff substrates (>7 kPa), oligodendrocyte

differentiation was favoured. This consequence indicated that matrix stiffness had effect on lineage choice and differentiation. In light of previous data that stiffness was a regulator of differentiation, Shih et al. further explore how matrix affects the osteogenic phenotype of MSCs [54]. They found that the matrix rigidity promoted osteogenic commitment through a α2-integrin-ROCK-FAK-ERK1/2 axis.

Fascinatingly, it was observed that mesenchymal stem cells all underwent osteogenic differentiation on both stiff and soft polydimethylsiloxane (PDMS) substrates; whereas, the osteoblast differentiation of the same cells was promoted on stiff polyacrylamide (PAAm) hydrogels, and more cell differentiated into adipocytes on soft PAAm hydrogels [55]. The different cellular responses to different substrates indicated that stiffness was not an independent stimulus for differentiation. Further data provided in this study suggested that the differentiation of human mesenchymal stem cells on PAAm was also regulated by the elastic modulus. Consistent with the previous study, Xue et al. reported that matrix elasticity was the main physical parameter directing stem cell differentiation at low cell density; with increased cell density, the cell–cell contact force and interactions took priority over the matrix elasticity [56]. Most notably, although cell differentiation was influenced by elastic modulus, recent discovery found that matrix-promoted adipogenic or osteogenic differentiation could not maintain when the cells were re-seeded into tissue culture plastic (TCP) [57]. Furthermore, global gene expression profiles and DNA-methylation profiles revealed no significant impact caused by matrix with different elasticity. These results indicated that matrix elasticity only exerted a transient influence on stem cell lineage commitment.

3.2.2. Ligands and ligand densities

When cells were seeded on ECM with different ligand densities, changes in stem cell viability, size, and shape provided the direct evidence that ligand immobilized to ECM could not be easily separated from the biophysical effects of matrix [58,59]. The spatial arrangement of ligands had a significant influence on MSC behavior [44]. Through manipulating of the ratio of polystyrene-block-poly (ethylene oxide) copolymers (PS-PEO-Ma) in mixtures of block copolymer and polystyrene homopolymer, the lateral spacing of RGD (arginine-glycine-aspartic acid) peptides was under control. With increased lateral spacing, osteogenesis of MSC was reduced while adipogenic differentiation was increased, which was consistent with the results of gene expression levels and alkaline phosphatase activities.

Moreover, the type of ligands covalently linked to ECM could also influence stem cell fate determination. The differentiation of MSCs on different composition of adhesion ligands with the same concentration was various. MSCs cultured on fibronectin or laminin matrices tended to undergo adipogenic differentiation; whereas, MSCs cultured on ECM containing collagen preferred to adopt a neurogenic outcome [60].

3.2.3. Macro/nano-scale topography

Recent development also demonstrated that macro/nano-scale of ECM was another important physical parameter that could not only change the shape of stem cells but also influence the

behaviour of stem cells. A preliminary study demonstrated Zyxin played an important role in nanotopographical feature-facilitated changes in stem cells [48]. On 350 nm grating, expression of Zyxin was down-regulated, which was associated with the accelerated speed of migration and the decreased intracellular tension. Likewise, McMurray et al. revealed that modification in surface nanotopography of thermoplastic polycaprolactone (PCL) would influence intracellular tension, which could maintain the multipotency of stem cells and diminish spontaneous differentiation of MSC [61]. Moreover, his current study further illustrated that nanoscale spatial organization of cell-adhesive ligands bound to ECM could affect lineage commitment of MSCs [62]. By using nanopatterning techniques, arginine-glycine-aspartate (RGD) was covalently linked to the surface of poly (ethyleneglycol) (PEG) hydrogels with different nanospacing. It was interesting to identify that large RGD nanospacing was beneficial for osteogenesis; small RGD nanospacing was conducive to adipogenesis.

4. ECM augments therapeutic effects of stem cell therapy

Many attempts at cell therapy have employed ECM to improve efficacy for the following reasons. First, the major obstacle to the application of stem cells, which is known as the extremely poor survival and engraftment of transplanted stem cells, could be minimized by co-transplantation stem cells with ECM [51,63,64]. Second, engineered ECM mimicking the natural stem-cell microenvironments could provide plenty of subtle and instructive cues to control the fate of both transplanted and endogenous cells, including stem cell self-renewal, differentiation, and migration [7,65,66]. Taken together, the development of engineered matrices is promising for the application of stem cells in regenerative medicine.

4.1. Enhance efficacy of transplanted cells

For both experimental studies and clinical applications, transplanted stem cells are commonly prepared for transplantation as single cells. During this process, interactions between cells and ECM are lost and adhesion-related survival signals are down-regulated, which could cause a decrease in cell viability and initiated apoptosis [67]. Fortunately, recent research discovered that the down-regulated molecules of detached cells could be regained in the presence of Matrigel [4], which provided a theoretical rationale for using ECM as a protective scaffold to enhance viability and to stimulate self-renewal of the transplanted cells.

In support of this finding, recent study demonstrated that biomimetic scaffold could protect the transplanted stem cells, and further promote functional and structural recovery from acute kidney injury (AKI). Through immobilization of the C-domain of insulin-like growth factor 1 (IGF-1C) to chitosan (CS) hydrogel (CS-IGF-1C), an artificial microenvironment for supporting growth of stem cells was synthesized. The pro-proliferative, anti-apoptotic, and pro-angiogenic effects of CS-IGF-1C were demonstrably beneficial for enhancing survival of transplanted stem cells, which could ameliorate renal function [47] (**Figure 5**).

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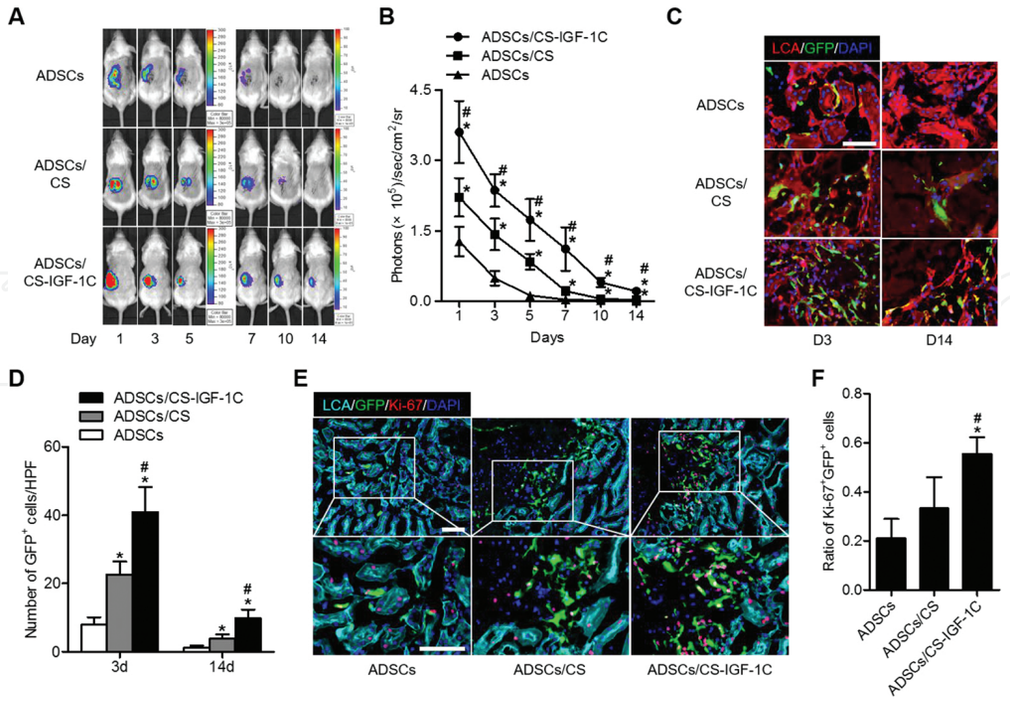

Figure 5. CS-IGF-1C hydrogel increases ADSCs viability in vivo. (A) The fate of ADSCs after transplantation was tracked by molecular imaging. Images are from representative animals receiving 1×10^6 ADSCs alone, with chitosan hydrogel or CS-IGF-1C hydrogel. (B) Quantitative analysis of BLI signals demonstrated that cell survival was improved by chitosan hydrogel and CS-IGF-1C hydrogel application at all time-points. CS-IGF-1C hydrogel group showed significantly better cell survival. Data are expressed as mean ± SEM. (C) Representative photomicrographs displayed the engraftment of ADSCs (GFP, green) within kidneys at day 3 and 14. Proximal tubular epithelial cells were stained by rhodamine-labeled lens culinaris agglutinin (LCA, red). (D) Quantitative analysis data revealed that chitosan hydrogel improved cell engraftment and CS-IGF-1C hydrogel further increased this effect. Data are expressed as mean ± SEM. *P<0.05 vs. ADSCs, #P<0.05 vs. ADSCs/CS. (E) Representative images showing the proliferation (Ki-67, red) of transplanted ADSCs (GFP, green) in the border regions 3 days after AKI. DyLight 649-labeled LCA staining (cyan) was performed to reveal renal structure. (F) Quantification of the proliferation index of ADSCs. Data are expressed as mean ± SEM. *P<0.05 vs. ADSCs, #P<0.05 vs. ADSCs/CS. (47). Reprinted by permission of the publisher.

Besides, we could attribute the efficacy of stem cell therapy partly to the pluripotency of stem cells [68,69]. A morphological study of MSCs in collagen type I (Col I) hydrogel and in interfacial polyelectrolyte complexation (IPC) based hydrogels containing Col I discovered that cells were neatly arranged and closely packed in IPC- Col I hydrogel [70]. This uniform arrangement results in notably enhanced commitment to the chondrogenic lineage of MSC, which could be an attractive source of cartilage equivalents for tissue engineering.

Recently, a variety of studies have demonstrated that decellularized matrix could provide tissue specific cues for cell growth and lineage commitment [71–73]. Decellularized myocardial matrix hydrogel, which keeps the original structure and natural heart ECM, is the most compelling example among these biomaterials. One of the most inspiring finding is that a mouse heart could contract and beat again after removing its own cells and repopulating the decellularized whole-heart ECMs (DC-ECMs) with multipotential cells that could differentiate in response to the signals from the DC-ECMs. Through repopulating decellularized mouse hearts with induced pluripotent stem cell (iPSC)-derived earliest heart progenitors, the

recellularized DC-ECMs exhibited myocardium, vessel-like structures, intracellular Ca^{2+} transients (CaiT), spontaneous heart contractions and significant response to numerous drug interventions [74].

4.2. Support the function of endogenous cells

MSCs could mobilize into circulating blood and be recruited to the injury site, which was consistent with the evidence that numbers of MSCs were increased considerably in peripheral blood [75]. Several approaches were used in an attempt to investigate this cell recruitment event. It was unexpected to find that ECM was indispensable for the homing of MSCs toward sites of injury [76]. The homing effect could be inhibited through adding inhibitor of serine proteases and leupeptin to ECM, which illustrated the key role of matrix remodelling in MSC migration. In addition, evidence also indicated that exposing MSCs to injury-associated ECM prior to transplantation could augment the efficiency of MSCs' intrinsic tropism for injury [77].

As resident stem cells and progenitor cells could be activated to participate tissue regeneration after injury [78,79], ECM designed for cell seeding should also benefit the growth of host cells and support the function of endogenous cells. Encouragingly, evidence suggested that host cells could respond to ECM in the site of injury in vivo. Firstly, immune responses were elicited in hosts, which was identified by the quickly infiltrated $CD68^+$ cells throughout the entire ECM within 3 days after implantation. Then there were indications of myogenesis in the muscle injury area, which was confirmed by morphology and myosin heavy chain positive staining [80].

Furthermore, accumulating data suggested that human mesenchymal stem cells (hMSCs) could modulate immune system response through their paracrine effect and then create a pro-regenerative environment in situ. Their paracrine effects could be optimized through encapsulating hMSCs into protective ECM [81]. The recruitment of endogenous macrophages and the M1/M2 polarization were modulated by the trophic factors secreted by hMSCs, which was possibly capable of counteracting the hostile environment and sustaining tissue regeneration. This cell-friendly microenvironment could also be established by administration of ECM alone. Increased stem cell tropism, revascularization, and improved cardiac function induced by chitosan-based ECM were observed in ischemic myocardium [82], which may be attributing to the mechanical support provided by ECM and the therapeutic biomolecules enriched by ECM [83–85].

5. Future perspectives

The ECM is not only a simple scaffold that provides physical supports for stem cells but also a dynamic and complex environment that is capable of regulating cell behaviours. Although the application of natural or synthetic ECM with the aim to enhance therapeutic effects of stem cells is highly appealing for promoting regenerative processes, issues related to efficiency and safety limit their translational use as regenerative medicine. Further identifying specific

biochemical and biophysical properties of ECM and understanding the interplay between stem cells and ECM will provide knowledge of stem cell biology and fuel the development of regenerative therapies based on stem cells.

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