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Review Article

Novel therapies using cell sheets engineered from allogeneic mesenchymal stem/stromal cells

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Mesenchymal stem/stromal cells (MSCs) have long been recognized to help regenerate tissues, by exploiting their intrinsic potentials for differentiation and secretion of therapeutic paracrine factors together with feasibility for cell banking. These unique MSC properties are attractive to provide effective new cell-based therapies for unmet medical needs. Currently, the infusion of suspended MSCs is accepted as a promising therapy to treat systemic inflammatory diseases. However, low cell engraftment/retention in target organs and off-target entrapment using conventional cell infusion must be improved to provide reliable localized disease treatments. Cell sheet technology offers an alternative: three-dimensional (3D) tissue-like structures can be harvested from culture using mild temperature reduction, and transplanted directly onto target tissue sites without suturing, yielding stable cell engraftment and prolonged cell retention *in situ* without off-target losses. Engineered MSC sheets directly address two major cell therapy strategies based on their *therapeutic benefits*: (1) tissue replacements based on mult-ilineage differentiation capacities, focusing on cartilage regeneration in this review, and (2) enhancement of tissue recovery via paracrine signaling, employing their various secreted cytokines to promote neovascularization. MSCs also have *production benefits* as a promising allogeneic cell source by exploiting their reliable proliferative capacity to facilitate expansion and sustainable cell banking for off-the-shelf therapies. This article reviews the advantages of both MSCs as allogeneic cell sources in contrast with autologous cell sources, and allogeneic MSC sheets engineered on thermo-responsive cell dishes as determined in basic studies and clinical achievements, indicating promise to provide robust new cell therapies to future patients.

Introduction

Medical treatments continuously advance with new technology developments. Newly introduced and approved biologic drugs provide increasing examples of improved efficacies and fewer side effects for biological molecules compared with conventional small-molecule synthetic drugs. Nevertheless, many clinical pathologies remain unaddressed, including diverse inextirpable degenerative diseases, such as neurodegenerative disorders, some cardiovascular diseases, osteoarthritis, and acute/chronic fibrosis, due to lack of effective therapeutic approaches.

In a healthy body, various cell types, including somatic stem cells, continuously orchestrate normal tissue and organ functions through appropriate proliferation–differentiation-aging processes to maintain homeostasis. Deteriorations of cellular function, especially stem/progenitor cell deficiencies, promote degenerative diseases since these normal maintenance pathways are disrupted or abnormal. Cell therapies have been arduously attempted to treat some of these intractable diseases. Currently, embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and somatic stem/progenitor cells, such as mesenchymal stem/stromal cells (MSCs) and hematopoietic stem cells, have been investigated

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as promising cell sources for new therapies. MSCs in particular have been widely applied to address various diseases by exploiting their known multipotency (e.g. bone, cartilage, and adipose) and abundant paracrine secretome [1]. MSCs also exhibit high growth potential that facilitates a reliable, sustainable cell banking and supply system amenable to produce substantial numbers of human doses [2,3]. However, few MSC-based therapies have progressed beyond preclinical studies to clinical trials evaluating their safety and therapeutic efficacy. One reason for this struggle is thought to derive from their intrinsically poor cell engraftment/retention in target tissues and substantial off-target loss of infused cells, which remains a daunting, significant hurdle for many MSC therapies [4–10]. To overcome this challenge, cell delivery systems with or without biomaterials are designed to produce tissue-like structures that are implanted or adherent locally at host target tissue sites, and improve cell retention without off-target problems, and therefore considered more efficient as culture-expanded cell-based therapies.

In this review, we discuss cell delivery to tissue sites exploiting 'cell sheet technology' allowing local cell transplantation with thermo-responsive cell cultureware that allows confluently cultured cells to yield readily harvested three-dimensional (3D) tissue-like sheet structures. Sheets are harvested with minor temperature reduction to below 32°C from 37°C (i.e. that of a normal cell culture incubator) (Figure 1). Cell sheets retain endogenous cell matrix and membrane proteins and enhance healthy cultured cell yields for immediate use as engineered patches [11,12]. This enables direct cell sheet transplantation onto target tissues without supporting scaffolds or suturing [13,14]. Decades of investigations developing cell sheet technology reveal that scaffold-free cell sheets exhibit both safety and efficacy in treating seven different organs using autologous cell sources in clinical settings: cornea, esophagus, heart, lung, middle ear, periodontal membrane, and cartilage regeneration [15–21]. Moreover, recent reports describing use of allogeneic cell sheet technology suggest a high potential for clinical utility, especially with MSCs isolated from adipose tissue, bone marrow, periodontal membrane, and cartilage [22–25]. This review discusses the advantages of combining cell sheet technology and MSCs; effective cell sourcing, expansion and cell delivery using cell sheets engineered from MCSs, and resulting numerous

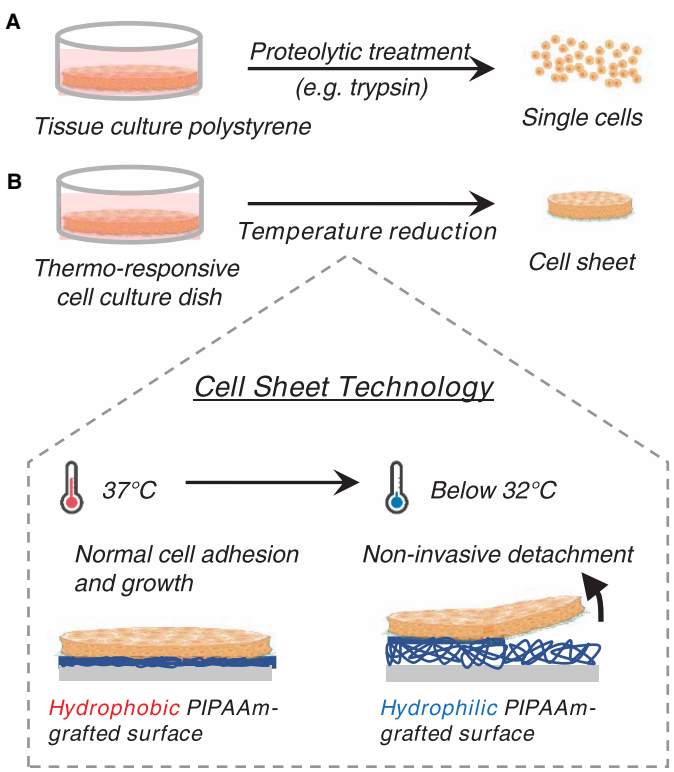

Figure 1. Cell sheet technology using TRCDs.

(**A**) Conventional single-cell recovery method by proteolytic treatment. (**B**) Non-proteolytic cell sheet recovery using thermo-responsive cell culture dish (TRCD). Mild temperature reduction facilitates spontaneous cell sheet detachment.

attractive features and reliable outcomes in preclinical and clinical studies. MSC sheet technology will offer new opportunities for diverse disease treatments in the near future. Furthermore, we discuss the scaling and economic benefits of forthcoming allogeneic MSC sheet-based regenerative therapies.

Allogeneic cell sourcing: MSCs with high proliferative capacity enabling sustainable cell banking for off-the-shelf sourcing as therapy

Clinically feasible cell-based regenerative therapies rely on consistent, available and potent cell sources. Several clinical trials of ES cells have been conducted, followed by recent iPS cell-based early-stage clinical trials [26–29]. Nonetheless, the crucial hurdles for commercialization of pluripotent stem cells, including costs of long-term cell culture, must be cleared [30] and reliable methods for tumorigenic cell elimination must be established and validated [31]. On the other hand, somatic stem/progenitor cells isolated from various tissues are valuable to replenish deficient host cells to restore homeostasis [32] by exploiting their lower possibilities of tumorigenicity compared with pluripotent stem cells [33,34]. Autologous somatic cell sourcing is an advantage when cells must avoid host immune rejection for stable cell engraftment and retention [35]. However, autologous cell therapy usually requires multi-stage surgeries for obtaining patients' own cells, expanding them *ex situ*, and transplanting the harvested cells back to targeted tissue sites. Additionally, variability due to patient individual difference is a major obstacle for product quality control and reliability [36–39].

To overcome these obstacles, allogeneic cell transplantation has frequently been attempted as a promising next-generation cell therapy. In this regard, MSCs are a primary allogeneic cell source amenable to cell banking due to their high proliferative potential [2,3]. This robust proliferative capacity allows use of a single qualified cell line showing high efficacy for expansion to accommodate substantial numbers of patient treatments. Moreover, MSCs show no/low-MHC class II expression, suggesting tolerance to host immune rejection [40,41]. Therefore, MSCs are frequently proposed as a strategy to overcome current major problematic issues surrounding allogeneic cell immune rejection while yielding large cell banks for therapeutic applications. Beyond cell banking advantages, MSCs exhibit unique multi-lineage differentiation both *in vitro* and *in vivo*, and therapeutic cytokine secretions to heal damaged tissues [1]. Based on these remarkable properties, MSC-based therapy can be a game-changing strategy to provide new treatments to address unmet medical needs.

Conventional cell delivery systems —advantages and disadvantages

Cultured MSCs are a major allogeneic cell source of current interest due to their immunomodulatory effects against excessive immunoreactions in systemic diseases such as graft-versus-host disease (GVHD), Crohn's disease, and severe acute pancreatitis [42–46]. While single-cell administration by intravenous infusion is advantageous to deliver cells and their paracrine factors to the entire body via the blood stream, this strategy is unsuitable to treat non-systemic, localized diseases due to low cell engraftment and survival rates at target sites and high off-target localization [4–9]. Intra-arterial and local injections show slightly better homing to target sites, though injected MSC accumulation is still high in lung early post-transplantation [10,47,48]. To date, both systemic and local injection of cell suspensions have been used to position MSCs in target organs for localized disease treatments, but insufficient MSC retention can limit local exposures to therapeutically beneficial paracrine factors and compromise the potency and duration of the desired therapeutic effect [5]. Increasing MSC dose to enhance therapeutic effects concomitantly expands the risks of embolism in lung and liver by off-target MSCs, exposing the difficulties of dose control [7,10]. This is one reason currently limiting clinical trials to small-cohort safety and efficacy studies [5]. Therefore, alternative cell delivery methods are needed to establish localized disease treatments.

To facilitate and improve cell engraftment in target host tissues, numerous biomaterial-based scaffold approaches using seeded MSCs are continuously reported. However, biomaterial designs often focus on manipulating a single parameter of the cell transplantation (e.g. biocompatibility, biodegradability, and donor cell viability or differentiation), and among myriad types of materials applied, few examples of knowledge transfer from *in vitro* experiments are translated successfully to *in vivo* cell transplantation experiments [49]. An alternative approach is scaffold-free tissue engineering utilizing biological cell–cell binding architectures as typified by spheroid/aggregate culture or cell-dense culture in non-adherent wells to facilitate 'self-assembly'.

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This approach can deliver cells at high density to target sites while avoiding interference by and rejection of scaffold biomaterials and their degradation products [50,51]. This strategy removes certain confounding cell-implant variables to enhance tissue regeneration and integration in host disease sites.

Cell sheet engineering —key enabling features of thermo-responsive cell cultureware

Among diverse cell sheet engineering methods more recently reported, Okano and co-workers originally invented the thermo-responsive culture dish, thermo-responsive cell dishes (TRCD), to produce the first scaffold-free cell sheets, enabling cultured cells to form native tissue-like structure compared with other scaffold-free methods (*cell sheet technology*, Figure 1) [52,53]. Culture plastic grafted with ultrathin layers of the temperature-responsive polymer, poly(*N*-isopropylacrylamide), transitions from hydrophobic in culture conditions, 37°C, to hydrophilic below its lower critical solution temperature of 32°C. Below 32°C, grafted poly (*N*-isopropylacrylamide) swells and hydrates, forming a swollen polymer layer between the culture surface and adherent cells, enabling harvest of cultured cells as a confluent sheet retaining instructive extracellular matrix (ECM) and cell-cell interactions (Figure 1) [12,52–55]. The approach yields transplantable tissue-like constructs that spontaneously adhere to target organs without sutures or complex procedures, safety concerns in transplanted cells, and show therapeutic effects [56,57]. In addition to TRCD-based methods, electro-responsive [58,59], pH-responsive [60], and magnetic-responsive systems [61–63] are employed to harvest cells from culture surfaces as cell sheets. However, the pioneering TRCD-based cell sheet technology has now been widely applied to treat diverse diseases with various cell types, requiring no additional devices in laboratory and clinical situations, limiting safety concerns caused by cell labeling systems or pH reduction methods that affect cell property changes when harvested as cell sheets. Significantly, several types of autologous human cell sheets have been successfully delivered to small numbers of patients using this TRCD approach to date, demonstrating clinical safety and efficacy in cell sheet therapies in seven tissue sites [15–21].

Extending this strategy to MSCs as a prospective allogeneic cell source, two major cell sheet-based strategies have been proposed in clinical and preclinical studies: (1) tissue replacement, especially focusing on cartilage regeneration in this review to introduce a unique allogeneic cell source, based on cell sheet differentiation into chondrocytes, and (2) tissue recovery employing cell sheet therapeutic cytokine production and paracrine signaling.

Tissue replacement: cartilage-derived MSC/chondrocyte sheet treatment

Cartilage regeneration has been an active target of diverse tissue engineering efforts due to the increasing incidence of cartilage injury, its lack of innate regenerative capacity [64], and outcomes from surgical bone marrow induction approaches (e.g. microfracture) that commonly result in fibrocartilage in contrast with hyaline cartilage found in native healthy articular surfaces [65–67]. Native cartilage contains self-renewing chondrocyte progenitors expressing MSC-related markers [68]. Isolated cells from cartilage de-differentiate into a fibroblastic morphology *in vitro*, and exhibit the capacities of colony-forming unit fibroblast (CFU-F) formation and multilineage differentiation [69,70]. Culture-expanded chondrocytes can re-differentiate into mature cartilaginous cells expressing cartilage-specific molecules under chondrogenic differentiation conditions [71,72]. These reports strongly suggest that cultured chondrocytes are one MSC type possessing strong chondrogenic capacity. Although nomenclature of cultured-expanded chondrocytes varies by schools (e.g. cartilage-derived stem/progenitor cells, chondroprogenitor cells, etc.), 'chondrocytes' in this review particularly denote *in vitro*-expanded cells derived from cartilage as one MSCs type, distinct from mature/differentiated chondrocytes existing *in vivo*.

Various tissue engineering methods, either combined with or without biomaterials, have been developed with myriad clinical studies ongoing seeking to reliably regenerate cartilage [73–75]. However, to date, most human studies utilize autologous cells, such as matrix-associated autologous chondrocyte implantation (MACI). No gold standard clinical practice for cell-based chondral defect treatment exists due to the various limitations of current approaches, including the necessity for and expense of multiple surgeries, inconsistent donor tissue availability, donor cell quality, and potency [76,77].

Cell sheet engineering for cartilage repair

MACI has demonstrated better outcomes compared with bone marrow induction approaches [78]. However, its cost compared with microfracture is high, and reported graft delamination and fibrocartilage formation [79,80]

may be attributed to low density, and dissociated chondrocyte colonization in the porcine collagen grafting matrix. Cell sheet technology can better address these clinical issues, grafting cell-dense scaffold-free patches directly to target tissue surfaces, preserving cell-cell communication and endogenous ECM presumed to promote chondrogenic re-differentiation based on previous basic studies [81,82]. Additionally, cell sheets conform to the shapes of various defects to better facilitate graft integration with host tissue, avoiding alignment and defect space-filling issues seen in grafts such as osteochondral allograft [83].

As one MSC type, cultured chondrocytes isolated from articular cartilage have been investigated as a primary cell source in autologous cell sheet therapy for cartilage regeneration. Articular cartilage-derived chondrocyte sheets secrete cartilage protective humoral factors [84]. The formation of human articular chondrocyte sheets from these cells enhances gene expression of aggrecan and type 2 collagen *in vitro* compared with conventionally cultured cells [85,86]. Interestingly, extended culture after layering of three human articular chondrocyte sheets enhances gene expression levels of type 2 collagen while suppressing type 1 collagen expression compared with single-cell sheets [85,86]. Human articular cartilage-derived cell sheets spontaneously engraft at transplanted tissue sites and regenerate cartilage tissue, expressing type 2 collagen in rat and rabbit xenogeneic transplantation models [87,88]. Moreover, the safety and efficacy of chondrocyte sheets is shown in large animal model studies using mini pigs [89] and rabbits [90]. Targeted biodistribution to the knee [91] and genomic stability of human-derived chondrocytes after *in vitro* cultivation were certified by G-band staining and array CGH [92]. Significantly, both safety and prominent clinical improvements were demonstrated in human patients transplanted to unloaded cartilage areas in eight autologous cases using patient-derived chondrocyte sheets combined with alignment surgery [21]. However, the two-stage surgical procedure and patient-specific cell quality variations still remain challenging issues in autologous cell-based approaches.

Juvenile cartilage-derived chondrocytes

Cultured chondrocytes as an MSC sourced from young donor cartilage show a higher proliferative ability and chondrogenic potential compared with adult donor cells [93,94]. One prominent juvenile cartilage cell source is from polydactyly surgical discards (incidence: approximately 1 per 1,000–2,000 live births [95,96], which can be easily isolated, expanded to a thousand times in a few weeks. Cryopreserved human allogeneic cell bank from one donor from both Japanese origin [25] and US origin (Kondo et al., submitted) can cover large populations of the applicable patients. Polydactyly-derived cell characteristics, including high transforming growth factor (TGF) beta secretion, in juvenile chondrocyte sheets, has been reported [25]. Hyaline cartilage regenerative capacity using human juvenile polydactyly cartilage-derived chondrocyte sheets was confirmed in an immunosuppressed rabbit osteochondral defect model [97] and nude rat focal chondral defect model (Kondo et al., submitted). The rat chondral defect model without intentional bone marrow induction particularly demonstrated that regenerated hyaline cartilage forms from human origins with no gaps found at host–donor tissue interfaces (Figure 2). This suggests the advantage of juvenile chondrocyte sheets for native tissue-like repair by exploiting highly proliferative and chondrogenic sheet characteristics. These reports support application of potent juvenile chondrocyte sheets to serve as next-generation single-stage cartilage regenerative therapy. Strategies that can reliably validate regenerative capacity and minimize variability of human juvenile chondrocyte sheets with proper cell/donor selection criteria will be critical for designing and conducting successful large-scale studies.

Tissue recovery: MSC sheets promote tissue regeneration in seven different organs via secreted paracrine factors

In addition to recognized intrinsic multi-lineage differentiation potential (e.g. osteogenic, adipogenic, and chondrogenic), MSCs also produce a remarkable array of paracrine factors that elicit both immunomodulatory effects and enhance tissue regeneration [98–100]. Cell sheets engineered from human MSCs (i.e. MSC sheets) have been studied *in vitro* [101,102] and *in vivo* to employ those functional properties. Significantly, cell sheet technology enables transplantation of tissue-like structures containing contiguous MSCs grown in their endogenous matrix onto target sites without off-target distribution. This results in stable cell engraftment and target site retention over time compared with single-cell administration [7,23,103,104]. Local MSC sheet application better facilitates direct, local therapeutic factor delivery to damaged tissue sites, resulting in continuous support for tissue recovery from various diseases.

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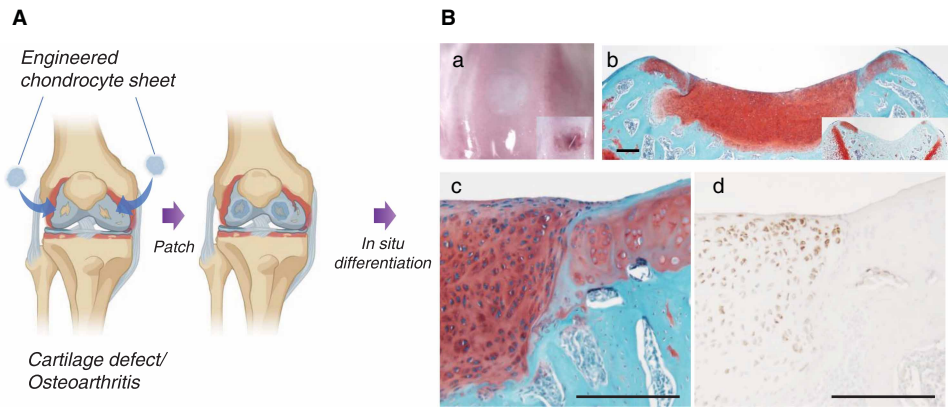

Figure 2. Cartilage regeneration with engineered human juvenile polydactyly cartilage-derived chondrocyte sheets.
(**A**) Schematic images of chondrocyte sheet transplantation to knee defects. (**B**) Repaired trochlear groove cartilage with human juvenile polydactyly cartilage-derived chondrocyte sheet. *In situ* hyaline cartilage maturation occurs within 4 weeks in rodent focal cartilage defect models. (a) Macroscopic image (right corner box shows defect only control). (b) Safranin O staining of nude rat trochlear groove (right corner box shows defect only control with fibrotic tissue formation). (c) Magnified image of Safranin O staining at regenerated cartilage and host tissue interface. Note no gap is observable at the interface. (d) Human vimentin antigen-specific immunostaining, suggesting that regenerated cartilage originates from transplanted human cells. Bars: 200 μm.

MSC sheet treatments in multiple organ disease models, such as heart [22], periodontal membrane [20,24,105–107], skin [108], bone [23], esophagus [109], intestine [110], kidney [7], artery [104], and brain [111], have been studied using the TRCD approach, and their details are summarized in Table 1. These studies reveal that MSCs isolated from bone marrow, adipose tissue, and periodontal ligament consistently exhibit common characteristics such as multi-lineage differentiation potential, adipogenesis and osteogenesis, and colony-forming ability *in vitro*. These isolated MSCs can be employed to prepare transplantable MSC sheets to address various cell culture conditions (Table 1). In addition, *in vivo* analyses show that MSC sheets remain localized on target tissue surfaces up to 2 months, depending on the target sites and/or their assays [7,20,22–24,104–111] (Table 1), although long-term studies are required to verify sheet safety and efficacy. Furthermore, prolonged MSC local engraftment in cell sheets distinguishes their capabilities to enhance therapeutic benefits compared with single-cell administration [7,23,104]; local MSC sheet transplantation is reported to improve organ functionality on seven different tissue/organ disease models. Notably, transplanted MSC sheets adhere spontaneously and directly to host tissue surfaces, influencing the cells in damaged and surrounding area through local autocrine and paracrine effects that promote both neovascularization and tissue regeneration with host cell recruitment (Figure 3) [7,22,23,104,108,109,111–113]. Interestingly, some reports using GFP-labeled cell tracking systems also indicate that transplanted MSCs from TRCD-prepared cell sheets migrate into the host tissue and express markers of endothelial cells, pericytes, and/or other cell types after transplantation, suggesting direct support for neovascularization in addition to indirect contributions from MSC-secreted paracrine factors in local tissue regeneration [22,108,111].

Based on distinct, promising therapeutic effects reported for TRCD-based MSC sheet transplantation (Table 1), a first-in-human clinical study to verify their safety and efficacy has demonstrated periodontal regeneration using autologous human MSC sheets [20]. To permit expanded patient treatments for diverse diseases, allogeneic MSC sources exhibiting broad histocompatibility and consistent paracrine factor secretion profiles are required to produce cell banks that yield validated, efficient MSC therapeutic properties. As a next-step to scaling more effective, local MSC therapy for economic off-the-shelf use, allogeneic human MSC sheet transplantation exploiting their innate high proliferative capacity and therapeutic efficacy is timely; yet, challenges defining MSC sheet critical quality and functional attributes still remain. Nonetheless, human allogeneic MSC sheet fabrication and transplantation is rapidly developing as a rational and economically attractive process, capable of possibly addressing both past issues with inconsistent cell therapy outcomes, and also diverse unmet

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Table 1. Preclinical models reporting TRCD-based allogeneic MSC cell sheet therapy in various tissue sites

		MSC sheet preparation and properties *in vitro*						*In vivo* observation	
Target site	**Disease model**	**Origin**	**Size**	**Seeding density in 35 mm dish**	**Culture duration**	**Total grafted cell number**	***In vitro* properties**	**Grafted MSC sheets**	**Therapeutic effects in host tissue**
Heart [22]	Rat myocardial infraction model	Adipose tissue	24 × 24 mm^2	7.8 × 10^5 cells/dish	3 days	1 × 10^6 cells/rat	VEGF and HGF secretion	High cell viability at 2-day post-transplantation Cell sheet retention at least for 4 weeks	Enhanced angiogenesis Suppression of fibrosis Improvement of cardiac function
Periodontal membrane [24,105,106]	Dog three-wall infrabony defect model	Periodontal ligament tissue	8.8 cm^2 (35 mm dish)	3–4 × 10^4 cells/dish	5 days	N.A. (trimmed triple-layered sheets)	Alkaline phosphatase activity Osteoblast/cementoblast markers Periodontal markers	Cell sheet retention at least for 2 months	Periodontal regeneration including alveolar bone, cementum, well-oriented fibers, and nerve filament
Skin [108]	Rat wound-healing model of type 2 diabetes and obesity	Adipose tissue	8.8 cm^2 (35 mm dish)	1.5 × 10^5 cells/dish	7–8 days	1.5 × 10^5 cells/rat	VEGF, HGF, TGF-ß1, IGF-1, EGF, and KGF secretion	Cell sheet retention at least for 2 months	Improved skin wound healing Enhanced angiogenesis
Bone [23]	Rat bisphosphonate-related osteonecrosis of the jaw model	Bone marrow	8.8 cm^2 (35 mm dish)	2.5 × 10^5 cells/dish	7 days	1.5 × 10^6 cells/rat	VEGF and HGF secretion Bone regeneration marker (RANKL and OPG) gene expression	Cell sheet retention at least for 2 months	Improved skin wound healing Bone regeneration Enhanced angiogenesis
Esophagus [109]	Porcine esophageal endoscopic submucosal dissection model	Adipose tissue	3.5 cm^2 (12 well plate)	3.8 × 10^6 cells/dish	12 h	1.2 × 10^7 cells/rat (double-layered sheets x4)	N.A.	Cell sheet retention at least for 3 days	Less alimentary trouble and higher weight gain Reduced stricture and fibrosis formation
Intestine [110]	Porcine intestial anastomosis delayed wound-healing model	Adipose tissue	8.8 cm^2 (35 mm dish)	2.4 × 10^6 cells/dish	4 days	2.4 × 10^6 cells/rat	Gene expression levels of FGF2 and TGF-ß1	Cell sheet retention at least for 1 week	Enhanced collagen synthesis Increased the stiffness of intestinal anatomosis
Artery [104]	Rat femoral artery injury model	Adipose tissue	8.8 cm^2 (35 mm dish)	1 × 10^6 cells/dish	1–2 days	6 × 10^6 cells/rat (triple-layered sheets x2)	N.A.	Cell sheet retention at least for 2 weeks	Artery reendothelialization Suppression of myofibroblast proliferation
Kidney [7]	Rat ischemia — reperfusion — injury model	Bone marrow	8.8 cm^2 (35 mm dish)	1.2 × 10^6 cells/dish	2 days	7.2 × 10^6 cells/rat	VEGF and HGF secretion	Cell sheet retention at least for 2 weeks	Enhanced angiogenesis Suppression of microvascular injury Suppression of fibrosis Improvement of renal function
Neuron [111]	Rat stroke model	Adipose tissue	8.8 cm^2 (35 mm dish)	1 × 10^6 cells/dish	2 days	3 × 10^6 cells/rat (triple-layered sheets)	IGF-1, HGF, VEGF, and TGF-ß1 secretion single-cell condition)	Cell sheet retention at least for 2 weeks	Enhanced angiogenesis Enhanced neurogenesis Behavior improvement

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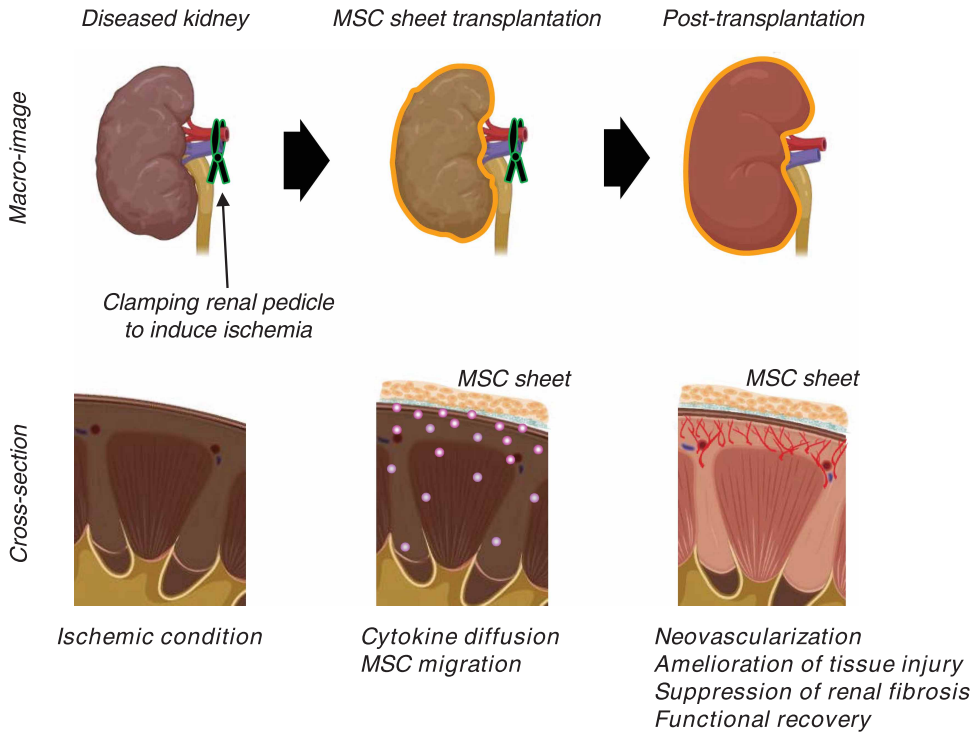

Figure 3. Enhanced tissue recovery after MSC sheet transplantation onto diseased kidney.

Schematic depiction of mesenchymal stem/stromal cell (MSC) sheet strategy. MSC sheets are transplanted over diseased kidney sites in an ischemia-reperfusion injury (IRI) model. Two-weeks post-transplantation, MSC sheets remain on kidney cortex surface and improve kidney functions as evaluated by levels of serum creatinine and blood urea nitrogen. In addition, renal tubule and epithelial cell injury in this IRI kidney model are ameliorated, and renal fibrosis, the final common product of chronic kidney disease, is significantly suppressed with enhanced neovascularization in MSC sheet transplantation group compared with non-treatment and single-cell administration groups. These findings suggest that MSC sheets might provide new therapy options for treating kidney disease.

medical needs in multiple organ diseases. Additionally, establishing human MSC sheet treatments and therapeutic mechanisms in one initial target disease could support expansion of related new MSC-based therapeutic strategies for other diseases in the future.

Conclusions

Cell sheet therapy strategies are well-established in numerous preclinical and clinical applications using diverse cell types. TRCD-based MSC sheet technology in particular is now described in diverse preclinical models and some early pilot human clinical reports. While autologous human MSC sources are first-in-human for cell sheet therapeutic use, allogeneic human MSC sources are currently more attractive to produce new scalable, affordable, histocompatible and widely distributable cell sheet-based therapies for tissue regeneration, exploiting their recognized differentiation potential, immunomodulatory capacity, and diverse paracrine secretome at transplanted specific organs. Moreover, allogeneic human MSC therapies are shown scalable due to readily accessible cell sourcing and cell banking systems, enabling phenotypic and genomic stability for safety and efficacy control and off-the-shelf cell sheet availability for broad clinical use. Thus, allogeneic MSC sheets represent an attractive cell therapy strategy to provide more reliable, novel therapies to address diverse unmet medical needs.

Summary

- Cell therapies in conventional injectable cell suspension forms currently lack sufficient homing, disease site retention, reliable potency, and durable therapeutic responses for local diseases.
- Human MSCs offer substantial therapeutic benefits if quality control features for allogeneic sourcing and disease use are known.
- Juvenile chondrocytes harvested from routine polydactyly surgical discards are an attractive source of MSCs amenable to scaling, banking and allogeneic cell sheet use to regenerate/replace the damaged human cartilage.
- MSC sheet transplantation rapidly engrafts and elicits therapeutic signaling *in situ* via secreted paracrine factors to provide a new strategy for tissue regeneration in various diseases.
- TRCD-based MSC sheet technology is a feasible approach as demonstrated by its safety and efficacy in multiple preclinical and clinical studies.

Competing Interests

Teruo Okano holds equity in CellSeed, Inc. (Japan) and is an inventor/developer designated on the patent for CellSeed's commercialized temperature-responsive cultureware. No other competing financial interests exist and all authors declare that they have no other competing interests.

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Author Contributions

S.K. and M.K. wrote the manuscript and equally contributed to this work. D.W.G. and T.O. reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Schematic representations were created with Biorender.com.

Abbreviations

ECM, xtracellular matrix; ES cell, embryonic stem cell; GFP, green fluorescent protein; iPS cell, induced pluripotent stem cell; MACI, matrix-associated autologous chondrocyte implantation; MSC, mesenchymal stem/stromal cell; TGF, transforming growth factor; TRCD, thermo-responsive culture dish.

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