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REVIEW

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Scaffold-free cell sheet therapies: clinical advances, global approval landscapes, and strategic directions to address regenerative medicine barriers

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Abstract

Cell sheet therapy has emerged as a transformative technology in regenerative medicine, providing scaffold-free constructs that preserve cell–cell junctions and extracellular matrix components. Compared with traditional cell delivery methods, cell sheets enable improved engraftment, survival, and integration after transplantation. Recent years have witnessed remarkable progress in clinical translation, with several products approved in Japan, the United States, and South Korea. This review summarizes the current landscape of cell sheet therapies approved worldwide, focusing on their fabrication technologies, cell sources, and clinical indications. We highlight representative products such as JACE®, Nepic®, Ocural®, JACEMIN®, HeartSheet®, Epicel®, Holoderm®, Kaloderm®, and ZEVASKYN™, emphasizing their technological foundations and regulatory trajectories. Advances in temperature-responsive culture surfaces, closed culture devices, and automated sheet manipulation have facilitated large-scale and standardized manufacturing. Furthermore, the establishment of cell banks, donor eligibility screening, and Good Manufacturing Practice (GMP)-compliant processes ensure product consistency and safety. In parallel, regulatory frameworks in Japan, the United States, and South Korea have shaped the development paths of autologous and allogeneic products, with different strategies for approval, reimbursement, and long-term monitoring. Cell sheet-based regenerative therapies have already demonstrated clinical and commercial viability, offering novel treatment options for burns, ocular diseases, vitiligo, and cardiac conditions. Despite encouraging outcomes, challenges remain in vascularization, large-scale production, cost-effectiveness, and equitable patient access. Continued progress will depend on addressing biological limitations, optimizing manufacturing logistics, and harmonizing international regulations. Collectively, cell sheet therapies represent a pivotal step toward broader adoption of regenerative medicine in routine clinical practice.

Keywords Cell sheet therapy, Regenerative medicine, Tissue engineering, Clinical translation, Regulatory approval, Global landscape

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Introduction

Cell therapy is rapidly advancing, and the global market is experiencing sustained growth. In 2022, its value was estimated at 297 million USD, with a projected compound annual growth rate of 16.8% from 2022 to 2027 [1]. Within this context, cell sheets have emerged as an innovative modality in stem cell-based regenerative medicine. Defined as continuous layers of cells engineered *in vitro* and transplanted as intact tissue-like structures, they preserve cell–cell junctions, extracellular matrix (ECM), and metabolic activity—features that confer superior engraftment and therapeutic efficacy compared with traditional cell suspensions.

The concept of cell sheets originated in early tissue transplantation studies of the 1950–1960s, when researchers such as Billingham and Reynolds reported the transplantation of epidermal sheets directly derived from donor tissue [2]. These constructs, however, lacked the hallmarks of engineered cell layers. The modern era began with Howard Green in the 1970–1980s, who demonstrated that cultured keratinocytes could form continuous epithelial sheets detachable with dispase without disrupting intercellular junctions [3]. This achievement provided the first proof-of-concept for in vitro engineered, intact cell layers. In the late 1990s, Okano and colleagues introduced temperature-responsive polymer culture surfaces like poly(N-isopropylacrylamide) (PIPAAm), enabling non-enzymatic detachment of intact sheets [4]. In 2004, they formally coined the term "cell sheet engineering," consolidating a unified framework for scaffold-free cell sheet fabrication and application [5]. In this review, the scope is confined to scaffold-free cell sheets, including early cultured epithelial autografts (CEAs) by Green and subsequent technologies developed by Okano and co-workers. Scaffold-dependent constructs relying on collagen or fibrin matrices are excluded, as they differ fundamentally from the scaffold-free concept discussed here.

Unlike conventional cell-scaffold-growth factor triad-based tissue engineering approaches, scaffold-free cell sheets provide intact, metabolically active cell layers with preserved ECM and intercellular junctions [6, 7]. Such characteristics not only maintain normal tissue function but also enhance immunomodulatory potential, as evidenced by the increased expression of IL-10, IDO-1, and PTGES2 [8]. Endogenous ECM ensures seamless integration while avoiding enzymatic detachment damage, minimizing immunogenicity and foreign-body reactions [9, 10]. Owing to these advantages, scaffold-free cell sheets have been successfully applied in ocular surface reconstruction [11], skin wound healing [12], bone and periodontal regeneration [13–16], neural repair [17], and myocardial restoration [18, 19].

While numerous reviews have extensively summarized the preclinical advances in cell sheet technology [20–22], the present review focuses specifically on clinical research, including both clinical trials and approved therapies, in order to delineate the current translational landscape and therapeutic efficacy in human applications. Clinically, scaffold-free cell sheet therapies address pressing unmet needs in ophthalmology, dermatology, and cardiology. For example, conventional autologous skin grafting for burns is limited by donor-site morbidity and slow wound closure [23, 24], whereas allogenic cell sheet therapies reduce donor reliance and improve healing [25]. Moreover, regulatory frameworks increasingly support these therapies. Japan has pioneered conditional and expedited approvals via its SAKIGAKE system, leading to approvals of scaffold-free autologous products such as JACEMIN®, Ocural®, Nepic®, and JACE® [26–29]. In parallel, the U.S. Food and Drug Administration (FDA)'s Regenerative Medicine Advanced Therapy (RMAT) designation and the European Medicines Agency (EMA)'s Priority Medicines (PRIME) scheme provide accelerated pathways for cell sheet translation [30, 31].

Taken together, the rapid progress of scaffold-free cell sheet therapy warrants a comprehensive assessment of its development, clinical translation, and regulation. This review aims to provide such an overview, covering: (i) diverse cellular sources and their biological mechanisms, (ii) the technical foundations and fabrication strategies enabling sheet production, (iii) the global landscape of approved products and regulatory frameworks, and (iv) current challenges and future directions. By constructing a multidimensional analytical framework, this review provides evidentiary foundations and strategic decision-making support for establishing industry technical standards, optimizing clinical treatment pathways, and enhancing regulatory science systems. Supported by an expanding body of clinical trial evidence and regulatory approvals, scaffold-free cell-sheet therapies are emerging as a clinically validated frontier in regenerative medicine.

Cell sources for cell-sheet therapies

To date, 70 clinical trials involving scaffold-free cell sheet therapies have been registered worldwide (Fig. 1). The selection of cell source critically influences the therapeutic efficacy, safety, and scalability of these engineered tissues. Clinically investigated cell types include epithelial cells, connective tissue/mesenchymal cells, induced pluripotent stem cell (iPSC)-derived cells, and mixed cell populations, each presenting distinct advantages and limitations that affect both treatment feasibility and long-term outcomes. The distribution of clinical trials across these cell sources is summarized quantitatively in Fig. 2, while Fig. 3 illustrates the corresponding anatomical targets, highlighting the tissue-specific application emphasis

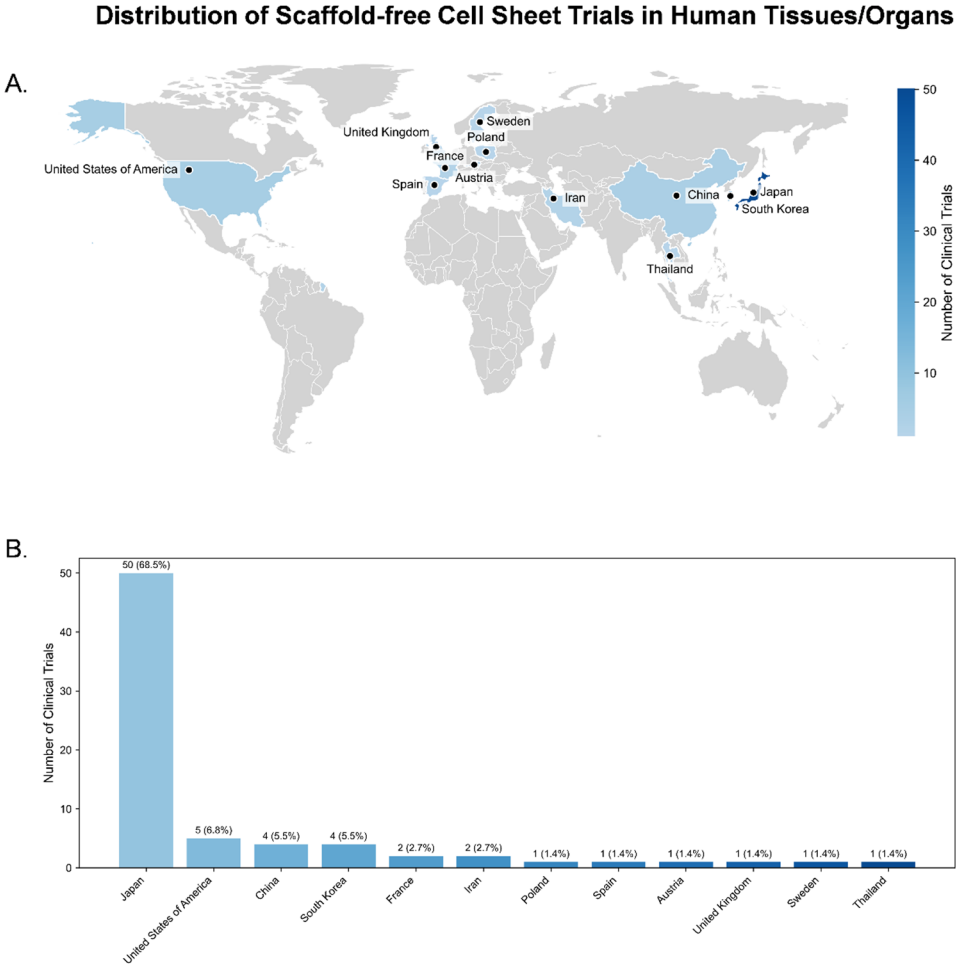

Fig. 1 The group of figures presents an overview of the global landscape of clinical trials on scaffold-free cell sheets. **A** is a world map illustrating the geographical distribution of these clinical trials, where darker shades of blue indicate a higher number of trials in a particular region. **B** is a bar chart that quantitatively shows the number of clinical trials in different countries and the proportion of clinical trials each country accounts for. Among them, Japan has 50 clinial trials, accounting for 68.5% of the total, followed by the United States of America with 6 clinical trials, while other countries have 4 or fewer trials

of this technology. In the following sections, we detail each cell source, discussing representative clinical trials and underscoring their key strengths and challenges.

Distribution of Scaffold-free cell sheet trials by cell type

Epithelial cells

Epithelial cell sheets are among the most established scaffold-free therapies, valued for their ability to restore barrier function, support epithelial regeneration, and ease of accessibility. Commonly studied sources include oral mucosal epithelial cells (OMECs), corneal limbal epithelial cells (LECs), nasal mucosal epithelial cells (NMECs), and keratinocytes. These cells have been applied in burns, corneal damage, esophageal defects, and middle-ear disorders, with corneal reconstruction and ulcer management currently representing the most frequent indications.

Oral mucosal epithelial cells

OMECs can be obtained through minimally invasive buccal biopsies and represent an attractive source for scaffold-free cell sheet therapy owing to their accessibility, proliferative capacity, and intrinsic immunomodulatory properties, such as pathogen recognition and regulation of inflammatory responses [32, 33]. These biological features support their application in ocular surface reconstruction and esophageal repair.

OMECs have been most widely investigated among scaffold-free approaches, with more than 20 clinical trials registered across 10 countries, targeting primarily bilateral limbal stem cell deficiency (LSCD) and esophageal lesions. In ophthalmology, cultivated oral mucosal epithelial transplantation (COMET) was developed as an autologous alternative when limbal tissue is unavailable. Unlike allogeneic limbal epithelial transplantation (ACLET), which requires systemic immunosuppression, COMET avoids this complication while

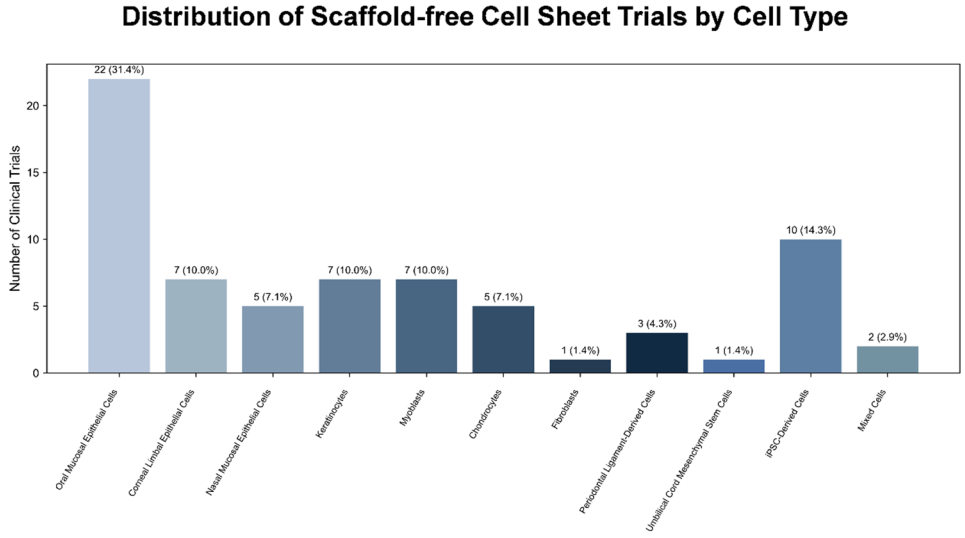

Fig. 2 This chart presents the distribution of the number of clinical trials on scaffold-free cell sheets for different cell types. To date, more than 10 kinds of cells have been applied in scaffold-free cell sheet clinical trials. Among them, oral mucosal epithelial cells have the largest number of clinical trials (22 cases), accounting for 31.4% of the total. iPSC-derived cells rank second, accounting for 14.3% of the total, while the proportion of clinical trials for other cell types is 10% or less

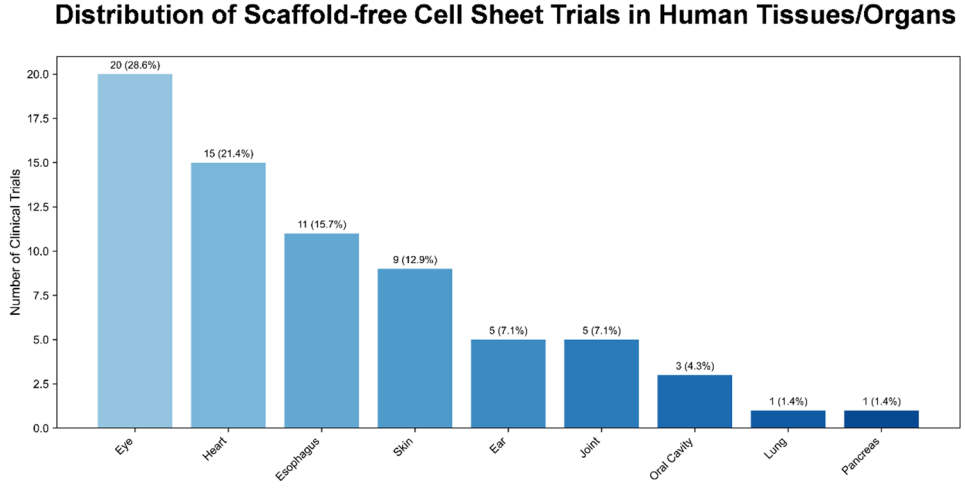

Fig. 3 This figure presents the distribution of clinical trials on scaffold-free cell sheets across different human tissues/organs. To date, 20 trials have been conducted for treating eye diseases, accounting for 28.6% of the total. Clinical trials for heart diseases rank second (21.4%), followed by those for esophagus diseases (15.7%), with all other tissues/organs categories having fewer than 10 trials

remaining clinically effective [34–36]. Two clinical studies confirmed safety and efficacy: in NCT02149732, six of eight treated eyes achieved stable epithelialization (mean 53.6 days), with 62.5% showing improved vision and no systemic adverse events [37]; in NCT02415218, chemical burn patients achieved restored vision and surface stability at one year, whereas those with Stevens–Johnson syndrome had poorer outcomes, likely due to persistent inflammation [38]. Scaffold-free OMEC sheets have also demonstrated superior corneal regeneration compared with human amniotic membrane (62.5% vs. 37.5%) [33].

In gastroenterology, the world's first clinical trial of OMEC sheets (UMIN000000473, 2006) showed that endoscopic transplantation after endoscopic submucosal dissection (ESD) prevented strictures in 89% of patients and shortened ulcer healing to three weeks, without transplantation-related adverse events [39–41]. However, refractory strictures may still develop in full-circumferential mucosal defects where sheet coverage is incomplete [42]. Subsequent work (UMIN000010251) confirmed the feasibility of long-distance sheet transport, with graft viability of 89–99% and stricture prevention in 60% of patients over two years [43]. More recently, OMEC sheets were tested in congenital esophageal atresia and stenosis (UMIN000034566). While some patients required repeated balloon dilations or surgery due to

fibrosis, others achieved durable benefit, remaining EBD-free for up to two years and maintaining normal diets [44–46].

Taken together, OMEC sheets represent one of the most clinically advanced scaffold-free platforms. Their abundant autologous availability and ability to circumvent systemic immunosuppression make them a valuable alternative for ocular surface reconstruction in bilateral LSCD, while clinical data also demonstrate consistent benefit in accelerating epithelialization and reducing stricture formation in esophageal disorders. Nonetheless, challenges remain in achieving durable efficacy across etiologies and in treating full-circumferential lesions, highlighting the need for improved delivery strategies and long-term follow-up.

Corneal limbal epithelial cells

LECs are situated at the limbus, the junction of the cornea and sclera, and act as stem or progenitor cells for the corneal epithelium. They exhibit strong proliferative capacity and the ability to differentiate into mature epithelial cells, thereby maintaining corneal homeostasis and transparency [47]. Damage or loss of these cells, due to trauma, genetic disorders, or inflammation, leads to LSCD, characterized by corneal opacity and visual impairment [48–50]. For therapeutic applications, LECs are usually harvested from the nasal, superior, or inferior limbal regions, balancing accessibility with minimal donor site morbidity [51].

To date, seven clinical trials have been initiated to evaluate autologous cultivated LEC sheets, although peer-reviewed outcome data are currently available from only one study. In the study UMIN000018969/UMIN000039994, a good manufacturing practice (GMP)-compliant autologous LEC sheet demonstrated clear safety and efficacy in unilateral LSCD. Corneal epithelial reconstruction was achieved in 60% of patients at one year and 70% at two years, significantly outperforming allogeneic transplantation. Additionally, 50–80% of patients reported improved visual acuity, corneal integrity, and quality of life, with no serious transplantation-related adverse events [52]. Based on these results, the product was granted regulatory approval in Japan, becoming the first cell- and tissue-based therapy for ocular regenerative medicine.

LEC sheets thus represent a clinically validated approach for restoring corneal transparency in LSCD. Their established safety and durable efficacy highlight their potential as a standard therapy, although imitations in donor tissue availability and further studies are needed to confirm long-term outcomes across broader patient populations.

Nasal mucosal epithelial cells

The nasal mucosa and middle ear mucosa share a common embryological origin from respiratory epithelium and exhibit striking structural similarities [53, 54]. This anatomical and histological affinity provides the rationale for using NMECs in middle ear regenerative therapy. Clinically, autologous NMECs are usually harvested from the inferior turbinate, where a small mucosal specimen (approximately 10×10 mm) can be obtained through a minimally invasive procedure. These cells show strong proliferative potential, and donor sites heal rapidly without major complications [55, 56].

To date, five clinical projects have investigated the application of NMEC sheets for adhesive otitis media and middle ear cholesteatoma, with outcome data reported from two studies. In the first-in-human trial (JMA-IIA00193), tympanoplasty combined with autologous NMEC sheet transplantation was performed in five patients. The procedure demonstrated a favorable safety profile, with uneventful donor site healing and an absence of recurrence or re-adhesion during follow-up. Moreover, most patients exhibited improvements in hearing function, although technical challenges in delivering sheets within the confined middle ear cavity were noted [55]. A subsequent study (PB3170011) involving six patients confirmed these findings: all sheets met quality control standards, transplantation was safe, and donor sites recovered well. Postoperatively, most patients maintained tympanic cavity aeration, and two-thirds achieved clinically meaningful hearing gains, attributed to enhanced mucosal regeneration and reduced fibrotic scarring [56].

Collectively, these studies indicate that NMEC sheets represent a safe and feasible autologous cell source for middle ear regenerative therapy. Their histological integration with middle ear mucosa, together with encouraging clinical outcomes, underscores their therapeutic potential. Nevertheless, refinement of sheet delivery techniques remains a critical requirement to facilitate broader clinical translation.

Keratinocytes

Keratinocytes, the predominant cells of the epidermis, play a central role in maintaining skin integrity through the synthesis and secretion of type VII collagen (C7), a structural component of the dermal–epidermal junction that stabilizes the skin via anchoring fibrils. They represent the earliest and most extensively studied cell source in skin tissue engineering. CEAs, pioneered for the treatment of extensive burn injuries, achieved graft survival rates of 72.7% and increased patient survival to 91%, establishing keratinocyte sheets as a lifesaving therapy in severe burns [57].

Building upon this foundation, both autologous and allogeneic keratinocyte sheets have been evaluated in clinical contexts. In Iran, the Royan Institute conducted a Phase I trial (IRCT20080728001031N31) demonstrating the safety and feasibility of allogeneic keratinocyte sheets, followed by a Phase II trial assessing their efficacy in deep second-degree burns. Outcomes included accelerated epithelialization, favorable graft survival, and improved scar profiles [58]. In Japan, a Phase I/II study (jRCTs052190079) tested dried allogeneic cultured epidermal sheets in six patients with burns or chronic ulcers, achieving wound closure rates of 73.4% by day 7 and 92.2% by day 14, with only one case of localized infection, thereby confirming safety [59]. Beyond acute injury, keratinocyte sheets have been genetically engineered for the treatment of inherited skin disorders such as recessive dystrophic epidermolysis bullosa (RDEB), caused by mutations in *COL7A1* leading to defective or absent C7 [60]. In this approach, keratinocytes harvested from unaffected skin are genetically corrected to restore C7 synthesis and anchoring fibril formation [61]. The Phase 1/2a trial of EB-101 (NCT01263379) showed durable wound closure, with 87% of treated sites achieving ≥ 75% healing at 3 months and 50% maintaining this at 12 months. C7 expression and fibril formation were confirmed in 90% of biopsies [62]. Long-term follow-up (up to 5.9 years) demonstrated sustained benefit, with 70% of sites maintaining ≥ 50% closure and no serious treatment-related adverse events, although larger chronic wounds showed reduced responsiveness [63, 64]. A subsequent Phase 3 trial (NCT04227106) further validated efficacy, reporting ≥ 50% healing in 81% of chronic RDEB wounds versus 16% in controls (standard of care) (mean difference 67%, $p < 0.0001$), alongside significant pain reduction and a favorable safety profile [65].

Together, these findings highlight the versatility of keratinocyte sheets. Initially developed as life-saving grafts for severe burns, they have since been applied to chronic wounds and advanced to gene-corrected therapies for genetic skin diseases. While outcomes in inherited disorders demonstrate durable correction and clinical benefit, further refinement is needed to improve responses in large chronic wounds and ensure scalable manufacturing for widespread application.

Connective tissue/mesenchymal cells

Connective tissue/mesenchymal cells contribute to tissue regeneration primarily through ECM secretion, modulation of inflammation, and angiogenesis. These cells, including myoblasts, chondrocytes, fibroblasts, periodontal ligament-derived cells (PDLCs), and umbilical cord mesenchymal stem cells (UC-MSCs), possess lineage-specific reparative capacity and paracrine activity. This enables them to facilitate structural restoration in load-bearing tissues and provide stromal support in vital organs. Scaffold-free cell sheet approaches leverage their inherent adhesive properties and microenvironmental interactions to achieve functional integration without exogenous scaffolds.

Myoblasts

Myoblasts, as precursors of skeletal muscle fibers, present several advantages for regenerative applications, including autologous availability, robust proliferative capacity in vitro, ease of handling, and a low tumorigenic risk attributable to their lineage-restricted differentiation potential [66]. They secrete growth factors such as hepatocyte growth factor and vascular endothelial growth factor (VEGF), which enhance neovascularization and myocardial repair [67]. Typically isolated from skeletal muscle biopsies (e.g., quadriceps), myoblasts can be expanded and engineered into scaffold-free sheets for cardiovascular applications [68, 69].

Clinical investigations in Japan have explored myoblast sheets across ischemic cardiomyopathy, dilated cardiomyopathy, and refractory heart failure. In UMIN000003273, 27 patients received autologous myoblast sheet transplantation, demonstrating safety and long-term benefit. One patient with end-stage dilated cardiomyopathy undergoing concomitant mitral valve replacement survived over six years with preserved function [70]. Five-year survival in ischemic cardiomyopathy reached 90.9% among responders [69, 71]. In UMIN000008013, seven patients with advanced ischemic heart failure received TCD-51073 myoblast sheets, with no severe adverse events observed and significant functional improvements noted: average left ventricular ejection fraction (LVEF) increased by 7.1% at 26 weeks, and six patients achieved an improvement of ≥ 1 New York Heart Association (NYHA) functional class [72]. Compared with propensity-matched cardiac resynchronization therapy, myoblast sheet recipients achieved superior LVEF improvement (33% vs. 23%, $p = 0.045$) and avoided cardiac deaths or left ventricular assist device (LVAD) implantation during two years of follow-up [73]. Additionally, the clinical study UMIN000000660 evaluated autologous myoblast sheets in four LVAD-supported patients with dilated cardiomyopathy, showing a favorable safety profile. Three patients exhibited ventricular remodeling, two were weaned from support, and histology confirmed angiogenesis and reduced hypertrophy, implicating paracrine-mediated recovery [74]. Findings from UMIN000012906 further demonstrated antifibrotic effects and reduced wall stress post-myocardial infarction [75]. Collectively, these trials support myoblast sheets as a safe and effective adjunctive therapy in severe heart failure.

Chondrocytes

Chondrocytes, the primary cells of cartilage, maintain biomechanical function by secreting ECM proteins such as aggrecan and type II collagen [76]. For therapeutic use, autologous chondrocytes are typically harvested from non-weight-bearing regions of the knee, while allogeneic sources often derive from discarded polydactyly tissue, providing a practical donor supply. The potential of chondrocyte sheets was first demonstrated in 2006 [77], and subsequent preclinical studies confirmed that transplantation can restore cartilage structure and function [78, 79].

Clinical translation has since advanced through both autologous and allogeneic approaches. In the UMIN000006650 trial, eight patients with knee osteoarthritis and advanced cartilage defects (Outerbridge grade III/IV) underwent autologous chondrocyte sheet transplantation combined with surgery. The procedure was well tolerated, with no sheet-related serious adverse events. Clinically, Knee injury and osteoarthritis outcome score (KOOS) and Lysholm knee scoring scale (LKS) scores improved significantly and were maintained for up to 54 months. MRI at three years showed complete defect repair in 62.5% of patients and full tissue integration in 75%, while biopsies revealed hyaline-like cartilage with type II collagen and Safranin O staining. Importantly, gene expression profiling identified predictive signatures correlated with functional outcomes, suggesting their potential as surrogate markers for clinical efficacy [80]. Building on this, UMIN000015205/jRCTa030190242 evaluated allogeneic polydactyly-derived chondrocyte sheets in 10 patients combined with high tibial osteotomy. Results confirmed safety and consistent functional improvement at 12 months, alongside imaging and histological evidence of hyaline-like cartilage regeneration. Moreover, molecular markers such as TGFB1 and ESM1 correlated with patient outcomes, reinforcing the translational value of biomarker-guided evaluation [81]. Currently, three additional clinical studies are ongoing: NCT01694823 in China (autologous chondrocyte sheets for knee cartilage lesions), jRCTb030190166 in Japan (autologous chondrocyte sheets for knee osteoarthritis), and NCT06549686 in the United States (allogeneic chondrocyte sheets for knee osteoarthritis).

Collectively, these studies highlight the clinical promise of chondrocyte sheet therapies. Both autologous and allogeneic products demonstrate favorable safety profiles and durable functional restoration, with emerging biomarker evidence offering a path toward precision patient selection and outcome prediction.

Fibroblasts

Fibroblasts, central to ECM remodeling and angiogenesis, proliferate rapidly and are easy to culture at low cost [82]. Clinically, autologous dermal fibroblasts can be harvested from a small (~ 1 cm^2) skin biopsy obtained at the planned surgical incision site on the lateral chest wall [83, 84].

The clinical study UMIN000022554 evaluated autologous dermal fibroblast sheets as a bio-artificial pleura during thoracoscopic lung resection in five patients with intraoperative air leaks. The sheets effectively sealed the leaks, which led to a reduced mean drainage duration of five days and, notably, no recurrence was observed during six months of follow-up [83]. Long-term follow-up confirmed durable closure in two patients monitored for 51 and 82 months, with no fibrosis or tumorigenesis [84]. These results support fibroblast sheets as a safe, durable solution for pleural repair.

Periodontal ligament-derived cells

PDLCs, including stem cells, fibroblasts, and progenitors, contribute to periodontal regeneration through collagen secretion, osteoblastic differentiation, and immune modulation [85, 86]. They are typically harvested from extracted third molars or retained deciduous teeth [87, 88].

In UMIN000005027, autologous PDLC sheets were transplanted in 10 periodontitis patients, with stable clinical attachment and defect closure observed over an average follow-up of 55 months, without adverse events [89]. Ongoing studies include NCT01082822 (autologous PDLSC sheets, China) and jRCT2090220391 (allogeneic PDLC sheets, TWP-0001, Japan). These results underscore the regenerative potential of PDLC sheets in periodontal therapy.

Umbilical cord mesenchymal stem cells

UC-MSCs, particularly those from Wharton's jelly, possess robust self-renewal, multipotency, and paracrine activity [90, 91]. Preclinical studies in porcine myocardial infarction models showed that UC-MSC sheets improved LVEF from 42% to 67% and reduced scar burden by 50% [92]. GMP-compliant UC-MSC sheets have since been developed for clinical use [93], supporting their translation into registered clinical studies. A Phase I/II trial (ChiCTR2400092039) is evaluating transplantation of UC-MSC sheets during coronary bypass surgery for ischemic cardiomyopathy. These advances underscore the feasibility of UC-MSC sheets as a regenerative therapy for cardiac repair.

Current therapeutic applications of connective tissue/mesenchymal cell-derived sheets remain predominantly restricted to their tissue-specific origins. However, emerging insights into embryological homology and the mechanistic basis of stem cell-mediated therapeutic effects—particularly their paracrine signaling and immunomodulatory capacities—are paving the way for

expansion beyond conventional indications. This evolving understanding may enable deployment to diverse anatomical sites, potentially unlocking synergistic therapeutic outcomes through conserved regenerative mechanisms.

iPSC-derived cells

iPSCs are generated by reprogramming somatic cells with defined transcription factors, such as Oct4, Sox2, Klf4, and Myc, into a pluripotent state resembling embryonic stem cells [94–96]. iPSCs exhibit virtually unlimited expansion capacity, genetic modifiability, and differentiation potential into most somatic lineages, enabling wide applications in disease modeling, drug screening, and regenerative therapy [97]. Multiple somatic sources have been explored for reprogramming, including fibroblasts [94–96], peripheral blood mononuclear cells (PBMNCs) [98], keratinocytes from plucked hair [99], urine-derived cells [100], and mesenchymal stromal cells from wisdom teeth [101].

iPSC-derived cell sheets are under clinical investigation for ocular, cardiovascular, and metabolic disorders. In ophthalmology, autologous iPSC-derived retinal pigment epithelium (RPE) sheets were transplanted after neovascular membrane removal in a patient with neovascular age-related macular degeneration (UMIN000011929). The grafts survived for more than 4 years, supporting photoreceptors and choroid without immune rejection or tumorigenesis, although visual acuity did not improve and cystoid macular edema persisted [102, 103]. In another trial (UMIN000036539/jRCTa050190084), four patients with LSCD received allogeneic iPSC-derived corneal epithelial cell sheets (iCEPSs). Over two years of follow-up, no serious adverse events such as tumorigenesis or rejection were reported. All treated eyes demonstrated improved LSCD stage, enhanced visual acuity, and reduced corneal opacity, with greater efficacy in patients receiving low-dose cyclosporin, confirming the safety and therapeutic potential of iCEPS transplantation [104].

In cardiology, seven clinical trials have been conducted in Japan since 2018, with outcomes reported for jRCT2053190081. This investigator-initiated trial evaluated allogeneic hiPSC-derived cardiomyocyte (hiPSC-CM) sheets for ischemic cardiomyopathy. The first-in-human case involved a 51-year-old male with end-stage disease who received three hiPSC-CM sheets transplanted onto the infarcted myocardium [105]. Subsequent reports described three additional patients treated under the same protocol, all of whom showed improved cardiac function at one year without tumorigenesis, arrhythmias, or immune rejection, confirming safety and preliminary efficacy [106]. Detailed follow-up of the initial patient further demonstrated favorable outcomes. Clinical-grade sheets, validated for cardiogenic phenotype and non-tumorigenicity in preclinical studies, were implanted onto the left ventricular epicardium under transient immunosuppression (prednisolone, tacrolimus, and mycophenolate mofetil for three months). At one year, no severe adverse events were observed, and efficacy endpoints included improved symptoms (NYHA class III to II), enhanced ventricular wall motion at the graft site, reduced end-systolic wall stress, normalized coronary flow reserves, and improved exercise tolerance. Positron emission tomography confirmed the absence of tumor formation [107]. Collectively, these results provide important early clinical evidence that hiPSC-CM sheet transplantation is a safe and feasible regenerative strategy for ischemic cardiomyopathy, with benefits likely mediated by paracrine angiogenic effects. Building on these outcomes, a new trial (jRCT2053230136) was initiated to evaluate hiPSC-CM sheets for non-ischemic dilated cardiomyopathy. Two patients have undergone transplantation, and their postoperative courses are under monitoring with formal safety and efficacy analyses forthcoming [108].

In metabolic disorders, iPSC-derived cells are under investigation as a therapeutic strategy for type 1 diabetes, with current efforts focusing on allogeneic iPSC-derived islet cell sheets. A Phase 1/1b trial (jRCT2053240146) is currently evaluating OZTx-410, an allogeneic iPSC-derived islet sheet product, to assess its safety and preliminary efficacy.

Collectively, these clinical studies underscore the broad therapeutic potential of iPSC-derived cell sheets across multiple disease contexts. Nevertheless, several challenges must be addressed before wider clinical adoption. Immunosuppression remains necessary, and transplant-specific anti-HLA antibodies have been observed post-therapy, with pre-existing anti-HLA-DQ associated with poorer outcomes, highlighting the importance of immune profiling in patient selection for iPSC-derived cardiomyocyte therapies [109]. To address this barrier, next-generation strategies employ CRISPR-Cas9-mediated knockout of HLA class I/II genes to develop hypoimmunogenic iPSC lines, creating universal "off-the-shelf" products with significantly reduced rejection risks that are poised to dominate regenerative medicine pipelines within this decade [110–115]. Additional barriers include the risk of tumorigenicity from residual undifferentiated cells, which requires stringent safety thresholds of ≤ 0.1% [116], along with high manufacturing costs [117], variability in differentiation efficiency across iPSC lines [118] and inconsistencies between production batches [119]. Addressing these immunological, safety, and manufacturing challenges in an integrated manner will be essential to unlock the full translational potential of iPSC-derived cell sheet therapies.

Mixed cell populations

While most cell sheet studies to date have focused on single-cell populations, emerging strategies explore the use of mixed cell sheets to better recapitulate the complex cellular interactions required for tissue repair. During tissue repair, different healing stages demand distinct biological functions, which can be addressed by combining multiple cell types into mixed sheets. Such constructs leverage synergistic effects: fibroblasts contribute to ECM synthesis and remodeling, while PBMNCs enhance angiogenesis, particularly after hypoxic preconditioning. Together, these functions accelerate wound closure and improve tissue quality, a concept supported by preclinical ulcer models demonstrating enhanced angiogenesis, granulation, and re-epithelialization [120–122].

Clinical evidence has begun to validate these findings. A registered trial in Japan (UMIN000031645/jRCTb060190034) evaluated autologous fibroblast–PBMNC sheets in six patients with refractory venous leg ulcers (VLUs). Three patients underwent transplantation, resulting in complete wound closure in two cases and partial reduction in one, all accompanied by pain relief. No serious transplantation-related adverse events were reported, confirming safety. However, three patients were excluded before treatment due to insufficient fibroblast proliferation or inadequate growth factor secretion, underscoring the practical challenges of relying solely on autologous cell sources [123]. Beyond venous ulcers, a clinical study in China (ChiCTR-INR-17010705) investigated autologous keratinocyte–fibroblast sheets for burn wounds and chronic ulcers. This work not only broadened the scope of mixed cell sheet applications but also reinforced the potential of fibroblast-based strategies in diverse wound-healing contexts.

Collectively, these studies highlight the promise of mixed cell sheets in addressing complex wound-healing requirements. At the same time, the observed variability in autologous cell quality suggests that integrating high-quality allogeneic sources may improve feasibility and scalability in future applications.

Brief summary

An overarching consideration across all cell types is the choice between autologous and allogeneic sources. Autologous cells, harvested directly from patients, offer intrinsic biocompatibility and circumvent immune rejection, which makes them particularly suitable for acute indications such as severe burns or LSCD. However, the application of such cell sheets relies on individualized manufacturing processes, which entail prolonged production timelines and elevated costs, thereby limiting large-scale clinical translation and widespread adoption [124]. In contrast, allogeneic mesenchymal stem/stromal cells allow for centralized, off-the-shelf manufacturing due to their high proliferative capacity and capability for cell banking, and they exhibit lower major histocompatibility complex (MHC) expression, potentially reducing immunogenicity [125]. However, for allogeneic cell sheets, a balance must be carefully weighed: while HLA matching or immunosuppressive strategies can reduce the risk of rejection, each approach carries inherent limitations and potential adverse effects [126–128]. Emerging strategies aim to bridge this gap. For instance, CRISPR-engineered hypoimmunogenic iPSCs—by knocking out MHC class I/II and overexpressing immune-checkpoint molecule CD47—have demonstrated the ability to evade immune rejection and survive long-term in fully immunocompetent allogeneic recipients, suggesting a route toward universal donor cell sheets [129]. Figure 4 summarizes the current distribution of autologous and allogeneic cell sheet clinical trials worldwide, illustrating how disease context and treatment urgency shape source selection. A comprehensive summary of all registered clinical trials of scaffold-free cell-sheet therapies is provided in Supplementary Table 1, detailing trial identifiers, registration dates, recruitment and clinical phase status, sample sizes, study objectives, donor characteristics, cell types, public and scientific titles, as well as associated countries, conditions, and target organs, thereby offering a global overview of the current translational landscape.

Fabrication of therapeutic cell sheets: from donor procurement to operative transfer

In this section, we present a current Good Manufacturing Practice (GMP)–compliant fabrication workflow for therapeutic cell sheets. The process encompasses donor procurement and tissue transport, isolation and expansion, controlled detachment and multilayer assembly, in-process and release testing, preservation and logistics, and finally, surgical application. Where feasible, quantitative detachment times and polymer compositions are provided, and process decisions are contextualized with recent clinical applications and regulatory approvals.

Standardization in cell banking: from donor screening to master cell banks

The establishment of cell banks represents a cornerstone for ensuring consistent and high-quality manufacturing of cell sheets. The process begins with rigorous donor eligibility assessment and screening procedures. For instance, both FDA and EMA guidelines mandate comprehensive testing for communicable diseases [130, 131]. In addition, ethical requirements, including informed consent and data privacy, must be strictly fulfilled to safeguard donor rights. Once eligible donors are confirmed, cells are isolated and expanded under GMP standards. Standardized protocols for primary cell isolation, such as deriving fibroblasts from skin biopsies or keratinocytes

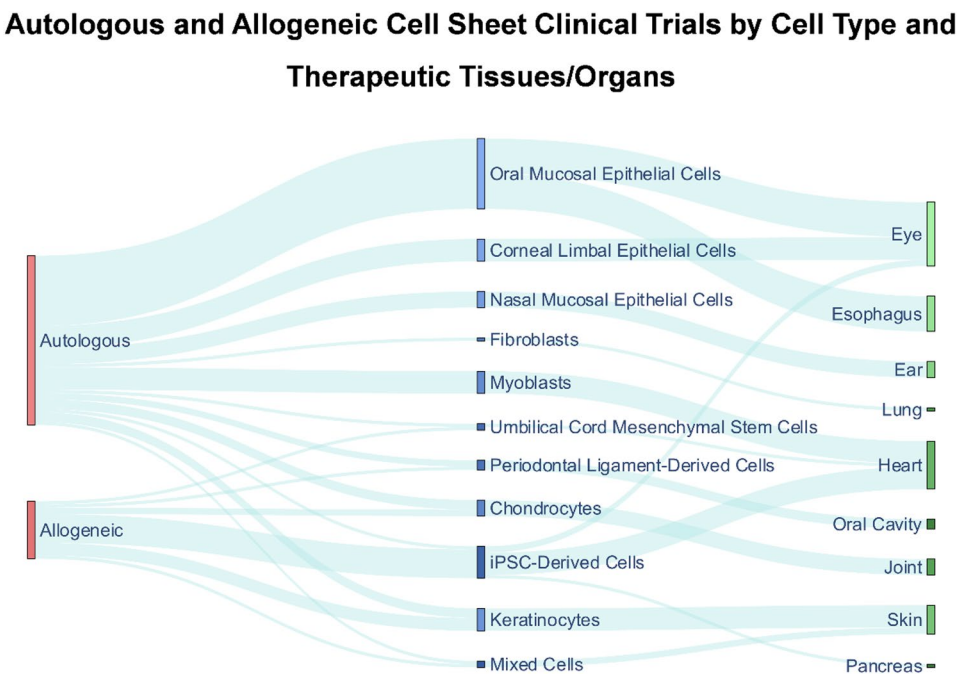

Fig. 4 This figure presents the distribution of scaffold-free cell sheet clinical trials according to cell source (autologous vs. allogeneic, left), specific cell types utilized (middle), and their clinical application sites (right). Autologous sources dominate current applications, particularly oral mucosal epithelial cells for esophageal and ocular repair, corneal limbal epithelial cells for ocular surface reconstruction, nasal epithelial cells, keratinocytes, or myoblasts for ear and skin indications. Allogeneic strategies, though fewer in number, primarily employ mesenchymal stem/stromal cells, chondrocytes, induced pluripotent stem cell (iPSC)-derived cells and keratinocytes, with applications covering the heart, oral cavity, joints, skin, and pancreas

from epidermal tissues, are essential to fulfill regulatory requirements [132].

Cryopreservation is another critical step. An optimal cryoprotectant (commonly dimethyl sulfoxide, often with additives) is selected to preserve cell viability during freezing and thawing [133]. Controlled-rate freezing—where temperature is gradually lowered at a defined rate—is widely used to minimize ice crystal formation and reduce cell damage [134]. Long-term storage in the vapor phase of liquid nitrogen ensures stable temperatures, preventing cell degradation over time. Post-thaw validation of cell viability and functional integrity is mandatory, with requirements consistent across regulatory bodies. For example, for pluripotent stem cells, assays to confirm retention of pluripotency markers like OCT4 and SOX2 are essential [135].

Quality control and characterization of cells in the cell bank are multi-faceted. Short tandem repeat profiling serves as the gold standard for identity authentication of human cell lines [136]. Additionally, for stem cells, markers of pluripotency and for differentiated cells, lineage-specific markers are used. In purity and safety testing, microbial contamination screening is vital. PCR-based methods are commonly employed to detect mycoplasma, a prevalent and difficult-to-detect contaminant that can significantly affect cell function. Multiplex real-time PCR is used for adventitious virus screening, ensuring that the cells are free from potential viral contaminant. Genetic stability assessment, including karyotyping to detect gross chromosomal aberrations and genomic sequencing for more subtle genetic changes, is required by regulatory guideline [137]. Potency assays, such as embryoid body formation for pluripotent stem cells or lineage-specific functional assays for differentiated cells, are designed with protocols harmonized across regions [138].

In conclusion, the establishment of cell banks for cell-sheet therapies is a complex and highly regulated process. From donor selection to quality control, each step is crucial in ensuring the production of safe and effective cell-sheet products. Future directions may involve the development of hypoimmunogenic cell banks, such as those using HLA-knockout iPSCs, to simplify cross-regional approval processes and improve the compatibility of allogeneic cell-sheet therapies [139]. Additionally, the integration of next-generation sequencing for more high-resolution genetic stability testing and digital droplet PCR for precise quantification of nucleic acids may further enhance the quality control measures in cell banks [140].

Culture medium composition

The choice of culture medium is crucial for cell sheet culturing, as it affects cell proliferation, ECM deposition, and sheet integrity [141, 142]. Designing culture media

for therapeutic cell sheet fabrication requires balancing cell-type–specific metabolism with preservation of junctional architecture and paracrine potency under clinically compliant conditions. Foundational choices begin with the base formulation aligned to metabolic demand and phenotype maintenance, followed by rational tuning of carbon sources (glucose/pyruvate), essential amino acids, and inorganic ions to keep osmolarity and electrophysiology within physiological windows. Media must simultaneously protect the sheet's structural integrity—tight junctions, desmosomes, and the nascent ECM—and sustain "therapeutic activity," including secretion of angiogenic and cytoprotective factors.

Early cell sheet culture methods commonly relied on serum-containing media because of their abundant growth factors and proteins. However, concerns about batch-to-batch variability and contamination risks prompted a transition toward serum-free, chemically defined media for clinical manufacturing [143]. These formulations not only provide more consistent and controllable conditions but also facilitate regulatory approval [144]. Furthermore, xenobiotic-free media have been shown to support cell growth without inducing significant DNA damage, highlighting their potential to minimize contamination risks and enhance safety in clinical applications [145]. Beyond the basal formulations, specific supplements are frequently incorporated to optimize cell sheet quality. For instance, ascorbic acid (vitamin C) serves as a cofactor for prolyl and lysyl hydroxylases, thereby enhancing collagen synthesis, strengthening ECM deposition, and improving the mechanical stability of the sheets [146, 147]. Likewise, the addition of growth factors is tailored to cell type: epidermal growth factor (EGF) is particularly critical for keratinocytes, while basic fibroblast growth factor (bFGF) promotes proliferation and matrix production in mesenchymal and cardiac cell sheets [148].

In conclusion, culture medium composition must be precisely tailored to the cell type and clinical objectives, balancing proliferation, ECM production, and cell functionality to ensure reproducible fabrication of high-quality cell sheets.

Noninvasive cell sheet detachment techniques used in clinical trials

Detachment of cells as an intact sheet is accomplished by selectively disrupting the adhesion between cells and the culture dish surface [149]. To preserve sheet integrity, various strategies have been developed, including enzymatic detachment, temperature-responsive surface-based detachment, and emerging stimulus-responsive technologies. Among these, enzymatic and temperature-responsive detachment remain the most widely adopted methods in clinical applications.

Enzymatic detachment technologies

Enzymatic detachment represented the first method for producing cell sheets. To create epithelial grafts for treating burns and similar skin injuries, autologous human epidermal cells were cultured. Once these cells achieved confluence, the sheet was enzymatically released from the culture dish. This involved incubation with 1% dispase at 37 °C for 15 min, followed by mechanical lifting using a cell scraper [3]. In the NCT02149732 trial [37], for instance, dispase was used to treat OMECs, yielding sheets for ocular surface reconstruction that integrated well with host tissue post-transplant without significant inflammation; similarly, in the UMIN000031645/jRCTb060190034 trial [123], dispase detachment of keratinocyte sheets for skin wound repair resulted in over 90% sheet viability and a 30% reduction in healing time compared to traditional skin grafting. Beyond dispase, collagenase has also been explored, such as in a study where a 0.2% collagenase and 0.1% dispase mixture was used for OMSC sheets, reducing impacts on cell viability and boosting ECM retention by 15% [150]. However, enzymatic detachment has drawbacks—prolonged incubation can degrade cell surface adhesion proteins—so clinical use now strictly limits incubation to no more than 20 min and includes viability checks like trypan blue staining to ensure cell quality.

Temperature-responsive culture surfaces

One of the cornerstones in scaffold-free cell sheet therapy is the development of enzyme-free detachment strategies, particularly those based on temperature-responsive culture surfaces coated with PIPAAm. These intelligent polymer surfaces enable non-enzymatic detachment of confluent cell monolayers when the culture temperature is reduced below the Lower critical solution temperature (LCST), approximately 32 °C. Upon temperature reduction, the surface becomes hydrophilic, weakening cell–substrate interactions and allowing intact sheet detachment while preserving cell–cell junctions and ECM components [151]. Among enzyme-free strategies, temperature-responsive systems are the most widely adopted and have been extensively translated into clinical practice. Most scaffold-free clinical applications employ thermo-responsive culture surfaces, particularly PIPAAm-coated dishes, which allow detachment of intact sheets at ~32 °C. This platform has enabled clinical translation of epithelial sheets for ocular and esophageal reconstruction, including trials addressing LSCD (NCT02415218) [38], esophageal ulcer repair (UMIN000000473) [39–41], and post-ESD stricture prevention (UMIN000010251 [43], UMIN000034566 [44–46]). In the cardiovascular field, autologous skeletal myoblast sheets have been applied to dilated and ischemic cardiomyopathies (UMIN000008013, UMIN000000660)

[72, 74], and more recently, iPSC-derived cardiomyocyte sheets have progressed into allogeneic transplantation (jRCT2053230136) [108]. Similarly, chondrocyte sheets for cartilage repair (UMIN000006650) [80] and periodontal ligament cell sheets (UMIN000005027) [89] rely on thermo-responsive systems to ensure viability and ECM integrity. Collectively, these studies underscore the central role of thermo-responsive culture technology in enabling reproducible and clinically translatable cell sheet therapies across diverse disease contexts. Beyond clinical trials, commercial products such as Nepic® have also employed this approach to generate corneal epithelial sheets for ocular surface reconstruction [52]. Currently, several commercially available temperature-responsive culture dishes are widely used for generating cell sheets, including Nunc™ UpCell™ Surface, RepCell®, and CellDETACH™. For detailed product specifications, see Table 1.

Emerging non-thermo-responsive enzyme-free detachment strategies

In addition to temperature-responsive surfaces, more and more enzyme-free alternatives that respond to mechanical force, pH shifts, light, magnetic or electric fields are being explored [149]. These approaches also enable the release of intact cell sheets, yet still remain at the preclinical stage.

Mechanical harvesting system Mechanical harvesting systems rely on three core tactics: peeling, scraping, and pipetting. Peeling is lifting the cell sheets up gently. In Guo et al.'s study, dental follicle and PDLC sheets cultivated for four weeks were peeled gently from the surface without enzymes and both regenerated periodontal-like tissues after transplantation into nude mice [152]. Similarly, hMSC sheets kept intact after peeling and exerted therapeutic effects in full thickness skin wound repair [153]. Scraping is usually performed by using a cell scraper, for example, porcine BMSC sheets and canine ADSC sheets were detached with cell scrapers, both maintaining continuous intercellular junctions [154, 155] Pipetting, on the other hand, applies mild lateral hydrodynamic force to release the sheet and the sheet is slightly smaller in diameter yet thicker, and the intact construct, along with its medium, can be aspirated and moved to a fresh dish [156]. These mechanical harvesting systems use only simple, commonly available materials for cell culture, avoiding any introduction of foreign reagents into the cell sheet. This ensures biosafety and keeps costs stay low. However, the handling is intricate, which limits its scalability and reproducibility. Moreover, the cell sheets must be sufficiently thick to resist tearing. Although the method is straightforward, inexpensive, and instrument-free, skilled operation is essential, as the sheets can easily tear during manipulation.

pH-responsive systems pH-responsive systems function by tuning surface chemistry and cell adhesion simply by adjusting acidity. Among the earliest proofs-of-concept, coatings that incorporate poly(2-vinylpyridine) or $CaCO_3$ particles release cultured cells when mild acid either protonates the polymer or dissolves the mineral. For instance, Sugiyama et al. showed that dissolving $CaCO_3$ particles at a mildly acidic pH detaches MSC sheets without tearing, while maintaining cell viability and structural integrity [157]. In addition to modifying the surface of the culture dish to help altering the acidity, directly adjusting the pH of the culture medium is also effective. For example, applying a sequence of media—first acidic (~ pH 6) to loosen adhesion, then basic (~ pH 8) to prevent clumping, and finally neutral to stabilize the shape—allows cells to reassemble quickly while preserving their ECM [158]. These successes rely on precise control of pH fluctuations, as even brief deviations can place stress on cells or disrupt the microenvironment they depend on.

Light-responsive systems Light-responsive systems detach cell sheets by illuminating light-sensitive materi-

Table 1 Comparison of temperature-sensitive cell culture surfaces: products, features and applications

Product name	Manufacturer	Material	Mechanism	Suitable cell types	Unique advantages
Nunc™ UpCell™ Surface	Thermo Fisher Scientific	Surface coated with covalently grafted poly(N-isopropylacrylamide, PIPAAm) polymer	cultures at 37 °C (adhesion), hydrophilic < 32 °C (release via swelling).	Various adherent cells	Enzyme-free detachment, preserves cell–cell junctions and cell surface proteins integrity
RepCell®	CellSeed Inc.	Surface coated with covalently grafted poly(N-isopropylacrylamide, PIPAAm) polymer	cultures at 37 °C, releases cells in 10–30 min at 20–25 °C.	Sensitive cells such as stem cells, dendritic cells	High cell viability, suitable for sensitive cells, supports single-cell isolation
CellDETACH™	Jet Life Science	Base materials: Polyolefins (polystyrene, polypropylene, polyethylene) or polycarboxylates (polycarbonate, etc.);Temperature-sensitive components: N-isopropylacrylamide, N-isopropylmethacrylamide or their oligomers	cultures at 37 °C, releases cells in sheets automatically in 10–40 min at 20 °C	Various adherent cells	Maintains cell surface receptors and antigens integrity, suitable for immunology studies

als at chosen wavelengths, which induce photochemical or photophysical changes, thereby modifying surface chemistry and disrupting integrin–ECM adhesion. One example is a near-infrared (NIR)-triggered dynamic wrinkling biointerface that rapidly releases cell sheets through a photothermal–mechanical cascade: the collagen support dissolves while interfacial shear forms, yielding viable, self-supporting sheets that accelerate wound healing in vivo [159]. Comparable NIR-patterned photothermal systems, built on Polydimethylsiloxane microlens arrays or gradient-thickness Poly(3,4-ethylenedioxythiophene) layers, can harvest areas exceeding 19 cm^2 with spatial selectivity, maintaining cell viability and ECM integrity above 90–95%, but require precise optical design and strict thermal control to prevent heat damage [160, 161]. Ultraviolet light (UV)-triggered alternatives employ TiO_2 nanodot-coated quartz, where a light-driven wettability switch enables MC3T3-E1 sheet detachment with > 90% efficiency and 97% viability, offering a reagent-free and biocompatible harvesting approach [162]. Despite their precision and biocompatibility, light-based systems remain constrained by substrate dependency, potential photothermal effects, and limited in vivo validation.

Magnetic assisted systems Magnetically assisted systems employ Fe_3O_4 nanoparticles or magnetic scaffolds, allowing external fields to trigger and guide the detachment of intact cell sheets. Ito et al. introduced the approach by culturing human keratinocytes on ultralow-attachment plates in the presence of cationic magnetite (Fe_3O_4) liposomes; withdrawal of the underlying magnet released a cohesive sheet that retained its architecture [163]. Later refinements, including magnetic cell sheet technology, combined Fe_3O_4 magnetic nanoparticles with human tendon-derived cells to build inflammatory models whose activity could be tuned by pulsed electromagnetic fields via the MAPK (ERK1/2) pathway [164]. Magnetically guided tissue engineering has likewise been used to fabricate porcine epithelial–mesenchymal composite sheets, enhancing odontogenic marker expression and epithelial–mesenchymal crosstalk [165]. Yet hurdles remain: ensuring nanoparticle biocompatibility, securing uniform magnetic responsiveness, and clearing residual magnetic material after detachment continue to constrain in vivo adoption and clinical translation.

Electrically triggered detachment Electrically responsive systems rely on external electric fields or other electric signals that change substrate surface properties such as surface charge, wettability and the conformation of adsorbed proteins,, thereby weakening or disrupting integrin–ECM-mediated adhesion. The approach falls mainly into two classes: ferroelectric surface regulation and electrochemical responsiveness. In the former, an electric pulse reverses the polarization of the ferroelectric substrate, flipping the surface charge from positive to negative; the resulting electrostatic repulsion encourages cells to let go [166]. For instance, a – 15 V pulse switches poly (vinylidene fluoride–trifluoroethylene) (PVDF–TrFE) from positive to negative, disrupting surface-protein interactions and releasing confluent fibroblast sheets [167]. In the latter, electrical input drives a reversible wettability shift from hydrophobic to hydrophilic, lowering ECM-protein adsorption and softening the substrate's grip so that cells detach gently. Conductive polymers such as biotin-modified polypyrrole have been used to capture and later release C2C12 sheets loaded with bone morphogenetic protein-2 when a negative potential is applied, an effect traced to conformational changes in adhesion-related proteins [168]. Moreover, pretreating gold substrates with UV has also been shown to accelerate electrochemical detachment, further improving the performance of electric-field-responsive biointerfaces [169]. Overall, this system enables rapid and controllable cell sheet detachment without additional reagents, but achieving uniform current distribution and consistent detachment across large culture surfaces remains challenging.

Ultrasound-responsive systems Ultrasound systems drive high-frequency mechanical vibrations into the culture medium, creating local microflows and shear that break the adhesion balance between the sheet and the substrate, so the cells lift off. Experiments have shown that ultrasonic vibration can harvest ECM-rich sheets; and 25 V released 95.6% of C2C12 sheets within 1 h with little biological penalty. However, voltages under 12.5 V left patches attached and intensities above 1 W cm^{-2} or exposures longer than 30 s cut viability to about 70% [170]. Another approach uses a piezoelectric PVDF composite containing 15 wt% $BaTiO_3$, which polarizes under ultrasound to modulate fibronectin conformation and cell adhesion, delivering a piezoelectric coefficient of 15.7 pC N^{-1} [171]. This method allows intact, proliferative sheets to be lifted off without damage, yet it requires accurate control of ultrasound parameters and involves increased fabrication complexity compared with uncoated culture vessels.

Formation of stratified cell sheets

Stratified cell sheets recapitulate the multilayered architecture of native tissues, making them particularly important for regenerative engineering [172]. One widely applied fabrication strategy involves temperature-responsive substrates, which enable synchronous detachment of multilayer sheets. Through layered culture, three-dimensional constructs such as chondrocyte or hepatocyte sheets can be generated, with all layers detaching together while preserving integrity. In

the UMIN000006650 trial [80], transplantation of triple-layered autologous chondrocyte sheets for knee osteoarthritis resulted in 1.2 mm greater hyaline cartilage regeneration and a 40-point KOOS score increase at 24 months compared with single-layer sheets. Similarly, stacking sheets detached from temperature-responsive dishes has enabled in vitro construction of functional three-dimensional tissues, including spontaneously contracting cardiac constructs. Incorporation of endothelial cells between layers further promotes capillary-like prevascular networks, laying the groundwork for functional tissue and organ models [173].

Another line of development focuses on corneal epithelial sheets and specialized culture devices. One strategy employs a closed-cell culture device ("cell cartridge") in which rabbit limbal epithelial cells are cultured on a porous membrane without 3T3 feeders. A 25-μm gas-permeable membrane was shown to yield multilayered corneal sheets with appropriate corneal marker expression [174]. Building on this concept, a novel temperature-responsive closed culture device [175], which applies thermo-responsive polymers to polycarbonate surfaces in an automated closed system, has been shown to fabricate transplantable corneal and oral mucosal epithelial sheets with quality comparable to traditional inserts, while ensuring superior sterility for clinical application.

Moreover, the cell sheet manipulator technique enables automated 3D tissue assembly with enhanced stacking precision, reduced structural damage, and improved fabrication efficiency. It is compatible with thermo-responsive systems for streamlined workflow integration [176]. Recent studies demonstrate that coupling this technique with biodegradable nanochannel membranes addresses key limitations of manual operation—including prevention of multilayer misalignment and preservation of cellular integrity. The integration with thermo-responsive systems further enables complementary cell retrieval strategies, broadens application potential, and supports the engineering of complex tissues for clinical translation [177].

Collectively, these strategies and device innovations have enabled the fabrication of complex, multilayered cell sheets that closely mimic native tissue structure and function. In summary, stratified cell sheet therapy provides a robust foundation for advancing tissue regeneration and clinical translation.

Transportation of cell sheets

In early applications, cell sheets were typically used immediately after preparation. They were mounted onto polyvinylidene difluoride (PVDF) membranes, grasped with endoscopic forceps, and delivered via EMR tubes directly to post-ESD esophageal ulcer sites [39–41]. As the field advanced, the feasibility of inter-institutional transport became a critical prerequisite for enabling the "centralized GMP manufacturing–multicenter clinical application" model, and both the safety and efficacy of shipped sheets have since been validated in clinical practice. OMEC sheets represent one of the most extensively studied examples in this regard. Inter-institutional transport was successfully demonstrated in the UMIN000010251 clinical study, in which autologous OMEC sheets were shipped from GMP facilities to surgical hospitals using a sterile, temperature-controlled device maintained at 2–8 °C. Quality assessments confirmed that viability (89–99%), intercellular junctions, ECM integrity, and epithelial-specific marker expression were preserved, without transport-related apoptosis or structural disruption, and the therapeutic outcomes were equivalent to those of freshly transplanted sheets [43]. Temperature-regulated approaches have further expanded the range of feasible strategies. In several studies, sheets were maintained at 37 °C during transport to preserve viability, followed by cooling to 20 °C prior to transplantation to facilitate detachment [44–46]. Similarly, autologous limbal epithelial cell sheets have been delivered from centralized facilities to multiple surgical sites using specialized sterile containers. These containers, featuring a three-layer composite structure with vacuum insulation, heat-retention materials, and ethylene oxide–sterilized dual chambers, maintained a stable 32–37 °C environment and sterility during long-distance air transport. For example, in shipments between Osaka University and Tohoku University (~650 km), 12-hour transport preserved sheet viability (77.3%), purity (87.9%), and marker expression (K3/76, p63) [52, 178]. Beyond epithelial cell sheets, other cell types have also been successfully transported. Dermal fibroblast sheets have been carried using CellShifter membranes as both carriers and manipulation substrates, ensuring sterility in transport containers or temperature-stable bags [83, 84]. Autologous skeletal muscle-derived stem cell sheets were directly transferred from clean benches or processing facilities to operating theaters in several clinical trials [69–71, 74]. For allogenic chondrocyte sheets, fresh transport was achieved by suspending sheets in DMEM/F12 supplemented with serum and antibiotics at room temperature, while cryopreservation methods, including conventional freezing with STEM-CELLBANKER™ at −180 °C and vitrification using cyclic freezing bags, enabled long-term preservation and distribution [81, 179]. More recently, iPSC-derived islet sheets have also been successfully transported to clinical centers using temperature-controlled devices. Taken together, these studies demonstrate that diverse transport strategies—from fresh handling to advanced cryopreservation—can maintain the structural and functional integrity of cell

sheets, thereby enabling their safe application across multiple clinical sites.

Nevertheless, the clinical success of transported sheets relies equally on rigorous quality control and standardized assessment in addition to technical feasibility. Case studies have demonstrated multiple strategies to validate both cell viability and functional integrity during inter-institutional transfer. For example, OMEC sheets have been evaluated prior to shipment using trypan blue exclusion assays to confirm viability ≥ 85%, together with immunofluorescence for keratin 3/76 and the tight-junction protein ZO-1 to ensure epithelial phenotype and barrier function. Post-transport evaluations further employed metabolic assays (e.g., MTT) to verify proliferative and differentiation potential, and clinical outcomes consistently demonstrated complete re-epithelialization without transport-related compromise [39]. Similarly, limbal epithelial sheets were required to meet pre-transport benchmarks of viability ≥ 80%, p63 positivity ≥ 30%, and continuous collagen IV expression, while post-transport analyses confirmed purity ≥ 90% and preserved capacity to reconstruct stratified epithelia in vitro [52]. In parallel, transport devices themselves have undergone validation. The study's vacuum-insulated vessel with phase-change materials (N-eicosane) kept temp stable (32–37 °C), over 35 °C for 24 h in 3–5 °C, meeting cell sheet transport needs [178].

Despite these advances, critical limitations remain. Certain contractile or metabolically active sheets, such as those derived from skeletal muscle or cardiomyocytes, can only be transferred under immediate, short-range conditions, restricting them to "on-site preparation and transplantation" protocols [74]. Similarly, corneal epithelial sheets exhibit pronounced humidity sensitivity, with detachment occurring at relative humidity < 40% even when temperature is tightly controlled, thus confining most applications to intra-institutional settings [52].

Collectively, these findings highlight both the remarkable progress and the current challenges in developing reliable, quality-controlled transport systems for therapeutic cell sheets. While epithelial and fibroblast-based products have demonstrated reproducible inter-institutional delivery, future research should focus on engineering transport platforms capable of supporting the unique bioenergetic and biomechanical demands of contractile or highly sensitive sheet types, or alternatively, the establishment of cryopreservation protocols that ensure both safety and biological activity maintenance, thereby broadening the clinical reach of regenerative therapies. Overall, these advances underscore the essential interplay between technical feasibility and rigorous quality assurance in the successful clinical translation of cell sheet transport.

Approved products around the world

The global landscape of approved cell sheet therapies illustrates diverse trajectories shaped by regulatory frameworks, clinical needs, and technological strategies. As summarized chronologically in Fig. 5 and in detail in Table 2 (which catalogues products, indications, cell sources, and approval years), Japan has established the most comprehensive approval record to date. In contrast, the United States and South Korea have advanced distinct product types—focusing on gene-modified

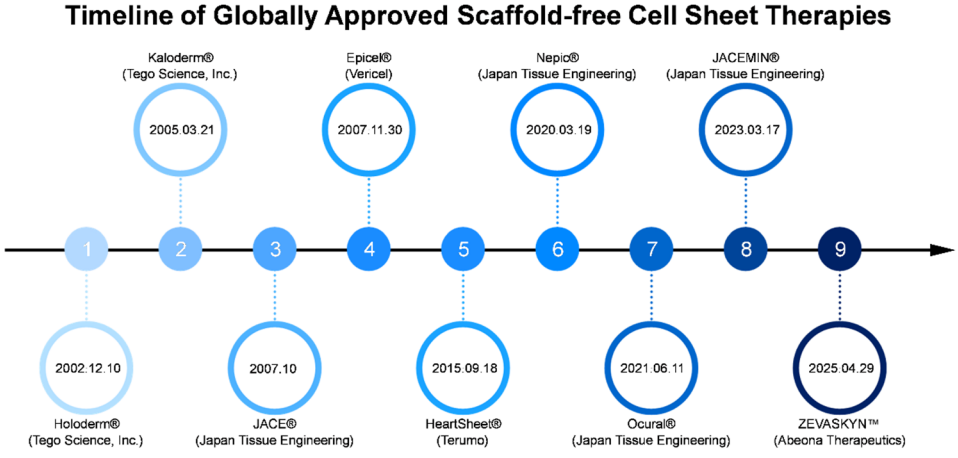

Fig. 5 This timeline illustrates the sequential global approvals of cell sheet therapies from 2002 to 2025. The first approval was Holoderm® (Tego Science, Inc., South Korea) in 2002 for skin regeneration, followed by Kaloderm® (Tego Science, Inc.) in 2005 and Epicel® (Vericel, United States) in 2007. Japan emerged as a leading country in this field, with multiple approvals including JACE® (Japan Tissue Engineering, 2007, for limbal stem cell deficiency), HeartSheet® (Terumo, 2015, for severe heart failure), Ocural® (Japan Tissue Engineering, 2021, for ocular surface disorders), Nepic® (Japan Tissue Engineering, 2020, for epithelial regeneration), and JACEMIN® (Japan Tissue Engineering, 2023, for corneal repair). Most recently, in 2025, ZEVASKYN™ (Abeona Therapeutics, United States) was approved, further expanding the scope of clinical indications. Together, these milestones highlight the progressive global landscape of scaffold-free cell sheet therapies, reflecting both technological advances and regulatory recognition across different countries and organ systems

Table 2 Global landscape of commercially approved cell sheet therapies

Product name (developer)	Approval date	Non-proprietary name	Cell source	Country	Clinical indication	Status
JACE (Japan Tissue Engineering)	2007/10	Autologous epidermal cell sheet	Autologous epidermal cells	Japan	Severe burns, large congenital melanocytic nevi, epidermolysis bullosa	On market (reimbursed and marketed since 2009)
HeartSheet (Terumo)	2015/09/18	Autologous skeletal myoblast-derived cell sheet	Autologous skeletal myoblasts	Japan	Severe heart failure due to ischemic heart disease (nonresponsive to standard care)	Withdrawn (approval revoked on July 25, 2024)
Nepic (Japan Tissue Engineering)	2020/3/19	Autologous limbal-derived corneal epithelial cell sheet	Autologous limbal cells	Japan	Limbal stem cell deficiency (corneal epithelial regeneration)	On market (approved and reimbursed)
Ocural (Japan Tissue Engineering)	2021/6/11	Autologous oral mucosa-derived epithelial cell sheet	Autologous oral mucosa	Japan	Limbal stem cell deficiency	On market (approved and launched)
JACEMIN (Japan Tissue Engineering)	2023/3/17	Autologous epidermal cell sheet containing melanocytes	Autologous epidermal cells	Japan	Vitiligo (refractory to non-surgical treatments)	On market (covered by National Health Insurance)
Epicel® (Vericel)	2007/11/30	Autologous epidermal cell sheet	Autologous epidermal keratinocytes	United States	Life-threatening deep dermal or full-thickness burns involving ≥ 30% of the total body surface area (TBSA)	On market (Humanitarian Device Exemption, expanded pediatric use approved, actively marketed)
ZEVASKYN™ (Abeona Therapeutics)	2025/4/29	Autologous gene-modified epidermal cell sheet	Autologous genetically engineered keratinocytes	United States	Recessive dystrophic epidermolysis bullosa (RDEB)	Approved (first FDA-approved gene-modified cell sheet therapy, pending commercial launch)
Holoderm® (Tego Science, Inc.)	2002/12/10	Autologous epidermal cell sheet	Autologous keratinocytes from patient	South Korea	Deep 2nd degree burns (> 30% TBSA), 3rd degree burns (> 10% TBSA)	On market (Approved in South Korea; reimbursed by Industrial Accident Insurance)
Kaloderm® (Tego Science, Inc.)	2005/03/21	Allogeneic epidermal cell sheet (allogeneic keratinocyte sheets from neonatal foreskin)	Allogeneic keratinocytes (infant foreskin)	South Korea	Deep 2nd degree burns; non-infected diabetic foot ulcers	On market (Ministry of Food and Drug Safety-approved; reimbursed by National Health Insurance in South Korea)

autologous applications and allogeneic off-the-shelf solutions, respectively. These developments underscore the heterogeneous yet convergent evolution of scaffold-free cell-based therapies across healthcare systems.

Japan

Japan maintains the most advanced regulatory framework for cell sheet therapies, with five products approved between 2007 and 2025. These therapies target niche indications with high unmet medical needs, all utilizing autologous cells to ensure immune compatibility and eliminate the need for immunosuppression. Four products obtained accelerated approval via Japan's orphan drug/regenerative medicine designation, reflecting a supportive ecosystem led by domestic companies—Japan Tissue Engineering holds four of the five approvals.

Ocular regenerative products include Nepic® and Ocural® [180, 181], both indicated for LSCD. Approved in 2021, Ocural® is the first product based on cultured autologous oral mucosal epithelial sheets for LSCD [182]. For dermatological conditions, JACEMIN® (approved March 2023) is an autologous epidermal sheet containing melanocytes, developed for treatment-refractory vitiligo and reimbursed under Japan's National Health Insurance [183]. The flagship product JACE®, initially approved in 2007, expanded its indications in 2016 and 2018 to include severe burns and epidermolysis bullosa [184], exemplifying the evolving clinical potential of these therapies.

HeartSheet®, the only myoblast sheet targeting internal organ regeneration, was initially approved in 2015 for ischemic heart failure but withdrawn in 2024 due to safety concerns. Clinical evaluation revealed a hazard ratio for cardiac death versus control of 1.9 (0.8–4.4) with adverse events in 70% of patients, including 11 deaths, six possibly treatment-related [185]. These outcomes highlight the logistical and clinical challenges of autologous skeletal myoblast therapy in cardiac applications [186].

In summary, Japan demonstrates a mature regulatory and clinical framework for autologous cell sheet therapies, particularly in ocular and dermatological

applications, while highlighting challenges in cardiac applications.

The United States

In the United States, two approved cell sheet products focus on skin and wound repair: Epicel® [187, 188] and ZEVASKYN™ [189], developed by Genzyme Tissue Repair and Abeona Therapeutics, respectively.

Epicel® is a scaffold-free autologous keratinocyte sheet expanded into 2–8 cell layers on growth-inactivated murine 3T3 feeder cells and delivered on petrolatum gauze (~50 cm^2 per sheet) [187, 188]. It received Humanitarian Use Device (HUD) designation in 1998 and Humanitarian Device Exemption (HDE) approval in 2007, with pediatric indications expanded in 2016 and regulatory oversight shifting from Center for Devices and Radiological Health (CDRH) to Center for Biologics Evaluation and Research (CBER) in 2013 [187, 188]. Clinical data indicate premarket graft survival > 86%, post-approval pediatric survival of 88.3%, and satisfactory graft integration and wound healing [190, 191], confirming its value in treating extensive burns.

ZEVASKYN™ (prademagene zamikeracel), approved April 29, 2025, is the first autologous gene-modified cell sheet therapy for RDEB [189]. It combines autologous keratinocytes with ex vivo gene correction using a replication-defective retroviral vector introducing *COL7A1*, restoring dermal–epidermal junctions and addressing RDEB-related skin fragility [189]. In the pivotal Phase III VIITAL™ study, ≥ 50% healing was achieved in 81% of chronic RDEB wounds at 6 months versus 16% in controls [192]. ZEVASKYN™ exemplifies next-generation personalized regenerative therapies for rare genetic skin disorders.

In summary, the United States demonstrates clinical translation of both conventional and gene-modified autologous cell sheet therapies, highlighting their critical role in treating severe and rare skin conditions.

South Korea

In South Korea, two approved cell sheet products focus on skin repair: Holoderm® and Kaloderm®, developed by Tego Scienc. Holoderm®, the country's first autologous cultured epidermal graft and the world's second, treats extensive second- and third-degree burns, achieving > 90% graft take in clinical trials and saving over 850 lives since 2002 [193]. It is fully reimbursed by South Korea's Industrial Accident Insurance. Kaloderm® is an allogeneic keratinocyte sheet for deep burns and diabetic foot ulcers, exemplifying a successful off-the-shelf therapy [194, 195]. Its use in complex trauma cases, such as salvaging necrotic fingertips, demonstrates clinical versatility and efficacy.

In summary, South Korea highlights the clinical translation of both autologous and allogeneic skin cell sheet therapies, emphasizing off-the-shelf solutions for extensive injuries and chronic wounds.

Current challenges and future directions

Despite remarkable clinical progress and increasing global approval of cell sheet products, their widespread adoption remains hindered by challenges in standardization and manufacturing, limited vascularization, and short product shelf-life, which collectively constrain scalability, reproducibility, and clinical translation.

Standardization and manufacturing challenges

The choice of cell source is critical, as both autologous and allogeneic cells entail distinct trade-offs in accessibility, safety, and potency. Future strategies should include stringent donor screening, genomic stability testing, and tissue-specific functional assays, together with standardized potency evaluations to ensure consistent therapeutic efficacy. Emerging approaches, such as single-cell transcriptomics and machine learning, hold promise for identifying optimal subpopulations with stable regenerative potential [196].

A major challenge in manufacturing lies in biological heterogeneity and batch-to-batch variability. Even when derived from the same donor under standardized protocols, cell sheets often display differences in viability, ECM composition, and functional activity [197]. Such variability complicates compliance with GMP standards and hinders reproducibility in clinical translation. To mitigate these issues, large-scale stirred bioreactor systems have been shown to reduce heterogeneity during iPSC differentiation into cardiomyocytes, producing consistent gene expression profiles and contractile performance across multiple lines [119, 198]. In addition, current workflows largely rely on manual, small-batch fabrication, which limits scalability and exacerbates batch variability. Future development should prioritize automated, closed-loop production platforms that integrate robotic handling, real-time sensor monitoring, and strict GMP compliance. Temperature-responsive harvesting systems combined with AI-driven quality analytics may further enhance reproducibility while reducing costs. Regulatory expectations also diverge internationally. For instance, Japan's Pharmaceuticals and Medical Devices Agency (PMDA) allows more flexible release criteria when clinical benefit is demonstrated [199], whereas the FDA and EMA require rigorous potency assays, such as quantifiable expression of functional markers and reproducible ECM metrics[200].

Overall, cell sheet translation is limited by donor variability, manufacturing challenges, and regulatory

differences, highlighting the need for standardized and scalable solutions.

Cell sheet vascularization

The limited vascularization of cell sheets, with diffusion restricted to ~100–200 μm, severely constrains their application in thick or metabolically demanding tissues, underscoring the need for establishing capillary networks in multilayered constructs [201–203]. Endothelial co-culture with cardiac cells promotes spontaneous formation of reticular networks and the secretion of angiogenic cytokines, including VEGF, which are crucial for neovascularization [204, 205]. Similarly, endothelial progenitor cells enhance the osteogenic potential of bone mesenchymal stem cell sheets by stimulating VEGF-mediated bone formation [206]. Exogenous factors such as fibroblast growth factor-2 (FGF-2) can further stabilize endothelial connections within multilayered skeletal muscle sheets, thereby extending vascular integration [207]. Collectively, such endothelialized constructs exhibit superior vascularization, nutrient exchange, and transplantation efficiency [208–210]. Complementing these biological strategies, bioengineering innovations have markedly improved cell sheet fabrication. Electrochemically desorbable peptide-modified gold membranes allow rapid, non-invasive detachment of intact sheets with preserved viability. Integration with microfluidic perfusion systems ensures continuous nutrient delivery and waste clearance, enabling the generation of thicker, more functional constructs. Collagen-assisted stacking further enhances interlayer adhesion, and pre-vascularized supporting layers provide perfusable networks that significantly improve graft survival in vivo [211].

These integrated biological and engineering approaches collectively address the long-standing limitations of vascularization, enabling the development of thicker, more functional, and clinically viable cell sheet constructs.

Prolonging shelf-life

A major limitation of current cell sheet therapies is their short usable window. For example, Holoclar® must be transplanted within 36 h of harvest, as indicated by regulatory stability data, creating significant challenges for multicenter trials and global distribution where centralized manufacturing is absent. At present, all clinical applications rely on freshly prepared sheets, which preserve high biological activity and structural integrity but are associated with high production costs, delayed quality control, rapid release requirements, and significant logistical barriers. These constraints severely restrict large-scale clinical translation and scalability.

Cryopreservation has emerged as a potential solution, enabling long-term storage and on-demand availability of cell sheets. Vitrification, which prevents ice crystal formation, offers an alternative to conventional cryopreservation [212, 213]. However, standard vitrification methods are technically complex and often rely on open systems, increasing contamination risk. To overcome this, Hayashi et al. developed a closed vitrification platform using microfluidic chips and VS83 cryoprotectant, which reduced contamination rates by 90% and achieved > 85% post-thaw viability in chondrocyte sheets [214]. Despite such progress, current preservation strategies still face critical challenges in maintaining the structural and functional integrity of cell sheets during freeze–thaw cycles. Further advances in cryoprotectant design, optimized freezing protocols, and post-thaw recovery methods will be essential to enable the development of practical, off-the-shelf cell sheet products for wider clinical use.

Together, these challenges emphasize that overcoming donor variability, limited vascularization, and short shelf-life will be critical for translating cell sheet therapies into widely accessible, clinically robust products.

Conclusions

Scaffold-free cell sheet therapy has emerged as a transformative platform in regenerative medicine, distinguished by its preservation of native cell–cell interactions, ECM, and paracrine signaling. Over three decades of technical innovation and clinical translation have validated its versatility across ophthalmic, dermatologic, cardiovascular, periodontal, esophageal, and otologic disorders, with regulatory approvals in Japan, the United States, and South Korea underscoring its therapeutic credibility.

Nevertheless, critical barriers remain. Standardization of manufacturing, vascularization of thick constructs, and reliable long-term preservation continue to limit broad clinical adoption. Furthermore, the balance between autologous and allogeneic approaches is unresolved, with emerging solutions such as hypoimmunogenic iPSC-derived sheets offering promise but requiring rigorous safety validation.

Looking ahead, the field must prioritize precision manufacturing, strategies for functional vascularization, long-term effective preservation, user-friendly applications, and global regulatory harmonization to ensure reproducibility, scalability, and patient safety. Addressing these challenges will allow scaffold-free cell sheet therapies to evolve from niche interventions into widely accessible regenerative solutions.

Abbreviations

ECM	Extracellular matrix
LSCD	Limbal stem cell deficiency
ESD	Endoscopic submucosal dissection
iPSC	Induced pluripotent stem cell
GMP	Good manufacturing practice
OMECs	Oral mucosal epithelial cells
LECs	Corneal limbal epithelial cells

NMECs	Nasal mucosal epithelial cells
CEAs	Cultured epithelial autografts
RDEB	Recessive dystrophic epidermolysis bullosa
UC-MSCs	Umbilical cord mesenchymal stem cells
PDLCs	Periodontal ligament-derived cells
hiPSC-CM	Human induced pluripotent stem cell-derived cardiomyocyte
iCEPS	iPSC-derived corneal epithelial cell sheets
VEGF	Vascular endothelial growth factor
FGF-2	Fibroblast growth factor 2
bFGF	Basic fibroblast growth factor
EGF	Epidermal growth factor
NIR	Near-infrared
UV	Ultraviolet light
PVDF-TrFE	Poly (vinylidene fluoride-trifluoroethylene)
LVEF	Left ventricular ejection fraction
NYHA	New York Heart Association
LVAD	Left ventricular assist device
HUD	Humanitarian Use Device
HDE	Humanitarian Device Exemption
CDRH	Center for Devices and Radiological Health
CBER	Center for Biologics Evaluation and Research
MFDS	Ministry of Food and Drug Safety
PMDA	Pharmaceuticals and Medical Devices Agency
FDA	Food and Drug Administration
EMA	European Medicines Agency
RMAT	Regenerative Medicine Advanced Therapy
PRIME	Priority Medicines
SAKIGAKE	Designation for Promising Innovative DrugsMedical Devices
KOOS	Knee injury and osteoarthritis outcome score
LKS	Lysholm knee scoring
COMET	Cultivated oral mucosal epithelial transplantation
ACLET	Allogeneic limbal epithelial transplantation
VLUs	Venous leg ulcers
PBMNCs	Peripheral blood mononuclear cells
PIPAAm	Poly (Nisopropylacrylamide)
LCST	Lower critical solution temperature
PVDF	Poly (vinylidene difluoride)
MHC	Major histocompatibility complex
TBSA	Total body surface area
C7	Type VII collagen

Supplementary Information

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Supplementary Material 1.

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The authors declare that they have not use AI-generated work in this manuscript.

Author contributions
AL was responsible for the conceptual design, literature collection, manuscript writing, and overall coordination of the review; SMY contributed to literature analysis, figure preparation, and manuscript revision; CL assisted with data interpretation and critical literature review; FJH contributed to the refinement of the manuscript and helped with reference management and formatting; CY and WDT supervised the entire project, provided critical revisions, and are the corresponding authors responsible for the final approval of the manuscript. The two co-corresponding authors contributed equally to this review and their collaboration ensured the scientific rigor and integrity of the work. All authors read and approved the final version of the manuscript.

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Declarations

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