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Review article

Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration

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ABSTRACT

Three-dimensional (3D) stem cell culture systems have attracted considerable attention as a way to better mimic the complex interactions between individual cells and the extracellular matrix (ECM) that occur *in vivo*. Moreover, 3D cell culture systems have unique properties that help guide specific functions, growth, and processes of stem cells (e.g., embryogenesis, morphogenesis, and organogenesis). Thus, 3D stem cell culture systems that mimic *in vivo* environments enable basic research about various tissues and organs. In this review, we focus on the advanced therapeutic applications of stem cell-based 3D culture systems generated using different engineering techniques. Specifically, we summarize the historical advancements of 3D cell culture systems and discuss the therapeutic applications of stem cell-based spheroids and organoids, including engineering techniques for tissue repair and regeneration.

1. Introduction

Progressing from two-dimensional (2D) monolayer cell culture to three-dimensional (3D) cell culture requires the use of cell based-models that mimic the function of living tissues and organs [1,2]. Moreover, 3D cell culture systems have great potential for narrowing the gap between cell-based methods and animal models for studying the development of novel drugs and therapeutics, as well as the repair and replacement of tissues and organs [3,4]. Previously, various cells have been cultivated in 2D monolayers on cell culture plates or other platforms and utilized not only for biological research but also for the development of therapeutic applications for damaged tissues and organs [5,6]. However, monolayer cells do not reflect the *in vivo* microenvironment because they lack exposure to the extracellular matrix (ECM) and physiological relevance, which may cause abnormal cell metabolism and protein expression [7,8]. To address this issue, 3D cell culture systems have attracted considerable attention as a strategy to better mimic *in vivo* conditions, such as the complex cell-cell and cell-ECM interactions [9]. Additionally, 3D cell cultures, such as aggregates, spheroids, and organoids, provide unique properties to guide the specific functions and growth of cells (e.g., embryogenesis, morphogenesis, and organogenesis). Thus, 3D cell culture is a tool for creating *in vivo*-like environments that enable basic research of various tissues and organs.

In 3D cell culture models, spheroids are simple clusters of a broad range of cell types including tumor spheroids, embryoid bodies, hepatospheres, neurospheres, and mammospheres [10]. Cell spheroids are widely used as multicellular 3D models that form because of the tendency of adherent cells to aggregate into higher cell densities. The multicellular spheroid, which allows for abundant chemical and mechanical interactions, was investigated in various *ex vivo* tissue models and presented gradients for nutrients, gases, growth factors, and signal factors [8]. Stem cells in the form of spheroids have great application potential in tissue engineering and regenerative medicine because of their extensive abilities, such as self-renewal, generation of differentiated progeny, and protein secretion [11–13]. For this reason, recently, stem cell-based spheroids have been utilized in tissue engineering and regenerative medicine as transplantable treatments targeting bone, cartilage, tendon, muscle, dental, nerve, blood vessel, and skin tissue. Furthermore, the application of various engineering approaches to forming or transplanting stem cell spheroids can improve the therapeutic effects [14–20]. The engineered stem cell spheroid is crucial not only for activating functions of stem cells residing in 3D niche

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microenvironments, in which the complex interactions between ECM molecules are induced, but also for overcoming the issue of biocompatibility in scaffolds and biomaterials [21,22].

Accurately controlling stem cell differentiation and formation of tissue *in vitro* is essential for development, organogenesis, and tissue homeostasis to achieve *in vivo*-like tissues or organs. Organoids, miniature organ models generated *in vitro*, are complex 3D stem cells [e.g., adult stem cell (ASC), embryonic stem cell (ESC), and induced pluripotent stem cell (iPSC)] culture systems that mimic the specific structural and functional characteristics of a native organ [23,24]. Recently, transplantable organoids have emerged as materials with the potential to replace and regenerate tissue or organs [23,25,26]. In modern medicine, it is possible to replace damaged or dysfunctional tissue with healthy tissue through homologous transplantation. Therefore, other types of tissue sources are required because of the limitations of providing healthy tissues and immune rejection response challenges. Organoids generated using stem cells derived from the patient tissue enable the formation of an isogenic tissue having identical genes. Specifically, in case of the pluripotent stem cell, utilizing the iPSCs derived from easily accessible human biological samples (e.g., skin tissue, blood, and urine) facilitates mass organoid production without the ethical issues surrounding human ESCs [27]. Thus, the development and production of organoids may result in improved replacements and regeneration tools for advanced therapeutic applications.

In this review, we focus on the recently advanced therapeutic applications of stem cells, spheroids, and organoids in 3D culture systems using engineering tools to improve tissue repair and regeneration. First, we introduce the timeline and historical origin of stem cell, spheroid, and organoid technology. We subsequently describe the therapeutic applications of stem cells in various tissues. We introduce and describe commonly used formation methods, engineering techniques, and therapeutic applications of stem cell spheroids for tissue repair and regeneration. We describe the recent advances in technology to guide matured organoids and therapeutic applications for tissue repair and regeneration. Finally, we discuss the future opportunities, challenges, and perspectives regarding 3D stem cell culture systems, including spheroids and organoids, for improving therapeutic applications.

2. Historical evolution of 3D cell culture systems

Although it has only been a few years since the full-scale development of 3D cell culture systems, 3D cell culture has a history of about 100 years (Fig. 1). In 1907, Wilson et al. described the first attempt of reorganization of an organism by demonstrating the ability of dissociated sponge cells to self-organize a whole organism [28]. That same year, the hanging drop method was established by Ross Harrison et al., while exploring ways to culture and maintain frog embryo nerve fibers *in vitro* [29]. Dissociation-reaggregation experiments were conducted by Holtfreter et al. with dissociated amphibian pronephros in 1944 [30] and by Weiss and Taylor with multiple organs from an embryonic chick in 1960 [31]. In 1971, multicellular spheroids with nearly perfect sphere-shaped aggregates were first established using Chinese hamster V79 lung cells grown in suspension in tissue culture [32]. In 1975, Rheinwald et al. showed that single cells of the keratinizing line grow into colonies, each consisting of a stratified squamous epithelium [33,34]. Martin et al. first reported the successful isolation and establishment of mouse ESCs, a pluripotent stem cell (PSC) line, from early mouse embryos in 1981, following which research related to stem cells accelerated [35]. Thomson et al. successfully isolated and cultured human ESCs derived from human blastocysts in 1998 [36]. The Yamanaka group first established iPSCs by reprogramming mouse and human fibroblasts, significantly influencing stem cell and organoid research from 2006 to 2007 [37,38]. In 2008, Eiraku et al. generated self-organized and polarized 3D cerebral cortical tissue from mouse and human embryonic stem cells [39]. In 2009, Sato et al. reported that 3D intestinal organoids formed by adult mouse intestinal stem cells isolated from primary intestinal tissue [40]. This impactful study is the first report on establishing 3D organoid culture derived from stem cells. After reporting this study [40], many researchers actively attempted to develop various organoids, such as those of the intestine (2011), stomach (2010, 2014), liver (2013), inner ear (2013), pancreas (2013, 2015), lung (2014, 2015), kidney (2013), thyroid (2015), brain (2013), retina (2012), breast (2016), taste bud (2014), heart (2018), blood vessel (2019), and skin derived from ASCs and PSCs [41–60]. The methods of developing organoids are well-established, and currently, efforts are being devoted to developing mature organoid systems. Recently, microengineering and nanoengineering techniques have attracted great attention. In the near future, applying microengineering- and nanoengineering-based platforms, such as particles, fibers, structural cues, fluidic chips, and 3D bioprinting, at the micro- and nanoscale may facilitate increased production, improved reproducibility, and development of highly matured organoid systems. Fig. 1 summarizes the various organoids formed by various stem cells and advances in microengineering and nanoengineering of 3D cell culture systems.

3. 2D-cultured stem cell for tissue repair and regeneration

Transplantation of stem cells can be utilized for the treatment of diseases, as well as the regeneration of tissues and organs, instead of using complex surgical procedures or tissue/organ transplantation [61]. Stem cells have several unique properties and capabilities, including continuous high self-renewal activity, multi-lineage differentiation capacity, re-population of a host, and secretion of reparative paracrine factors, making them an ideal cell source for cell-based therapy, regenerative medicine, and tissue engineering [12,62]. In recent decades, tissues engineered using a variety of stem cells have been applied to epithelial surfaces (e.g., skin, cornea, and mucosal membranes) and hard tissues (e.g., bone, tooth enamel, dentin, and cementum) [12]. Because of their limitless therapeutic potential, stem cells continue to receive tremendous public, scientific, and clinical interest. In this section, we provide an overview of a range of stem cell-based therapeutic applications for tissue regeneration and repair that are applied as the transplantation of only stem cells with controlled functions or engineered scaffolds combined with stem cells.

Various engineering approaches are used to provide the environment and conditions to improve adhesion, proliferation, differentiation, migration, and paracrine factor secretion of stem cells used in the regeneration of various tissues (e.g., bone, cartilage, tendon, muscle, nerve, tooth, and skin). Microengineered and nanoengineered platforms and biomaterials have been utilized to modulate the behaviors of stem cells and enhance their function by providing them with specific microenvironments (Fig. 1 and Table 1) [63–65]. For example, micro- and nano-scale structured materials and hydrogel matrix have been proposed as effective controllers for the modulation of cellular behaviors and functions [66–68].

3.1. Bone tissue regeneration

Bone regeneration is one of the major targets of stem cell-based therapy in orthopedic fields. The restoration of extensive bone loss and defects remains an unresolved challenge in modern medicine [69]. Clark et al. synthesized poly(ethylene glycol) (PEG) hydrogels based on 4-arm PEG macromers with terminal maleimide groups (PEG-4MAL) presenting GFOGER, a peptide targeting the α2β1 integrin (Fig. 2a) [70]. Human bone marrow-derived stem cells (BMSCs) encapsulated in PEG-4MAL hydrogels presenting GFOGER showed enhanced cell adhesion, paracrine secretions, and osteoblastic differentiation. Compared with the effects of other combined peptides in murine bone defects, the hydrogels promoted increased survival, retention, and osteo-reparative function of encapsulated BMSCs. Regarding bone tissue regeneration, the hydrogels improved the inflammation inhibition, vascularization, gene expression, and bone formation *in vivo*. Synthetic hydrogel systems

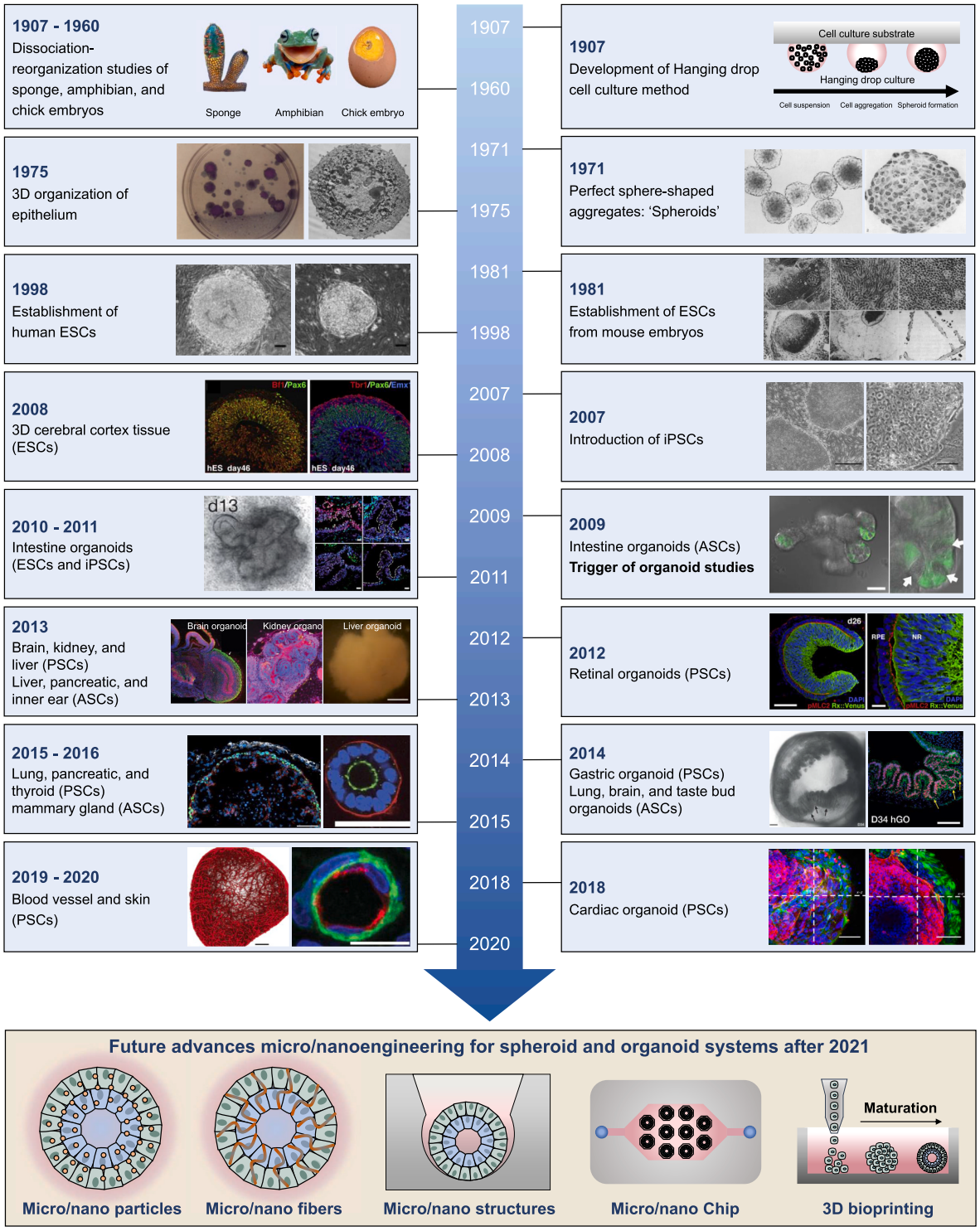

Fig. 1. Timeline of historical advancements and future advances in 3D cell culture systems. A summary of representative studies and breakthroughs leading to the establishment of various 3D culture systems has been presented. Reproduced with permission from Ref. [28–60]. Reorganization of dissociated sponge cells, amphibian pronephros, and multiple organs from an embryonic chick was first conducted. Reproduced with permission from Ref. [28–30]. The hanging drop method was established. Reproduced with permission from Ref. [29]. Perfect sphere-shaped aggregates were first established. Reproduced with permission from Ref. [32]. 3D organization of epithelium was conducted. Reproduced with permission from Ref. [33,34]. ESCs from mouse embryos were established. Reproduced with permission from Ref. [35]. Human ESCs were isolated and cultured. Reproduced with permission from Ref. [36]. Human and mouse iPSC were first established. Reproduced with permission from Ref. [37,38]. 3D intestinal organoids were formed by mouse intestinal stem cells. Reproduced with permission from Ref. [40]. Many researchers actively attempted to develop various organoids, such as intestine (2011), stomach (2010, 2014), liver (2013), inner ear (2013), pancreas (2013, 2015), lung (2014, 2015), kidney (2013), thyroid (2015), brain (2013), retina (2012), breast (2016), taste bud (2014), heart (2018), blood vessel (2019), and skin derived from ASCs and PSCs. Reproduced with permission from Ref. [41–60]. In the near future, applying microengineering- and nanoengineering-based platforms may facilitate increased production, improved reproducibility, and development of highly matured organoid systems.

Table 1
Stem cell therapy for tissue repair and regeneration.

Target tissue	Cell type (cell source)	Transplantation method	Material	Outcomes	Ref.
Bone	BMSC (rat)	Collagen sponge vehicle loaded with BMSCs	Treatment with N-acetyl-L-cysteine	Enhanced new bone formation via treatment with N-acetyl-L-cysteine after 5 weeks	[73]
	BMSC (human)	Integrin-specific hMSC-encapsulated hydrogel	4-arm PEG macromers with terminal maleimide groups (PEG-4MAL) and GFOGER peptides	Enhanced inflammation inhibition, vascularization, and bone formation	[70]
	DPSC (human)	Transplantation of nanospike array loaded with DPSCs	PEGDMA nanospike	Significantly promoted the regeneration of cranial bone defect	[63]
	Periosteal stem cell (mouse)	Implantation of periosteal stem cells in Matrigel	Matrigel	Enhanced periosteal bone formation and normal cortical architecture	[74]
	BMSC (murine)	Injection of microporous hydrogel loaded with BMSCs	GelMA hydrogel	Bone tissue volume/total tissue volume >30%, bone mineral density >500 mg/cc, trabecular thickness >200 μm, and trabecular separation/spacing <0.5 mm	[72]
Cartilage	BMSC (rabbit)	Injection of thermosensitive hydrogel loaded with BMSCs	Copolymers of PA-PEG-PA and PAF-PEG-PAF	Transparent tissue filling with smooth and consecutive surface ICRS macroscopic score: 9.19 Histological score: 9.92	[80]
	ADMSC (human)	Injection of ADMSC-loaded microbot with electromagnetic field control	PLGAMicroscaffold with ferumoxytol and chitosan	Low expression of proinflammatory genes and significant increase in COLII expression	[246]
	MSC (human)	Injection of MSCs encapsulated in hydrogel	Hyper-branched polyPEGDA and thiolated hyaluronic acid (HA)	ICRS macroscopic score: 12.68 ± 2.11 Reduced defect area: 0.148 ± 0.074 mm^2 Total histological score: 20.05 ± 1.73	[81]
	ASC (rabbit)	Implantation of cartilage extracellular matrix (ECM)-derived particles loaded with ASCs	Decellularized porcine knee articular cartilage	High ICRS macroscopic score, contact stiffness, reduced modulus, histological score, and bone volume	[82]
Tendon	BMSC (dog)	Engineered tendon-fibrocartilage-bone composite and BMSC cell sheet	Decellularized canine patellar tendons	Improved collagen fiber organization and increased new fibrocartilage formation	[86]
	BMSC (rat)	Aligned collagen fiber scaffold loaded with BMSCs	Collagen	Improved scoring, thickness, and weight of tendon; normal Achilles functional index; high quality of repair, as per histological score	[88]
	Tendon stem cell (TSC) (rat)	Implantation of scaffold loaded with TSCs	Biomimetic parallel-aligned collagen scaffold	Compact regeneration; smooth structure; more distributed structure; massive, spindle-shaped, tenocyte-like cells; and aligned collagen fibrous structure	[65]
	BMSC (rabbit)	Extracellular matrix scaffold and BMSC sheet	Decellularized bone-fibrocartilage-tendon tissue of rabbit	Bone tissue volume/total tissue volume >50%, trabecular thickness >35 μm, trabecular number <15/mm, high histological score, high failure load, and high stiffness	[87]
Muscle	Muscle stem cells (MuSCs)	Transplantation of + decellularized muscle tissue 3D scaffold loaded with MuSCs	3D scaffold (decellularized muscle tissue)	Bioconstruct made with human MuSCs and MRCs can generate functional muscle tissue in VML model.	[90]
	iPSC-CM (human), MSC (human)	hiPSCs-CMs injected intramyocardially, and implantation of 3D-printed scaffold loaded with MSCs	PCL and porcine heart-derived decellularized extracellular matrix bioink	Improved cardiac function and capillary density, and reduced scar formation	[91]
Nerve	Neural crest stem cell (NCSC) (human)	Injection of NCSCs	Electrical stimulation	Promoted axon regeneration and myelination	[95]
	ASC (rat)	Implantation of ASC sheets	–	Improved the functional recovery, improved reinnervation, and prevented atrophy	[94]
	Periodontal ligament stem cell (PDLSC), gingival mesenchymal stem cell (GMSC)	Implantation of stem cell-encapsulated hydrogel	Alginate and hyaluronic acid hydrogel	Higher expression levels of neurogenic-related genes, higher cell densities, and greater number of cell colonies	[96]
	iPSC derived NSC (murine)	Implantation of stem cell encapsulated- hydrogel	GelMA hydrogels	Reduced cavity areas, lesser collagen deposition, decreased inflammation, and promoted axonal regeneration	[97]
Tooth	DPSC	Implantation of tooth slice and scaffolds loaded with DPSCs	Human DPSCs culture conditions containing human serum (DPSCs-HS)	DPSCs-HS produced a robust angiogenic response and regeneration of dentin equivalent to DPSCs-FBS.	[101]
	DPSC	Implantation of human tooth root canal with DPSC constructs	Scaffold-free 3D cell constructs composed of DPSCs	Pulp-like tissues with rich blood vessels were formed within the human root canal 6 weeks after implantation	[102]
	Deciduous pulp stem cell (human)	Implantation of hDPSCs	–	Increased the length of the root, reduced the width of the apical foramen, and regeneration of dental pulp tissue containing sensory nerves (human patients)	[103]
	DPSC (human)	Implantation of injectable hydrogel encapsulating hDPSCs	Alginate and laponite hydrogel microspheres	Regeneration of rich microvessels and neotissue	[100]
Skin	Gingival MSC (human)	Transplantation of 3D-printed scaffolds	Medical grade polycaprolactone	Least contraction, least scar area, accelerated wound closure, and most differentiated epithelium	[106]

(*continued on next page*)

Table 1 (*continued*)

Target tissue	Cell type (cell source)	Transplantation method	Material	Outcomes	Ref.
	ASC (human and mouse)	Injection of hydrogel encapsulating ASCs	Hyperbranched PEGDA and thiolated gelatin-based hydrogel	Accelerated chronic wound closure, enhanced neovascularization, and reduced inflammation in diabetic wound model	[105]
	ASC (rat)	Injection of hydrogel encapsulating ADSCs	Hyperbranched multi-acrylated PEG macromers and thiolated hyaluronic acid	Inhibition of inflammation, promotion of angiogenesis, and re-epithelialization in diabetic wound model	[107]
	MSC (human)	Transplantation of pre-vascularized hMSC cell sheets	–	Smallest contraction, best preservation of skin appendages, highest number and area of microvessels, lowest inflammatory reactions, and a morphology that more closely resembles normal skin	[108]
	ASC (human)	Transplantation of ASC sheets	–	Enhanced immunomodulatory and antifibrotic capabilities, and reduced scar formation	[109]

are potentially suitable as *in vivo* stem cell carriers because of their biocompatibility, injectability, and versatility in presenting bioactive functionalities [70,71]. To improve the bone regeneration capacity, a system using gelatin methacrylamide (GelMA) hydrogel with porous microspheres, BMSCs preconditioned by N-acetyl-L cysteine, a poly (ethylene glycol) dimethacrylate (PEGDMA) polymer-based hydrogel nanopillar array, and periosteal stem cells was developed and investigated as an engineered stem cell carrier and stem cell therapy tool [63,72–74]. In addition, it has been reported that not only simple nanoscale structures, but also nanostructural scaffolds combined with various nanocomposites significantly promote the stem cell differentiation and the bone regeneration [75–77]. Although stem cell and engineered biomaterials have great potential for the treatment of bone loss and defects, maintaining therapeutic efficacy by delivering the microenvironment with stem cells currently remains a major challenge [69,78].

3.2. Cartilage tissue regeneration

Cartilage tissue is difficult to self-repair after injury due to its avascular structure and low metabolic activity [79]. Stem cells with exogenous biochemical or mechanical stimulations, and the engineered scaffolds in stem cell-based therapies, have demonstrated significant advances in cartilage regeneration. Go et al. developed a magnetic microrobot system (size: 357.55 ± 18.57 μm) with 3D porous structure (size: 43.85 ± 13.39 μm) by combining a poly(L-glutamic acid) (PLGA) microscaffold, ferumoxytol, and chitosan to achieve targeted stem cell delivery for knee cartilage regeneration (Fig. 2b) [64]. Human adipose-derived mesenchymal stem cells (ADMSCs) were loaded into the microbot systems after incubating for 24 h. Most ADMSC-loaded microbots were delivered and positioned to the targeted defect area of the rabbit knee cartilage by controlling the electromagnetic actuation system. The expression of COLII was significantly increased after injection of the hADMSC-loaded microrobot compared with the non-injected group, at 2 and 3 weeks after injection. Recently, engineering approaches for cartilage regeneration have been widely studied, including local delivery platforms for stem cells such as thermosensitive polypeptide poly(L-alanine-co-l-phenylalanine)-block-poly(ethylene glycol)-block-poly(L-alanine-co-*l*-phenylalanine) hydrogels, hyper-branched poly(ethylene glycol) diacrylate (PEGDA)/hyaluronic acid (HA) hydrogel, and decellularized porcine knee articular cartilage ECM-derived particles [80–82]. Although the cartilage therapeutic effect of stem cell-based scaffolds has shown good efficacy in repairing tissues, approaches that improve angiogenesis, anti-inflammatory, tissue formation potential, and increase cell viability when compared to individual cells are still needed.

3.3. Tendon and muscle tissue regeneration

Tendon injuries are treated by the conservative treatment which aims to relieve pain and surgical repair [83,84]. Stem cell-based therapies provides great promise for tendon regeneration due to their high proliferative, synthetic and immunomodulatory activities, as well as their potential to differentiate into target cell types [85]. Liu et al. reported combining novel biomaterials with decellularized canine patellar tendon ECM including tendon-to-bone interfaces and BMSC sheets for tendon-to-bone healing of the rotator cuff (Fig. 2c) [86]. The composite of BMSC sheets and decellularized tendon ECM implanted in canine non-weight-bearing models promoted rotator cuff healing, as demonstrated by anatomic structure, collagen organization, and biomechanical strength [86]. Recently, decellularization engineering has offered a more natural approach (i.e., using tissue-derived ECM) to mimic the morphology and composition of bone-tendon inserts. Tang et al. developed decellularized rabbit bone-fibrocartilage-tendon scaffolds combined with autologous BMSC sheets for tendon-to-bone healing by regenerating a robust fibrocartilage layer and collagen fibers, arranged in the direction of traction [87]. For functional regeneration and repair of tendons, biomimetic scaffolds, such as parallel-aligned collagen fiber scaffolds loaded with recombinant periostin and collagen fiber membranes of various orientations (established using counter-rotating extrusion), have been used [65,88,89]. Quarta et al. suggested a 3D bioconstruct scaffold composed of decellularized tibialis anterior muscle tissue cultured with muscle stem cells and other muscle resident cells using a perfusing bioreactor to improve the treatment of volumetric muscle loss injury [90]. Park et al. developed porcine heart-derived decellularized ECM bioink for fabricating 3D-printed scaffolds that allowed seeding of human mesenchymal stem cells (MSCs). The authors proposed a concomitant method that exploits the advantages of cardiomyocytes derived from human iPSCs and human MSC-loaded scaffolds to improve cardiac repair in a rat myocardial infarction model [91]. Biomimetic scaffolds combined with stem cells provide enhanced muscle tissue of the largely defected esophagus [89]. Despite tremendous efforts and advances in tendon regeneration, it presents clinical difficulties because it does not respond well to degenerative tendon treatment and requires long-term rehabilitation [92].

3.4. Nerve tissue regeneration

Nerve damage can cause great morbidity in people who suffer from loss of sensation, loss of movement, chronic pain, or a combination of deficits [93]. Hsu et al. proposed the use of stem cell sheets comprising adipose-derived stem cells (ADSCs) generated by constructing a Cre/loxP-based hybrid baculovirus vector, which enabled intracellular formation of an episomal DNA minicircle for effective transduction of cells and prolonged expression of functional glial cell line-derived neurotrophic factor capable of recruiting Schwann cells (Fig. 2d) [94]. Implantation of the hybrid baculovirus-engineered stem cell sheets significantly improved nerve repair, as indicated by the enhanced functional recovery, nerve reinnervation, electrophysiological functionality, Schwann cell proliferation and infiltration, axon regeneration, myelination, and angiogenesis [94]. In addition, strategies for functional

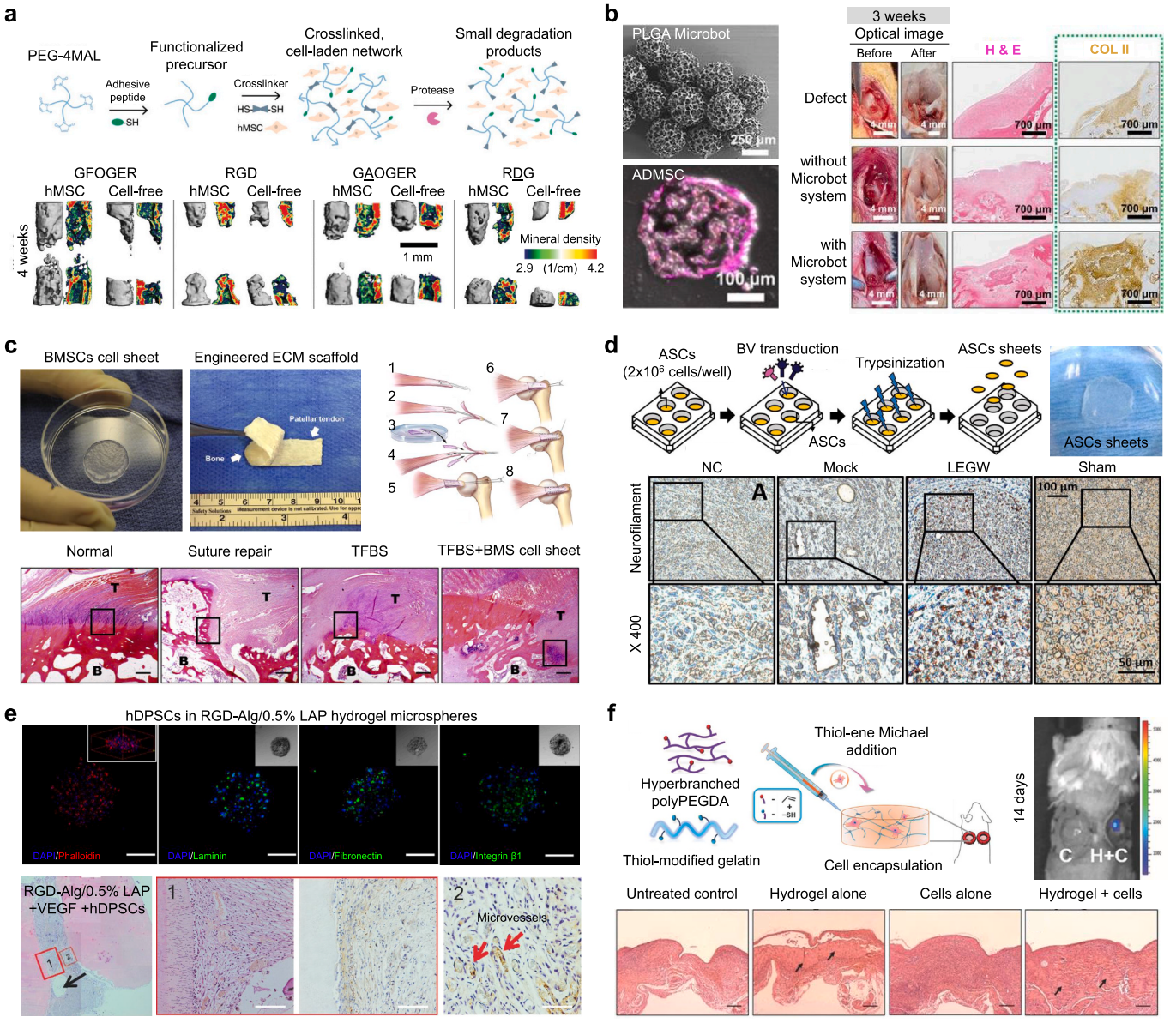

Fig. 2. Stem cell therapies for regeneration and repair of various tissues. **(a)** Bone tissue regeneration and repair using hBMSC-encapsulated 4-arm poly (ethylene glycol) (PEG) maleimide hydrogels. hMSC in GFOGER-conjugated hydrogels showed higher levels of new bone formation (live animal μCT). Reproduced with permission from Ref. [70]. **(b)** Cartilage tissue regeneration and repair using human adipose-derived mesenchymal stem cell (hADMSC)-loaded magnetic PLGA microrobot systems (SEM image of microbot system and Confocal images of hADMSC-microrobot). Microrobot system improved the cartilage regeneration by enhancing the delivery of the hADMSC-microrobot (optical images, H&E staining, and Collagen type II staining). Reproduced with permission from Ref. [64]. **(c)** Tendon tissue regeneration and repair using decellularized tendon extracellular matrix (ECM) combined with BMSCs cell sheet (images of the cell sheet, dECM scaffold, and Surgical procedure). These scaffolds demonstrated well-orientated collagen, fewer inflammatory cells and, spindle-shaped nuclei (H&E staining). Reproduced with permission from Ref. [86]. **(d)** Nerve tissue regeneration and repair using baculovirus vector -transduced adipose-derived stem cell (ADSC) sheets (ADSCs sheet image). Neurofilament protein was significantly deposited. Reproduced with permission from Ref. [94]. **(e)** Dental tissue regeneration and repair using the injectable hybrid RGD-alginate/laponite hydrogel microspheres by encapsulating human dental pulp stem cells (hDPSCs) (confocal images). The hydrogel improved neotissue regeneration (H&E, DMP-1, and CD31 staining). Reproduced with permission from Ref. [100]. **(f)** Skin tissue regeneration and repair using the ADSC-encapsulated injectable PEGDA/thiolated gelatin hydrogel. ADSC-encapsulated hydrogel promoted the host cell filtration and granulation tissue remodeling (H&E staining). Reproduced with permission from Ref. [105].

nerve regeneration using human neural crest stem cell differentiation controlled by electrical stimulation platforms, human periodontal ligament- and gingiva-derived mesenchymal stem cells encapsulated in alginate/hyaluronic acid hydrogels, and iPSC-derived neural stem cells encapsulated in GelMA hydrogels have been reported [95–97]. However, three-dimensional nerve regeneration still requires therapies that more accurately mimic the structure of autologous nerve grafts and efficiently form blood vessels to maintain the viability of transplanted cells [98].

3.5. Dental tissue regeneration

The restoration of tooth defect and loss are treated by replacing the tissues with biocompatible materials such as metal, ceramic, resin, and titanium implant, but these materials have limitations for perfectly reproducing the functions of teeth [99]. Zhang et al. developed injectable hybrid RGD-alginate/laponite hydrogel microspheres to co-encapsulate human dental pulp stem cells (DPSCs) and vascular endothelial growth factor (VEGF) (Fig. 2e) [100]. After subcutaneous

implantation with tooth slices in a mouse model for 1 month, the human DPSC-laden RGD-alginate/laponite + VEGF microspheres significantly promoted the regeneration of pulp-like tissues, in addition to the formation of new microvessels [100]. To regenerate dentin and dental pulp tissue, some studies demonstrated the potential of DPSCs isolated and expanded in human serum, DPSC constructs, and autologous human deciduous pulp stem cells [101–103]. Since dental tissue is exposed to significant blood flow, fluid movement, and physical stimuli, it is necessary to effectively implant stem cells into the treatment site while immobilizing them for vascular regeneration and tissue repair [104].

3.6. Skin tissue regeneration

Skin tissue-related diseases are the easiest and most frequent diseases in the human body. Dong et al. developed an injectable PEG–gelatin hydrogel with highly tunable properties from a multifunctional PEG-based hyperbranched polymer and a commercially available thiolated gelatin for encapsulating murine ADSCs (Fig. 2f) [105]. The proposed *in situ*-formed hydrogel significantly improved cell retention, enhanced angiogenesis, and accelerated wound closure in a murine wound healing model. The engineered hydrogels, 3D-printed scaffolds, and stem cell sheets were utilized for efficient wound healing [106–109]. The future challenges for stem cell-based skin tissue regeneration are rapid healing, scar-free and comprehensive regeneration of skin appendages including hair follicles and sweat glands [110].

Currently, the most commonly proposed stem cell therapy in clinical trials is performing *in vitro* expansion of a sufficient number of autologous stem cells isolated from the patient and then injecting them into the target site [61]. However, the stem cells transplanted into the target site have critical limitations, such as low efficiency (i.e., cells can be easily washed out of the target tissue) and failure to maintain viability and function (e.g., self-renewal, multi-lineage differentiation, recruitment, and paracrine secretion) in the host tissue. This has resulted in limited success in restoring damaged tissues and organs, especially in large-scale tissue repair or regeneration. Therefore, effective delivery systems that regulate the survival, behavior, and function of stem cells are required to efficiently transplant stem cells to the target tissue sites. Recently, many studies demonstrated that the 3D-cultured stem cells enhanced viability, differentiation, paracrine secretion, and tissue regeneration compared to 2D-cultured stem cells [111–117]. Cardiac stem cell spheroids promoted the secretion of growth factors and the expression of cardiomyocyte-specific markers [112]. The spheroids improved cell engraftment and survival within the myocardium and enhanced neovascularization and myocardial regeneration, resulting in myocardial infarction recovery compared to single cell. Therefore, 3D-cultured stem cells can be good alternatives to stem cell transplantation due to more realistic biochemical and biomechanical microenvironments compared to 2D-cultured stem cells.

4. 3D cell culture for stem cell spheroids and organoids

Spheroids formed by aggregating stem cells are the result of the self-assembly behaviors of single cells in suspension due to embryogenesis, morphogenesis, and organogenesis. The formation of spheroids involves complex homogeneous and heterogeneous binding of cell adhesion molecules, ECM proteins, and integrins [118]. Stem cell spheroid assembly occurs in multiple steps [8]. First, single cells are drawn closer to form loosely adhesive cell spheroids because ECM fibers and complementary binding of the peripheral cell surface to integrins encourages preliminary aggregation. Next, cadherin on the cell membrane surface induces tight connections between the aggregated cells because of the homophilic cadherin binding of peripheral cells. Finally, early cell assemblies formed by both pathways generate contractile forces via rearrangement of actin stress fibers, leading to compression and the formation of mature spheroids. After these processes, strong, compact multicellular spheroids are formed. 3D cell culture systems can be classified into three major organization and structural types of spheroids, multicellular spheroids, and organoids depending on their complexity [119]. Spheroids are generally considered as 3D cell aggregates generated from a single cell type or various cell types but cannot completely mimic complex contacts with other cell types [120]. Stem cell-derived organoids have a similar phenotype to *in vivo*, which has higher tissue complexity than spheroids. Although the differences between spheroids and organoids are vaguely defined and inconsistent among researchers, stem cell spheroids are usually close to the meaning of simple cell aggregates and can be utilized in a series of processes for developing organoids from stem cells [121,122]. Especially, whereas internal developmental processes drive organoid formation, spheroids develop primarily through cell-to-cell adhesion. Stem cell-based organoids can be formed by providing the appropriate physical and biochemical signal for differentiation and development of organ-like phenotype or embryoid body after the formation of stem cell-based aggregates and spheroids.

In the following contents, we reviewed the engineering techniques and therapeutic applications for tissue regeneration and organ repair of stem cell spheroids and organoids. Engineered platforms for stem cell spheroid culture require providing the environments that promote aggregation of stem cells and for cellular self-organization of the aggregated cells. Furthermore, it is important to high-throughput fabrication of spheroids with defined size and composition. Stem cell spheroids formed by engineering tools could maintain the stemness properties of stem cells and enhance their multilineage differentiation capacities. Organoids differ from spheroids in that they must have the characteristics of a fully differentiated organ. Therefore, the organoid culture platforms should provide the environments that promote and regulate the differentiation of stem cells to produce mature organoids like native organs. Spheroids produced under optimal culture conditions should promote regeneration by improving the proper differentiation of stem cells and paracrine effects when transplanted into tissues or organs. Otherwise, mature organoids can be inserted into a part of an organ to replace organ functions and be applied to tissue regeneration. Spheroids are mainly applied to tissue regeneration, and organoids are mainly applied to organ function recovery.

5. Engineering techniques for formation of stem cell spheroids

The generation of stem cell spheroids is essentially based on the common principle of self-assembly. The process of cell self-organization occurs within the test tube if the cells cannot attach to the substrate surface, and thus, must interact with each other [10]. The tight connection, cell-cell communication, and cell-ECM communication within cell spheroids contributes to the control of stem cell behaviors and functions (e.g., viability, stimuli responsiveness, and protein secretion), leading to considerable differences compared to monolayer cell cultures [123]. In addition, the high-throughput nature of generating stem cell spheroids with uniform size and composition is an important factor in fabrication [6]. To maximize the functionality of stem cells and cell-cell and cell-ECM connections when culturing 3D spheroids, various platforms based on commonly used spheroid formation methods (e.g., hanging drop, well plate, spinner flask, hydrogel matrix, magnet, and microfluidic chip) have been developed. In this section, we introduce the stem cell spheroid formation methods and platforms that have been developed and improved upon (Table 2 and Fig. 3). 3D cell culture system can be classified into two main types of scaffold-based system (e.g., hydrogel matrix, well plate, and microfluidic chip) and scaffold-free system (e.g., hanging drop, rotation methods, and magnetic force). Since scaffold-free systems do not use biomaterials or ECM components to support cell-to-cell adhesion and migration, cell-to-cell connectivity must be considered. Scaffold systems should consider the composition of various biological and synthetic materials with different porosity, permeability, surface chemistry, and stiffness to mimic the microenvironment of a specific tissue to promote

Table 2
Engineering techniques for stem cell spheroid formation.

Formation method	Culture platform	Platform materials	Structure	Features	Stem cell(Derived species)	Ref.
Hanging drop	Lid of a Petri dish	PolystyreneSolution coating	Square wettable surface	Superhydrophobic patternwith wettable regions	Adipose-derived stem cells (human)	[126]
	Automated microfluidic device	Water white glass substrates	Through-hole and well	2.4-mm wells	BMSCs (mouse)	[127]
	Pressure-assisted network for droplet accumulation system	Polycarbonatesheet, polyethylene terephthalate	Through-hole well plate	Balanced control of the internal and surrounding pressure	MSCs	[128]
	Bio-inspired superhydrophobic substrate	Silicon (Si)	Vertically aligned nanowires	Length: 25 μm	ADSCs (Human)	[17]
	Cell printer	–	–	2 nL or fewer than five cells	ESCs	[129]
Hydrogel scaffold	Sponge-like hydrogel	Gellan gum, silk fibroin	Micropore structures	Porosity: 90 ± 0.7% Pore wall thickness: 6.6 μm	ADSCs (human)	[150]
	Sandpaper-embossed microstructure	Tetronic-tyramine	Microstructure	Surface roughness from 100 to 200 μm	ADSCs (human)	[148]
	Porous microstructure	Poly(L-glutamic acid), polyethylene glycol	Porous microstructure	Pore size: 275–375 μm	ADSCs (human)	[149]
	3D bioprinted matrix	Chitosan methacrylate and polyvinyl alcohol hybrid microparticle ink	Biomimetic microstructures	Scaffold pore size: 250 μm Particle size: 197 μm	BMSCs (rat)	[147]
Microwells	Microstructure	Tet-TA polymer	lotus seedpod-inspired microwell	200 or 400 μm in width with various depths	ADSCs (human)	[133]
	Microstructure	Alginate	Hexagonal well array	100-, 200-, and 400-μm wells	MSCs (mouse)	[134]
	Microstructure	PEG hydrogel	Inverted-pyramidal opening well	Diameter: 200 μm	MSCs (human)	[135]
	Microstructure	Agarose	Round-bottom microwells	Diameter: 2 mm	iPSCs (human) ESCs (human)	[132]
	Microstructure	Collagen, Matrigel	Micro-honeycomb structures	100- and 300-μm holes	ESCs (mouse)	[20]
Spiner and rotational methods	Multi-trap acoustic levitation	PDMS, ultrasonic transducer	Cylindrical cavity	Diameter: 5 mm Height: 10 mm	ADSCs (human)	[139]
	Pellet culture with nanofiber	Poly(L-lactic acid) (PLLA)	Mineralized fragmented nanofiber	Diameter: 1–2.5 μm Length: 60 μm	ADSCs (human)	[247]
	Pellet culture with nanofiber	Poly(L-lactic acid) (PLLA)	Fragmented nanofiber	Diameter: 400–800 nm Length: 50–100 μm	MSCs (human)	[248]
	Pellet culture with nanofiber	Poly(L-lactic acid) (PLLA), platelet-derived growth factor (PDGF), bio-mineral	Fragmented nanofiber	Diameter: 5.4 μm Length: 100–150 μm	ADSCs (human)	[249]
Microfluidics	Hydrogel encapsulation	Poly(vinyl alcohol) (PVA)	Microcapsule	Smaller than 200 μm	BMSCs (human)	[162]
	Droplet-microfluidic platform, micro anchors hole	Agarose	Encapsulation	Diameter: 680 μm	MSCs (human)	[18]
Magnetic field	Magnetic nanoparticles	Iron oxide core (Fe_3O_4) coated in polydimethylamine	Nanoparticle	Diameter: 200 nm	BMSCs (human)	[15]
	Magnetic nanoparticles	Iron salts	Nanoparticle	Diameter: 8 nm	ESCs (mouse)	[19]
	Magnetic nanoparticles	Gold and iron oxide nanoparticles crosslinked by poly-L-lysine	Nanoparticle	Size: 50 nm	Neural crest-derived mesenchymal stem cells (human) DPSCs (human)	[157]

cell growth and migration.

5.1. Hanging drop methods

The hanging drop method is a well-established method commonly used for the formation of 3D cell spheroids that utilizes gravitational force to induce cellular assembly. Single cells are aggregated at the tip of small to medium droplets that are hung on the cell culture substrate using the hydrophilic and surface tension properties of the liquid. Hanging drop methods have several advantages including simplicity, consistency, lack of matrix requirements, scalability for high throughput production, and the ability to produce spheroids in a small number of cells [124]. Due to advances in liquid automated handling systems and robotics, the combination of automated systems and hanging drop platforms enables the simultaneous fabrication of large numbers of 3D cellular constructs [125]. However, the hanging drop method has some limitations in replacing cell culture medium without any negative effects on spheroids and using large droplets of the medium. In addition, the hanging drop methods combined with automated systems have disadvantages in that it requires high cost and complex processes. Based on these characteristics, bio-inspired superhydrophobic surfaces having vertically aligned silicon nanowires coated with a palladium layer using sputter deposition have been developed via aqueous electroless etching of silicon (Fig. 3a) [17]. The developed superhydrophobic surfaces have an extremely large water contact angle (>150°) in both ambient air and hydrogen, enabling the control of the size of hADSC spheroids. Compared to spheroids generated using spinner flask suspension culture and hanging drop culture on the lid of a petri dish, the spheroids fabricated on the developed superhydrophobic surfaces exhibited a more uniform size distribution. Utilizing a patterned superhydrophobic surface with polystyrene with wettable regions has been proposed for the production and culture of hADSC spheroids [126]. A hanging drop culture system for producing stem cell spheroids was engineered using a digital microfluidic device, coated with indium tin oxide, parylene-C, and Cytop, having an automated liquid-handling system that facilitated *in situ* cell spheroid culture [127]. The pressure-assisted network for droplet accumulation systems was proposed to form a consistent and uniform hanging drop array for quick and increased production of

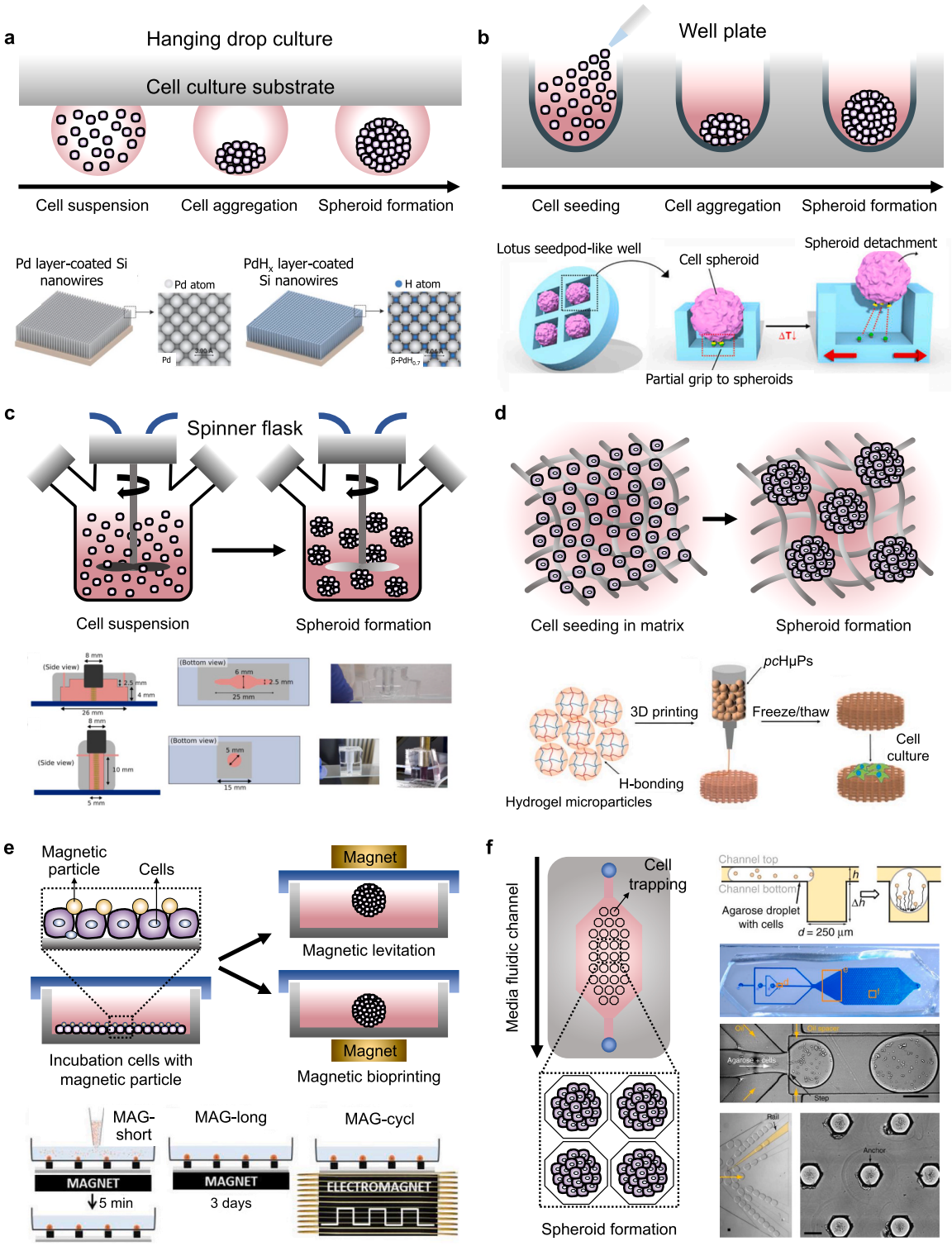

Fig. 3. Commonly used formation methods and advanced engineering formation methods for stem cell spheroids. **(a)** Representative schematic of hanging drop culture method and engineered hanging drop methods using bio-inspired superhydrophobic surfaces with palladium layer onto vertically aligned silicon nanowires. Reproduced with permission from Ref. [17]. **(b)** Representative schematic of microwell plate formation method and engineered microwell plate using lotus seedpod-structured Tet-TA polymer hydrogel coated with fibronectin. Reproduced with permission from Ref. [133]. **(c)** Representative schematic of spinner flask levitation culture method and engineered levitation formation method using multi-trap acoustic levitation platforms using a PDMS chip and an ultrasound transducer. Reproduced with permission from Ref. [139]. **(d)** Representative schematic of hydrogel matrix formation method and engineered hydrogel matrix formation method using the 3D-printed matrix of chitosan methacrylate and polyvinyl alcohol hybrid hydrogel microparticles. Reproduced with permission from Ref. [147]. **(e)** Representative schematic of magnetic force-derived formation method and engineered magnetic-based formation method using microfabricated magnetic patterns and magnetic nanoparticles. Reproduced with permission from Ref. [19]. **(f)** Representative schematic of microfluidic chip-based formation method and engineered method using a droplet-trapping microfluidic chip. Reproduced with permission from Ref. [18].

human umbilical cord blood-derived MSC spheroids [128]. The valve-based bioprinting process was reported to maintain hESC viability, be accurate enough to produce spheroids of uniform size, and allow printed cells to maintain their pluripotency [129].

5.2. Well plate methods

Well plates can be easily handled without complex processes for spheroid formation and can control the uniform size and cell composition compared to other spheroid formation methods [130]. The surface of commonly used low cell attachment plates has modified hydrophobicity to promote strong and fast aggregation of cells compared with traditional cell culture plates [131]. Regarding commercial low cell attachment well plates for stem cell aggregation, a lack of control over the homogeneity of the environmental factors to which individual cells are exposed makes them unsuitable for scalable mass production [132]. In addition, they can form large aggregates that adversely affect cell proliferation and differentiation and may lead to cell death due to the hindrance of mass transport. To address these issues, engineered well platforms with various microstructures have been proposed for efficiently controlling spheroid formation. Microwell platforms with microstructures fabricated by microengineering can be developed of various structures that can finely control the spheroid size and cell composition. Microwells, inspired by lotus seedpod-structured hydrogels with temperature-response and cell-interactivity, have been developed for culture and delivery of hADMSC spheroids (Fig. 3b) [133]. The bio-inspired square microwells spontaneously formed spheroids with high viability, and fibronectins conjugated to the hydrogel successfully gripped the spheroids, similar to the funiculus gripping seeds in the lotus seedpod. Dissolvable microwell scaffolds generated using alginate hydrogels were utilized to allow cells to form self-organized clusters and then be gently released, resulting in highly reproducible multicellular structures that can be produced on a large scale [134]. The PEG hydrogel-based microwell array, containing 1225 wells (200 μm in diameter) composed of cylindrical microwells with inverted-pyramidal openings, was fabricated to prevent cell loss during the mass-production of hMSC-spheroids [135]. Agarose hydrogel-based round-bottom microwells having low cell attachment, fabricated using Teflon stamps, were used to host the dissociated single-cell suspension of hiPSCs [132]. Shape-tunable, densely packed, soft micro-honeycomb structures created using natural ECM hydrogel (i.e., collagen and Matrigel) as the microwell array were developed to fabricate a 3D liver model consisting of compact cell spheroids [20].

5.3. Spiner and rotational methods

Levitation culture methods involve using stirring and rotating mechanisms to prevent cell adhesion onto the surface; continued agitation promotes spheroid formation in suspension [136]. The spinner spheroid culture method involves continuous mixing of a cell suspension in a spinner flask bioreactor vessel [137]. In rotation culture, reconstruction of microgravity is achieved via a constant circular rotation. In levitation culture, stem cell expression factors can be manipulated by altering the fluidic flow, mass, and rotation speed in the containers [10]. Especially, the spinner and rotation systems have the advantage of being able to produce a large amount of spheroid at the same time as the relatively uniform shape and can produce spheroids of various sizes by controlling rotational speed and cell concentration of the cell suspension. However, if the rotational speed of the rotating culture vessel becomes too fast, the shear force becomes stronger, which can affect the physiological response of the cell. In addition, the spinner and rotation systems have limitations in providing an appropriate environment for producing spheroids of a very uniform size or containing various types of cells [138]. Multi-trap acoustic levitation culture platforms, fabricated using polydimethylsiloxane (PDMS), combined with an ultrasonic transducer were used to generate a controlled cell culture environment (Fig. 3c) [139]. The acoustic forces that act on the suspended cells by clustering and trapping them in multiple disk-like layers in acoustic levitation are positioned at the different pressure nodes of a resonant cylindrical cavity. An hiPSC-specific suspension culture unit consisting of a fully monitored continuous stirred tank reactor system integrated into a custom-designed and fully automated incubator, primarily constructed using 3D-printed polylactic acid (PLA) filament, was developed [140].

5.4. Hydrogel matrix-based formation methods

Hydrogel is a complex network of physically or chemically cross-linked polymer molecules that expand in aqueous media [141]. Hydrogel matrices are designed with a wide range of compositions, biophysical properties, and biological functions in order to resemble various features of native ECM to induce ECM-cell interaction [141]. The hydrogel matrix can mimic the microenvironments of the native ECM which include ECM-like viscoelasticity, interstitial flow, high-water content, and diffusive transport characteristics [142]. Spheroids are formed in the 3D environment in which cells are embedded in a porous hydrogel that serves as unique properties of the native extracellular matrix [143]. The cells can migrate and proliferate within the hydrogels and specific proximity to adjacent cells can spontaneously form the multicellular aggregates to spherical culture. The single cells proliferated and incubated in the porous hydrogel matrix are formed to the relatively loose aggregates due to the action of the integrins of cells [144]. And then, the epithelial cadherin is expressed and accumulated, and the aggregates enter the delayed phase of compression arrest [145]. Consequently, the cell aggregates form the multicellular spheroids under the strong hemophilic interaction of epithelial cadherin by forming cadherin-cadherin binding. For these reasons, hydrogel matrices are frequently used in 3D culture environments that mimic the scaffolding provided by the ECM [24]. An engineered biomaterial-based hydrogel matrix for spheroid formation provides physical support for self-assembly of stem cells [146]. Various natural polymers [e.g., alginate, collagen, gelatin, fibrin, hyaluronic acid (HA), Matrigel, and chitosan] and synthetic polymers [e.g., PEG, poly(L-lactic acid) (PLLA), PLGA, and polyvinyl alcohol (PVA)] are commonly used for 3D stem cell spheroid generation because of their specific biophysical and cell-adhesive properties. Spheroids after cell aggregation are cultured on the hydrogel matrix or cells are mixed with the liquefied hydrogel and then embedded through gelation to form spheroids. Although the natural polymer contains the biological components of ECM from natural tissues *in vivo*, the natural polymer-based hydrogel matrix has weak mechanical properties, can be rapidly degraded, and can elicit an immune response. On the other hand, hydrogels fabricated using synthetic polymers can control their biochemical and mechanical properties relatively easily, thus improving the mimicry of ECM. The hydrogel matrix system has disadvantages in producing large amounts of spheroids, controlling the uniform size of spheroids, and scaling up. Self-healable and cross-linked hydrogel microparticles composed of chitosan methacrylate (CHMA) and PVA hybrid hydrogels were developed and used as bioink for fabricating a 3D bioprinted hydrogel matrix with high fidelity and biocompatibility (Fig. 3d) [147]. The 3D bioprinted hydrogel matrix facilitates the fabrication of constructs with precise structures, high aspect ratios, and shapes mimicking the blood vessel, ear, and bone. A hydrogel matrix with an embossed surface formed by a replica molding mimicking commercially available sandpaper surfaces was proposed as a platform for hADSC spheroid formation that can enable the rapid formation and culture of a large quantity of size-controllable stem cell spheroids [148]. A PLGA-based porous hydrogel matrix was developed and cross-linked with cystamine (which contains disulfide bonds) to form a porous hydrogel that could realize “gel–sol” transition via the reduction effect of glutathione [149]. A gellan gum and silk fibroin-based hydrogel matrix with sponge-like structure was created by regulating the blending

ratios of polymers; the resulting device acts as a scaffold for stem cell spheroid formation [150]. The thermoreversible hydrogels composed of PNIPAAm-PEG were utilized to confirm the improved expansion, differentiation, and 3D-directed differentiation of the human iPSC into multiple lineages [151]. This 3D thermoreversible hydrogel system demonstrated that the single cells in this 3D environment showed greatly increased viability, pluripotency, and uniform cell proliferation than cells cultured in 2D. In addition, the PNIPAAm-PEG hydrogel culture systems for fabricating hPSC spheroids showed a quick and efficient approach to achieve the rapid fusion capability of tissue spheroids [152]. Using this approach, the authors demonstrated that the PNIPAAm-PEG hydrogel induced a rapid fusion rate of PSC spheroids and matured differentiation of the cortical neural tissue spheroids.

5.5. Magnet-based formation methods

Magnet-manipulated stem cell spheroids are formed by rapidly and compactly aggregating magnetic-labeled stem cells using magnetic nanoparticles [153]. A magnet can control the position of the magnetized stem cell aggregate and move stem cells to the upper (i.e., levitation) or lower part of the culture plate containing the culture medium [154]. The spatial positions of stem cell aggregates or spheroids with magnetic particles, such as biocompatible iron oxide nanoparticles, are maintained because the magnetic force applied overcomes the gravitational force [155]. This spheroid formation system facilitates the induction of rapid cell aggregation, maintains good control over spheroid size, and requires minimal handling. In addition, this method enables the co-culturing of various cell types and the promotion of cell-cell connections. The formation rate of aggregate and growth rate of spheroid is high compared to the more commonly used methods for spheroid formation [156]. The aggregates and spheroids by formed magnetic force can be transferred to the desired location using magnetic tools [154]. However, this method is not very suitable for generating large amounts of aggregates or spheroids. Magnetic patterns with 100 μm diameter microtips composed of 30 × 30 nickel dots were developed using photolithography to guide cardiomyogenesis of embryonic stem cells (Fig. 3e) [19]. The embryonic stem cells incorporated with iron magnetic nanoparticles were aggregated into embryoid bodies, and the spheroids were exposed to a magnet for 5 min per day for 3 d via a cyclically stimulated electromagnet on the cylindrical magnetic patterns. The magnetic nanoparticles having a 200-nm diameter, composed of an iron oxide core coated with polydimethylamine, were used for magnetic levitation culture of human BMSC spheroids within type I collagen gel [19]. The magnetic 3D bioprinting biofabrication system was proposed for generation of spheroids of neural crest-derived mesenchymal stem cells and hDPSCs labeled with NanoShuttle (commercial magnetic nanoparticles) [157].

5.6. Microfluidic-based formation methods

Microfluidic-based engineering has evolved rapidly and has a variety of applications in cell-based analysis, tissue engineering, molecular diagnosis, and drug screening [158]. Microfluidic technology allows for precisely controlling the fluids in micro-scaled channels, which can be applied to 3D culture models [159]. In addition, microfluidic-based platforms enable the formation of a high number of uniform and size-controlled multicellular spheroids. The important functions of a microfluidic-based chip for stem cell spheroid formation include efficiently supplying nutrients and removing waste, providing a suitable chemical concentration gradient, positioning and trapping the cells, having low reagent consumption, and maintaining continuous perfusion and precise control of the pressure and shear stress on the cells [160]. Microfluidic technology also enables accurate replication of cell-cell contact, substrate properties, biochemical and mechanical signals, and stimuli. Microfluidic systems by including miniaturized size and array via microengineering can be used for high-throughput production of spheroids. Microfluidic-based engineering devices for spheroid formation and culture can have continuous-flow with single (e.g., perfusion) and multiple phases (e.g., T-junction, flow-focusing, and double emulsion) [161]. A droplet microfluidic chip based on modulating droplet confinement was proposed for trapping MSCs and guiding spheroid formation into grooves and holes (i.e., anchors) patterned in the device surface (Fig. 3f) [18]. Chips with 500 anchored droplet arrays within 2 cm^2 were used to perform quantitative characterization on the population scale; they contained thousands of individual spheroids with hundreds of thousands of cells within each spheroid, correlating functionality with cellular location within the spheroid. The use of PVA-based microgels fabricated via a high-throughput microfluidic technique has been proposed to co-encapsulate stem cells and growth factors for spheroid formation and culture [162].

6. Therapeutic applications of stem cell spheroids

As previously explained, stem cells have been widely applied for various tissue repair and regeneration applications, owing to their ability to differentiate into different types of cells, growth factor secretion ability, and homing effect. However, stem cells implanted into target tissues or organs using local delivery methods exhibit low transplant efficiency, limited survival rate, and rapid loss of transplanted stem cells. Consequently, efficient platforms for integrating the transplanted stem cells into host tissues are needed in order to achieve the goal of tissue repair and regeneration. Although various scaffolds, encapsulation materials, particles, and sheet forms have been proposed as delivery platforms, the construction of cells bound to the platforms has limitations that make it difficult to mimic the *in vivo* 3D cell-cell and cell-ECM interactions [163]. In contrast, 3D structures of stem cell spheroids showed enhanced biological properties including cell viability, stable morphology, polarization, increased proliferative activity, and physiological metabolic function compared to 3D cell culture systems [21]. Thus, stem cell-based spheroids are used in the field of tissue engineering because of their ability to differentiate along chondrogenic, osteogenic, adipogenic, and neurogenic lineages. In this section, we summarize the applications of stem cell-based spheroids in various tissues for regeneration and repair (e.g., bone, cartilage, tendon, nerve, blood vessel, tooth, and skin) (Table 3 and Fig. 4).

6.1. Bone tissue repair and regeneration

Bone is a dynamic and multifunctional tissue that exhibits control of mineral homeostasis, blood formation, and mechanical structure in response to changing physical stress and physiological needs [164]. In the process of bone formation, mechanical and structural stimulation, cell differentiation, and the generation of mineralized organic matrix are synchronized to create hybrid hierarchical structures [165]. In the process of active bone formation and remodeling, the cells (e.g., stem cells and osteoblasts) produce the collagen-based ECM structures and then mineralize them to form new layered bone tissues according to the differentiation of the cells [166,167]. Therefore, when generating functional spheroid implants for bone defects, it is important to improve cell-to-cell interactions and the regeneration capacity for efficient remodeling of bone ECM.

PLLA electrospun nanofiber fragments coated with adenosine and polydopamine were developed to create a 3D stem cell instructive microenvironment for generating stable spheroids using hADSCs (Fig. 4a) [168]. The adenosine-coated nanofibers combined with spheroids significantly improved the expression of adenosine 2b receptor; osteogenic genes such as Runx2, OPN, OCN, and OSX; and mineral deposition. Implantation of engineered spheroids enhanced bone formation in a calvarial defect mouse model. These results indicate stem cells promote differentiation into osteoblasts and the role of A2bR receptors in regulating bone inflammation. Platelet-derived growth factor (PDGF)-coated and bio-mineral-coated nanofibers were proposed as

Table 3
Therapeutic applications of stem cell spheroids for tissue repair and regeneration.

Tissue	Stem cell	Formation method	Spheroid size	Seeding density	*In vivo* model (Functional evaluation)	Paracrine factors	Ref.
Bone	hADSC	Microfibers, centrifugation	1.2 mm^2	2×10^4 cells/100 μL	Calvarial bone defect (Bone regeneration: 42.48 ± 10.84%)	VEGF	[169]
	hADSC	Nanofibers, centrifugation	0.425 mm^2	4×10^4 cells	Calvarial bone defect(Bone regeneration: 59.97 ± 18.33%)	Non-analysis	[168]
	BMSC	Ultra-Low Attachment plates	530 μm	1×10^4 cells	Calvarial bone defect(Bone regeneration: 77.1 ± 4.1%)	Non-analysis	[170]
	BMSC	Nonadherent microwell plates	300 μm	1×10^4 cells	Segmental bone defect(Bone volume: 58 mm^3)	VEGF	[171]
Cartilage	ADMSC	Non-fouling scaffold	80–110 μm	5×10^7 cells/mL	Articular cartilage defect(Collagen type II: 92%GAG: 89%)	Non-analysis	[175]
	hADSC	Porous scaffold	100–200 μm	5×10^7 cells/mL	Articular cartilage defect(Collagen type II: 83%GAGs: 82%)	TGF-β1	[176]
	MSC	Ultra-Low Attachment plates	291.3 μm	1×10^4 cells/well	Osteochondraldefect(Cartilage tissue regeneration: 74.0 ± 5.9%)	Non-analysis	[178]
	ASC	Porous scaffold	80–110 μm	5×10^7 cells/mL	Osteochondraldefect(Collagen type II: 81%,GAGs: 91%)	–	[177]
	ADSC	Membrane	~90 μm	8×10^4 cells/well	Articular cartilage defect(High GAG expression)	SDF-1	[179]
Nerve	hGMSC	Ultra-Low Attachment plates	~500 μm	4×10^4 cells/well	Facial nerve(High β-tubulin IIIand S-100 β expression)	Non-analysis	[188]
	ADMSC	Sulfonated chitosan well plate	–	2.8×10^4 cells/cm^2	Nerve conduits(90% differentiation into immature Schwann cells; great peak amplitude and nerve conduction velocity)	HGF, NGF, BDNF, GDNF, and SDF-1	[117]
Blood vessel	hAMSC	Porous hydrogel	200–300 μm	5×10^6 cells/mL	Ischemic limb(Promoted angiogenesis, healthy myofiber, and regeneration of myofiber)	VEGF and IGF-1	[192]
	hAMSC	Porous scaffold	220 μm	5×10^7 cells/mL	Subcutaneous vascular ingrowth, and adipose tissue regeneration	VEGF and FGF-2	[190]
Tooth	hDPSC	Agarose microwell	~300 μm	2.6×10^5 cells/190 μL	Regenerated and vascularized dental pulp-like tissue	Non-analysis	[194]
	hDPSC	Ultra-Low Attachment plates	~60 μm	3.3×10^5 cells in 3 mL of pellet	Regenerative dentin and neurovascular-like structures that mimicked the native teeth	VEGF and TGF-β	[195]
Skin	hADSC	Hanging drop	200–300 μm	8000−10000 cells/25 μL	Skin wounds(Wound regeneration: 100%epithelium thickness: 150 μmcollagen density: 40%CD31 expression: 35%)	IL-10 and TGF-β1	[200]
	hADSC	Ultra-Low Attachment plates	1.2–1.5 mm	7.5×10^4 cells/cm^2	Skin wounds(Rapid wound closure andhigher histological score)	VEGF, FGF, and HGF	[199]
	Human placenta-derived MSCs	Hanging drop	100 μm	5000 cells/droplet	Skin wound(Fast wound reepithelization and closure,enhanced microvessel density, andhigher expression of paracrine factors)	VEGFa, SDF-1, and Ang1	[197]
Heart	hBMSC	Hanging drop	260 μm	3×10^5 cells	Myocardial infarction(Enhanced vascularization, enhanced Cx43 expression, and improved cardiac function	VEGF, FGF, and HGF	[250]
	Human cardiac stem cell	Ultra-Low Attachment plates	95 μm	5×10^5	Myocardial infarction(Enhanced neovascularization, decreased fibrotic area, increased left ventricle thickness, and improved cardiac function)	FGF1, FGF2, FGF4, FGF5, FGF7, FGF16, IGF1, NGFβ, NRG4, ZEP91, ANGPT4, EFNA1, EFNA5, EFNB3, PDGFC, VEGFC, GH1, IFNA1, and IFNE1	[112]
	Human cardiac stem cell	6-well tissue culture plates coated with poly-2-hydroxyethyl methacrylate	75 μm	1052 cells	Myocardial infarction(Improved cardiac function and decreased fibrotic area)	SDF-1α and MCP-1	[201]
Kidney	hMSC	Ultra-Low Attachment plates	241 μm	1500 cells	Hypertensive kidney disease(Reduced proteinuria, improved glomerular permselective function)	VEGFA, HGF, IGF, and SDF-1	[204]
	hADMSC	Hanging drop	200 μm	25,000 cells	Ischemic kidney(Decreased creatinine and BUN levels, reduced tissue apoptosis and damage, and enhanced vascularization)	VEGF, EGF, IGF, bFGF, HGF, and TSG-6	[113]
Liver	hADMSC	Hanging drop	500 μm	1×10^6 cells	Hepatic fibrosis(Reduced hepatic fibrosis, decreased collagen I and collagen III expression, and improved liver function	IGF-1, HGF, and IL-6	[114]
	Human teeth-derived Stem cells	Ultra-Low Attachment plates	–	1×10^5 cells	Hepatic fibrosis(Reduced hepatic fibrosis and reduced bleeding)	TGF-β1	[206]
	human umbilical	Rocker system	111 μm	1×10^6 cells		IFN-γ, IL-6, and TNF-α	[251]

(*continued on next page*)

Table 3 (*continued*)

Tissue	Stem cell	Formation method	Spheroid size	Seeding density	*In vivo* model (Functional evaluation)	Paracrine factors	Ref.
	cord-derived -MSC				Acute liver failure(Activated liver regeneration, reduced necrosis, and improved regeneration of hepatocytes		
Lung	hADMSC	PDMS-based microwell	150 μm	1×10^5 cells	Emphysema(Recovered alveolar damage and enhanced growth factor production)	FGF-2, VEGF, and HGF	[115]

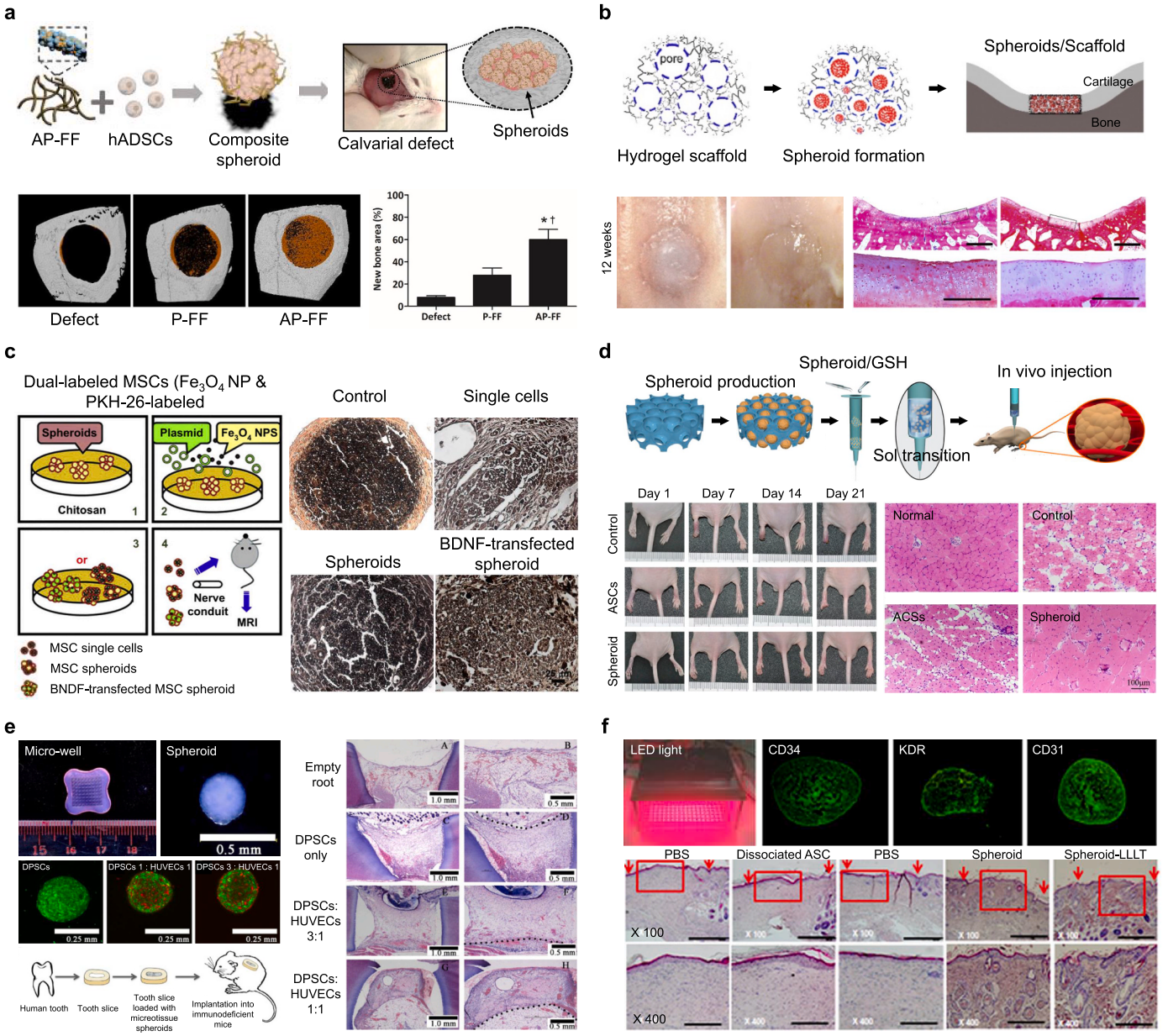

Fig. 4. Therapeutic applications of stem cell-based spheroids for tissue regeneration and repair. **(a)** Bone tissue regeneration and repair using human adipose-derived stem cell (hADSC) spheroids formed by adenosine/polydopamine-coated electrospun nanofiber fragments. Transplantation of spheroids into the calvarial defect promoted significant bone regeneration (image of micro-CT). Reproduced with permission from Ref. [168]. **(b)** Cartilage tissue regeneration and repair using ADSC spheroids formed by PLGA/chitosan scaffolds with porous structures and surfaces. The defect was repaired with the ADSC spheroids/scaffold (H&E staining). Reproduced with permission from Ref. [175]. **(c)** Nerve tissue regeneration and repair using BDNF-transfected ADMSC spheroids formed by Fe_3O_4 nanoparticles and chitosan scaffolds. BDNF-transfected spheroids enhanced the middle portion of the nerve conduit (H&E staining). Reproduced with permission from Ref. [117]. **(d)** Blood vessel tissue regeneration and repair using ADSC spheroids formed by PLGA-cystamine-PEG monomethyl ether porous hydrogels. Injection of spheroids/hydrogel solved by glutathione promoted angiogenesis and muscle regeneration (H&E staining). Reproduced with permission from Ref. [192]. **(e)** Dental tissue regeneration and repair using dental pulp stem cell (DPSC) spheroids formed by engineered gelled agarose microplate. Pulp-like tissue regenerated by DPSC spheroids (H&E staining). Reproduced with permission from Ref. [194]. **(f)** Skin tissue regeneration and repair using ADSC spheroids irradiated by an LED light source. The spheroids enhanced re-epithelialization and granulation (H&E staining). Reproduced with permission from Ref. [199].

tools for forming stable stem cell spheroids for bone and blood vessel formation in mouse calvarial defects [169]. Implanted spheroids showed improved regeneration capacity by promoting the expression of VEGF and von Willebrand factor. The bone marrow-derived MSC spheroids generated using low attachment round-bottom culture plates were implanted into rat calvarial bone defects and significantly induced the regeneration of bone filled with matured collagenous fibers [170]. Upregulated expression of RUNX-2, OSX, OPN, and BSP genes in spheroids induced accelerated bone formation. Spheroids formed by bone marrow-derived MSCs preconditioned in short-term hypoxic culture were used to increase the cell survival, trophic factor secretion, and bone tissue formation *in vivo*. The stem cells were preconditioned in 1% O_2 in monolayer culture for 3 d before spheroid generation, and hypoxia-preconditioned stem cell spheroids were generated via entrapment in alginate hydrogels [171]. The preconditioned spheroids were transplanted into rat critical-sized femoral segmental bone defects and were shown to promote an increase in angiogenesis, bone volume, bone mineral density, and stiffness compared to the control groups. The enhanced expression of VEGF induced by hypoxia had an effect on the regeneration effect.

6.2. Cartilage tissue repair and regeneration

Cartilage tissue has the limited self-healing capacity, and the damaged cartilage is difficult to repair because of poor blood supply and neural control [172]. Despite advances in the use of stem cells for the repair of the structure and function of damaged cartilage, the regeneration of fibrocartilage and hyaline cartilage expressing collagen type II still remains a challenge [173,174]. Zhang et al. generated rabbit adipose-derived stem cell spheroids using a non-fouling scaffold with porous structures and surfaces using amide-bonded PLGA and chitosan (Fig. 4b) [175]. The spheroids formed in scaffolds promoted chondrogenic differentiation by increasing the expression of glycosaminoglycans (GAGs) and collagen type II. The spheroids implanted into full-thickness articular cartilage defects in rabbits promoted enhanced expression of GAGs and collagen type II and suppression of collagen type I; the tissue had Young's modulus equal to 95% of that of native cartilage. The PLGA-based porous scaffolds were developed with tunable inner surfaces that could sequentially realize cell-scaffold attachment and detachment, as well as facilitate *in situ* spheroid formation [176]. The porous scaffolds with the spheroids were implanted into full-thickness articular cartilage defects in rabbits and enhanced the expression of GAGs and collagen type II and suppressed the collagen type I; the resulting tissue had a compressive modulus that reached 80% of that of normal cartilage tissue. Another porous scaffold possessing two different regions for forming ADSC spheroids was fabricated, and the porous scaffolds with spheroids resulted in significantly increased formation of cartilage and subchondral bone tissue by expressing TGF-β1 and IGF-1 [177]. Magnetic-responsive spheroids co-cultured with MSCs and magnetic nanoparticles were generated to target cartilage defects with high efficiency [178]. The spheroids showed the upregulated expression of collagen type II, SOX9, and Aggrecan resulted in the regeneration of cartilage tissue. Magnetic-responsive spheroids target-delivered into *ex vivo* cartilage defects of porcine femur osteochondral discs using an electromagnetic actuation system promoted regeneration of the defect area. ADSC spheroids formed by membrane-type scaffolds with chitosan and hyaluronan have been utilized for repairing cartilage defects through enhanced secretion of SDF-1 [179]. Recently, autologous chondrocyte implantation (ACI) using 3D cell spheroid form developed by CO.DON AG (Berlin, Germany) as a cell therapy is being utilized for the treatment of focal cartilage defects in the European Union [180,181]. These chondrocyte spheroids were prepared by culturing 1×10^5 and/or 2×10^5 cells in agarose hydrogel-coated 96 well plates [182]. Patients Implanted with less than 70 spheroids showed substantial improvement in various clinical outcomes [180,183–185]. Based on these clinical results, it is expected that the treatment efficiency of limited cartilage can be improved if stem cell-based spheroids are used as cell therapy by replacing chondrocytes in this treatment technology with stem cells.

6.3. Nerve tissue repair and regeneration

Because neural networks form complex 3D interwoven structures, mimicking the 3D microenvironment is critical, and this involves cellular, biochemical, mechanical, and topographical cues for the regeneration of target tissues [186]. Recently, it was reported that 3D self-assembled spheroids exhibited mature neuronal electrophysiology and expressed markers of multiple neural cell types [187]. The transplantation of stem cell spheroids improved the induction of nerve tissue regeneration while maintaining neural functions within the complex neural networks. For peripheral nerve regeneration, spheroids of ADMSCs labeled with superparamagnetic nanoparticles or transfected with the brain-derived neurotrophic factor gene were developed on chitosan scaffolds (Fig. 4c) [117]. The spheroids induced the axonal phase of the nerve repair by secretion of SDF-1, NGF, BDNF, and GDNF. Implantation of the engineered spheroids into rats resulted in short gap bridging time, largely regenerated nerves, and a thick myelin sheath. In addition, spheroids of human gingiva-derived mesenchymal stem cells were transplantable into bridge facial nerve defects in rats and improved regenerated nerve diameter, compound muscle action potential recovery, and aligned axonal regeneration by expressing β-tubulin III and β-tubulin III [188].

6.4. Blood vessel repair and regeneration

The development of new vessels is a dynamic process requiring strict regulation by pro- and anti-vascular factors and relies on the close interaction of endothelial cells with perivascular cells and ECM compounds [189]. Stem cell spheroids improved angiogenic efficacy *in vivo*, according to the hypoxia-induced paracrine secretion of high amounts of VEGF and FGF-2 [190]. Therefore, the implantation of spheroids with cells that have been preconditioned in ischemic environments could be a powerful tool to improve angiogenic efficacy [191]. Hong et al. formed ADSC spheroids using PLGA-based porous hydrogels with gel-sol transition properties for easy injection to treat hind limb ischemia (Fig. 4d) [192]. The injected spheroids improved paracrine secretion, resulting in the promotion of angiogenesis and muscle regeneration. The significant therapeutic effect on limb ischemia is due to the secretion of VEGF and IGF-1 related to pro-angiogenic and immunomodulatory factors by stem cells to support angiogenesis. In addition, porous hydrogel scaffolds based on PLGA activated by EDC and cross-linked by adipic dihydrazide were proposed for inducing the formation of ADSC spheroids. The scaffolds combined with spheroids were implanted into the dorsum of nude mice and significantly promoted subcutaneous vascular ingrowth and adipose tissue regeneration due to enhanced [190]. Spheroids of increasing size are described to induce hypoxia and upregulate VEGF and FGF-2 expression. Therefore, the induction of hypoxia condition in stem cell spheroids is effective to enhance the secretion of angiogenic factors.

6.5. Tooth repair and regeneration

Tooth regeneration has evolved with the aim of regenerating pulp-dentin complexes and repairing damaged functions due to pulp injury and/or inflammation [193]. Establishing an appropriate microenvironment that enables angiogenesis and vasculogenesis development and innervation is a challenge because of the unique anatomical limitations of dental structures. To mimic the microenvironment of the ECM of natural dental pulp tissues, microtissue-concepted spheroids of DPSCs pre-vascularized by human umbilical vein endothelial cells (HUVECs) were established for the regeneration of dental pulp tissues (Fig. 4e) [194]. The spheroids inserted into the canal space of tooth-root slices *in*

vitro were implanted under the skin of immunodeficient mice. The spheroids enhanced the well-vascularized and cellular pulp-like tissues of tooth-root slices, which contained odontoblast-like cells organized along with the dentin. Histological observation demonstrated the expression of human mitochondria and containing odontoblast-like cells organized along with the dentin, as assessed by immunostaining for nestin and dentin sialoprotein. Chen et al. identified multipotent dental pulp regenerative stem cells from mouse dental papilla and showed that the stem cell-based spheroids promoted the osteogenic and odontogenic differentiation for regenerating the pulp-dentinal complex-like tissue and neurovascular-like structures *in vivo* [195]. Expression of Sp7 as a key transcription factor influenced the formation of dentin, blood vessels and nerve tissue caused the Self-renewal of the stem cells.

6.6. Skin tissue repair and regeneration

Skin wound healing is a complex physiological process involving multiple cell types and bioactive factors, such as chemokines and cytokines, leading to cell proliferation, neovascularization, and granulation tissue generation, followed by epidermal formation [196,197]. However, chronic wounds caused by diabetes, burns, and inflammation can induce serious damage to skin tissue due to improper wound healing processes [198]. Treatment with stem cell-based spheroids has been proposed for stimulating granulation and epithelialization and improving collagen fiber deposition, organization, and thickness. In the implantation of matured spheroids for skin tissue regeneration, ADSC-based spheroids were treated with low-level light irradiation using an LED light source (Fig. 4f) [199]. The LED light-exposed spheroids exhibited up-regulated expression of VEGF, basic fibroblast growth factor (FGF), and hepatocyte growth factor (HGF). Implantation of the spheroids into excisional wound splinting model mice resulted in differentiated endothelial cells and enhanced density of vascular formation and skin tissue regeneration at the lesion site. Enhanced expression of VEGF and FGF induced by the hypoxia condition accelerated the re-epithelialization and neovascularization to promote the wound healing capacity. To efficiently deliver the spheroids into ear skin tissue, ADSC spheroids were encapsulated into microscale alginate beads fabricated using an electrospray system. After injection of hydrogel combined with alginate beads encapsulating the spheroids, it was confirmed that wound healing effectiveness was improved, as demonstrated by granulation, re-epithelialization, well-organized collagen fibrils, increased expression of α-smooth muscle actin (α-SMA), and high expression of angiogenesis [200]. IL-10 and TGF-β1 expression of the spheroids provides the environment for re-epithelization by improving fibroblast proliferation, secretion of matrix proteins, tissue granulation, and α-SMA.

6.7. Organ function repair and regeneration

Stem cell-based spheroids are increasingly being employed In vivo therapeutic applications for recovery of organ functions beyond the specific tissue repair and regeneration. Transplantation of spheroids formed with stem cells is a promising strategy for the repair of cardiac function with infarcted myocardium [14]. Park et al. reported the MSCs in spheroids incorporated with reduced graphene oxide nanomaterials showed improved expression of connexin 43, a gap junction protein, which can attenuate postinfarct arrhythmia [14]. In addition, spheroids engineered with the graphene nanomaterials highly expressed the VEGF, FGF-2, and HGF which are major growth factor that induces angiogenesis, which plays an important role in heart repair. The spheroids transplanted into mouse myocardial infarction models improved the vascularization in the infarcted myocardium, expression of connexin 43 in repaired cardiac area, and cardiac function with reduction of fibrosis. The existence of the nanomaterials in the spheroids enhanced cardiac repair and cardiac function restoration by promoting the expression of angiogenic growth factor and Cx43 of MSC. Cardiac stem cells designed in cell spheroid formulations improved the therapeutic efficacy of ischemic heart disease [112]. The cardiac stem cell spheroids significantly increased expression of the growth-related genes, angiogenic factors, anti-inflammatory factors, cardiopoietic factors, and cardiomyocyte markers after cardiomyocyte differentiation compared to the cardiac stem cell group. Transplanted cardiac stem cell spheroids in the infarcted myocardium induced significant decreases in the infarction size and functional improvement of the infarcted heart by promoting neovascularization. Another group reported the genetically engineered cardiac stem cell spheroids improved cardiac function and decreased fibrotic area [201]. The authors described that the genetically engineered cardiac stem cell spheroids improved cardiac function after acute myocardial infarction by increasing the mRNA expression of SDF-1α and CXCR4.

Stem cell-based therapy has been reported to be effective in reducing glomerular fibrosis and restoring kidney function [202,203]. However, Spheroid-assisted transplantation is promising due to the lack of a three-dimensional (3D) context that mimics the microenvironment of the native glomerulus. Yang et al. reported that the hybrid cell spheroids composed of podocytes, MSCs, and HUVECs promoted the repair of damaged kidneys by confirming the effective reduction of proteinuria in mice with hypertensive nephropathy [204]. MSCs in the hybrid spheroid contributed generation of environment for uniform spheroid constructs because it promotes cell-cell and cell-ECM interactions. In addition, MSCs increased the secretion of laminin and fibronectin and promoted the function of podocytes by highly expressing VEGFA, HGF. Another group reported that spheroids composed of MSCs improved therapeutic effects of acute kidney injury [113]. Spheroids produced higher levels of collagen I, fibronectin, and laminin, and exhibited stronger anti-apoptotic and anti-oxidative capacities. In addition, the spheroids improved reduced cell apoptosis, less tissue damage, increased vascularization in the kidney tissue by promoting secretion of VEGF, FGF, EGF, HGF, IGF, and TSG-6.

MSC-based therapy has been reported to be a promising treatment approach for liver regenerations and functions [205]. Zhang et al. demonstrated the treatment effect of hepatic fibrosis the spheroids derived ADMSCs [114]. The authors described that the enhanced expression of insulin growth factor 1 (IGF-1), interleukin-6 (IL-6), and hepatocyte growth factor (HGF) of spheroids protected the hepatocytes from cell injury and apoptosis. The spheroids improved liver functions by inhibiting hepatic fibrosis due to the reduction of expression levels of collagen I and collagen III. It is undisputed that 3D spheroids composed of stem cells promote the paracrine secretion of beneficial cytokines compared to 2D cultured MSCs. This effect suggested an important role in protecting hepatocytes from cell damage and fibrosis formation as well as liver function and fibrosis. Takahashi et al. developed spheroids using stem cells derived from human exfoliated deciduous teeth for improving liver function due to fibrosis effects [206]. The spheroids transplanted into chronic liver fibrosis of mouse model suppressed the progression of liver fibrosis and structural disarrangement by reducing Acta 2, collagen type I, matrix metalloproteinase 2, Mmp9, tissue inhibitors of matrix metalloproteinase 1, and transforming growth factor beta 1. In addition, the spheroids showed significant anti-hemorrhagic. These studies suggest that they show improved results not only for small-scale tissue regeneration but also for organ tissue regeneration and functional recovery.

7. Engineering techniques for maturation of stem cell organoids

Organoids are formed according to a self-organizing process in which early homogeneous populations of stem cells voluntarily break symmetry and undergo *in vivo*-like environment formation [207]. However, controlling the maturation of organoids in order to generate higher levels of tissue organization and functionality compared to other commonly used tissue engineering strategies, such as scaffolds, is challenging [208,209]. Developing mature stem cell organoids that possess

the functions and physiological properties of native organs is a critical challenge for therapeutic applications, drug screening, and basic biology studies. In this section, we introduce recently developed engineering tools to enhance the maturation of stem cell-based organoids (Table 4).

7.1. Microwell-based maturation methods

The microwell array contains concave portions with diameters on the microscale, which allows cells to form aggregates with defined shapes and sizes [210]. Microwell-based 3D multicellular formation plates, commonly fabricated using lithography or micropatterning, provide a method for controlling the formation of cell aggregates, such as spheroids and organoids [207]. Microwell array-based platforms are high-throughput with the properties of high-scalability, cost-effectiveness, easy operation, and ability to generate cell aggregates with uniform size and structure [211]. To control the quality of organoid production, Brandenberg et al. developed microwell arrays with a U shape using a PEG hydrogel via soft-lithography-based methods in combination with replica PDMS molding (Fig. 5a) [212]. The developed microwell arrays were fabricated on conventional multi-well plates with 121 microwells, with a diameter of 400 μm per well. The microwell arrays promoted homogeneity and reproducibility of organoids and enabled high-throughput and high-content organoid-based drug screening. The omni-well-array with a U-bottom-shaped microwell plate (500 μm diameter and 400 μm depth of each dimple) was fabricated by employing a molding machine process using resin molds [213]. The omni-well-array plates enabled scalable clinical-scale production of liver bud organoids, efficient differentiation into liver-bud-forming triple progenitors from human iPSCs, and differentiation by stimulating human iPSCs. The mPEG-coated PDMS microwell arrays with highly curved bottoms for human cerebral organoid culture were fabricated by 3D printing reverse molds [211]. The microwell arrays were used to form mature human cerebral organoids with the desired sphericity, organoid size, wrinkling index, lumen size and thickness, and neuronal layer thickness. To form and gently harvest the well-organized organoids, Rossen et al. developed dissolving alginate-based hydrogel microwell arrays [134]. The dissolving hydrogel microwells were used to produce a large number of vascularized organoids with reproducible size and structure. The generated organoids promoted the restoration of perfusion and muscle fiber regeneration upon injection into an ischemic hindlimb.

7.2. Microfluidic-based maturation methods

Microfluidic-based platforms provide an efficient system for formation and maturation of organoids because they enable the continuous infusion of nutrients and growth factors, as well as precise replication of cell-cell contacts, matrix characteristics, biochemical and mechanical cues, and stimuli [124]. Microfluidics enabled the creation of organoids associated with early human development on a scale that is not possible using conventional cell culture methods [214]. To achieve considerable advances in organoid models, organoid-on-a-chip combined with microfluidic systems can be used for more advanced and accurate drug and treatment studies by combining multiple organs on the same platform. Pump-free perfusion-based microfluidic culture devices were developed for generating matured human cerebral organoids from human iPSCs (Fig. 5b) [215]. Cerebral organoids were developed by reconstructing 3D brain-imitating microenvironments with decellularized human brain tissue-derived extracellular substrates and dynamic microfluidic systems. As a culture platform for intestine organoids, hybrid microchip systems were developed by combining tubular hydrogel scaffolds in a microchannel with an *in vivo*-like anatomical intestine structure [216]. The hybrid microchip promotes the self-organization of intestinal stem cells into a functional intestinal epithelium and induces the formation of organoids with the diverse cell types observed in intestinal systems *in vivo*. A retina organoid-on-a-chip was proposed as a microphysiological model of the human retina, integrating more than seven different essential retinal cell types derived

Table 4
Engineering tools for maturation of organoids.

Engineering tool	Materials	Features	Stem cell (Derived species)	Organoid type	Ref
Microwell	PEG	400 μm (diameter)	Intestinal stem cells (mouse)	Gastrointestinal organoids	[212]
	Polystyrene	500 μm (diameter), 400 μm (depth), and 30 μm (dimple)	iPSCs (human)	Liver bud organoids	[213]
	PDMS	800 μm (diameter)	Embryonic stem cell (human)	Cerebral organoids	[211]
	Alginate	800 μm (diameter)	Adipose-derived MSC (human)	Vascularized organoids	[134]
Microfluidic	PDMS, collagen type 1, Matrigel,	Two external medium reservoirs and two inlet and outlet reservoirs	Intestinal stem cells (human)	Intestinal organoids	[216]
	PDMS	Pump-free microfluidic device	iPSCs (human)	Brain organoids	[215]
	PDMS and hyaluronic acid	Vasculature-like perfusion channel	iPSCs (human)	Retinal organoids	[217]
	PDMS	Five parallel functional channels	iPSCs (human)	Brain organoids	[252]
Matrix	Allyl sulfide photodegradable hydrogel	Improved rate of degradation	Intestinal stem cells (mouse)	Intestinal organoids	[227]
	RGD-functionalized PEG	Mechanically dynamic matrices	Intestinal stem cells (mouse and human)	Intestinal organoids	[226]
	Decellularized porcine small intestine mucosa	Biochemical signature of tissue-specific ECM	Intestinal stem cells (mouse)	Endodermal organoids	[118]
	PEG-4MAL hydrogels	Well-defined structure, stoichiometric incorporation of biofunctional groups, and tunable reaction timescales for *in situ* gelation	ESCs and iPSCs (human)	Intestinal organoids	[228]
	Alginate	Minimally supportive hydrogel with no inherent cell instructive properties	ESCs and iPSCs (human)	Intestinal organoids	[229]
	Amikagels	Improved stiffness, protein adsorption, and surface biochemistry/amine content	ESCs (human)	Islet organoids	[230]
Bioreactor	Miniaturized spinning bioreactor	12-well size	iPSCs (human)	Brain organoids	[221]
	NASA-designed rotating-well vessel bioreactors	Rotation of 4, 24.3, 44.5 rpm	ESCs (mouse)	Retinal organoids	[222]
	Simple spinner-flask bioreactor	125 mL spinner flask and rotation of 90 rpm	iPSCs (human)	Kidney organoid	[223]

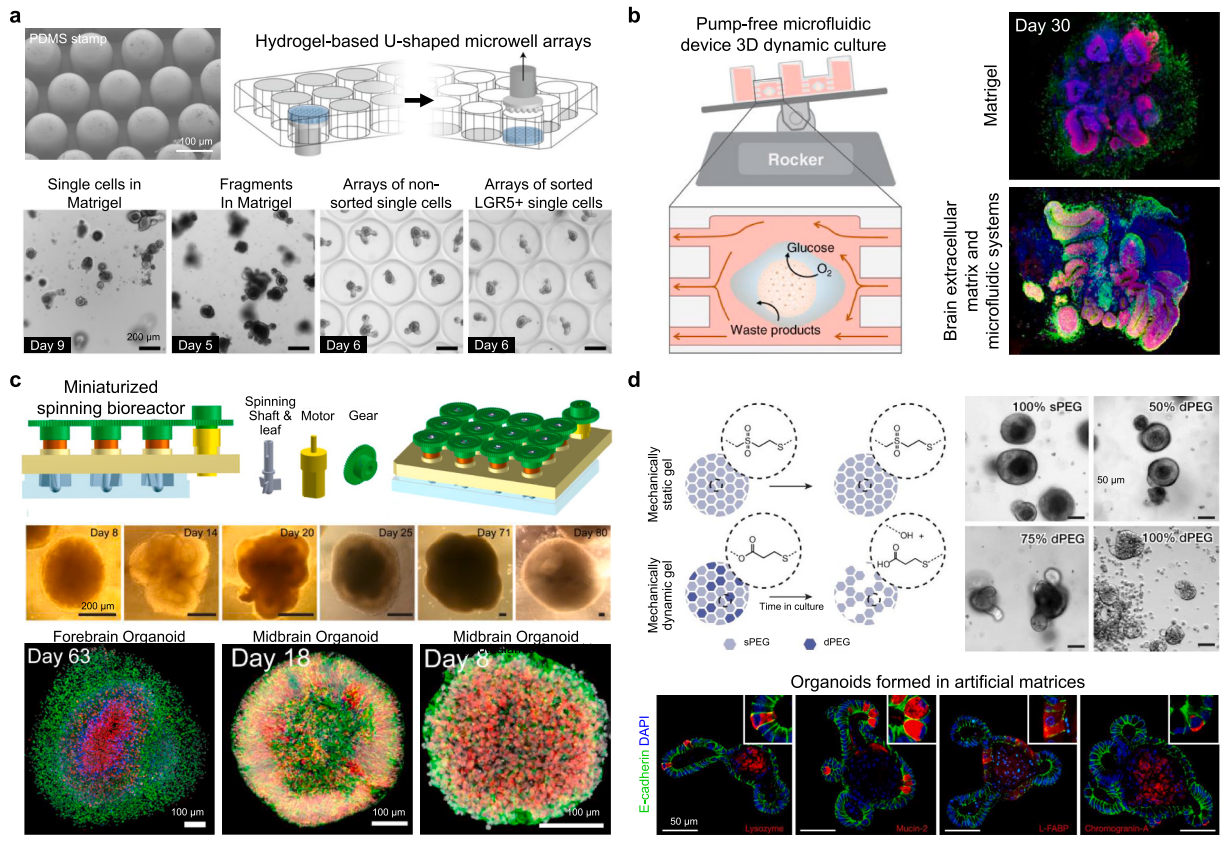

Fig. 5. Various engineering techniques for maturation of specific stem cell-induced organoids. **(a)** Microwell plate maturation method using microwell arrays with U shape using poly(ethylene glycol) (PEG) hydrogel for generating the intestinal organoids. Reproduced with permission from Ref. [212]. **(b)** Microfluidic-based maturation method using perfusion-based pump-free microfluidic culture devices for generating cerebral organoids. Microfluidic devices reproducibly improved the organization of progenitor zones and cortical layers in brain organoids. Reproduced with permission from Ref. [215]. **(c)** Bioreactor and spinner platform-based maturation method using miniaturized multiwell spinning bioreactor systems of 12 well size for generating cerebral organoids including the forebrain, midbrain, and Hypothalamic organoids. Reproduced with permission from Ref. [221]. **(d)** Hydrogel matrix-based maturation method using synthetic hydrogel networks and mechanically dynamic PEG polymer matrix for generating intestinal organoids. Organoids formed in PEG hydrogels contain differentiated intestinal cells. Reproduced with permission from Ref. [226].

from hiPSCs [217]. The developed organoid chips consisted of four identical micro-tissues connected through microchannels, two PDMS layers, and porous PET membranes with transparency and biocompatibility. The retina organoid-on-a-chip improved the formation of outer segment-like structures and the establishment of *in vivo*-like physiological processes, such as outer segment phagocytosis and calcium dynamics. Multilayer micro fluidic devices with a through-hole PDMS layer and polycarbonate porous membrane were developed for generating the islet organoids [218]. The microchip devices provided the easy integration of controlled embryonic body formation, *in situ* differentiation, and generation of heterogeneous islet organoids and enabled long-term 3D culture of islet organoids with cell viability- and islet-specific functions, owing to the continuous perfusion system.

7.3. Bioreactor system-based maturation methods

Suspension culture with a rotating bioreactor provides enhanced diffusion of oxygen and nutrients to support the growth and expansion of 3D tissues despite the absence of vascular structures [219]. Rotating-wall vessel culture is a relatively easy method for generating and maintaining organoids compared with other methods because improved nutrient exchange allows for consistent formation of organoids with superior morphology and unique organotypic gene expression [220]. The bioreactor system slowly rotates the liquid-filled cylinder on the axis and gently drags the fluid and all the particles in a perfectly circular path. Bioreactor systems facilitate the rapid generation of large and highly viable organoids and allow for high-throughput organoid generation for drug discovery, personalized medicine, and tissue therapy [220]. Miniaturized spinning bioreactor systems using 12-well cell culture plates were applied to generate cerebral organoids from human iPSCs (Fig. 5c) [221]. The organoids generated using the proposed bioreactor system improved human cortical development, including multi-layer progenitor zone organization, neurogenesis, gene expression, and a distinct human-specific outer radial glia cell layer. NASA-designed rotating-wall vessel bioreactors were utilized for generating retinal organoids using mouse-derived pluripotent stem cells. The rotating wall vessel promoted a well-defined morphology and increased proliferation and differentiation capacity of neurons including ganglion cells and S-cone photoreceptors [222]. Przepiorski et al. generated numerous kidney organoids from human iPSCs using 125-mL spinner flask bioreactors, which allowed for large-scale organoid production using a cost-effective protocol. The generated organoids formed nephrons containing podocytes, proximal and distal tubule segments, presumptive collecting ducts, and endothelial cells [223].

7.4. Hydrogel matrix-based maturation methods

Development of the hydrogel matrix and integration with microscale technology and stem cell biology has provided great opportunities for model systems of tissues and organs to be more realistic [224]. The organoids generated using other common methods are typically formed in the absence of essential components of *in vivo* microenvironments,

such as the ECM [225]. The hydrogel matrix, with high biocompatibility and tunable properties, such as permeability, elasticity, stiffness, and chemical reactivity, exhibits similar properties to the microenvironment of native ECM. Various natural and synthetic hydrogel-base materials have been extensively used as alternatives to Matrigel, a hydrogel commonly used for guiding the formation of matured organoids. The modular synthetic hydrogel networks were developed using a mechanically dynamic PEG polymer matrix for the well-defined intestinal organoid formation systems from mouse intestinal stem cells (Fig. 5e) [226]. Furthermore, in the case of switching intestinal stem cell colonies to intestinal organoids, the mechanically dynamic PEG polymer matrix exhibited stronger induction of differentiation of intestinal cells, including high YAP activation, compared with the stable PEG matrix. The allyl sulfide photodegradable hydrogel based on PEG macromer was used to achieve rapid degradation through radical addition-fragmentation chain transfer (AFCT) reactions, to support routine passaging of intestinal organoids [227]. The synthetic hydrogel matrix based on a four-arm PEG macromer with maleimide groups at each terminus was developed for growth and expansion of human intestinal organoids [228]. The developed hydrogel matrix induced the matured intestinal tissue structure and was used as the delivery platform of intestine organoids via injection into murine intestinal mucosal wounds. A natural polymer, decellularized ECM, provides structural support and delivers biochemical signals that are fundamental for assisting the regeneration process for organoid formation. ECM hydrogel matrix derived from decellularized porcine small intestine mucosa was used for forming gastric, hepatic, pancreatic, and small intestinal tissue [118]. Unmodified native alginate polymer crosslinked by calcium was utilized as a simple hydrogel system to induce human intestinal organoids for transplantation into immunocompromised mice [229]. Intestinal organoids formed by the alginate hydrogel systems exhibited a level of engraftment and maturation similar to that of organoids grown under the Matrigel matrix. As a novel hydrogel matrix for 3D cell culture systems, a hydrogel platform based on amine-glycidylether coupled hydroxyl linkages was used for generating mature pancreatic organoids [230].

8. Therapeutic applications of stem cell organoids

The need for organ development research models and the limitations of donor sources for organ transplantation have triggered the study of the development of functional substitutes for organs [231]. Organoid systems have spatially organized biomimetic cellular structures on both microscopic and macroscopic scales, as well as diverse cell phenotypes and extracellular environments [232]. There is a growing interest in using various organoids developed and matured by advanced engineering in regenerative medicine [233]. The use of organoids can advance regenerative medicine because transplants with organ-specific tissues could be more successfully integrated into the patient's own tissues compared with stem cells [234,235]. For regenerative medicine, obtaining mature organoids is crucial to achieve vascularization, minimize the chances of unwanted differentiation after transplantation, and promote function. Organoids contain stem cells and are likely to develop into an entire organ; hence, organoids can provide a source of autologous cells and tissues for transplantation. In this section, we introduce the recent therapeutic applications of various organoids generated from stem cells for organ therapy (Table 5 and Fig. 6).

Stem cells derived from human periosteum have been used for the scalable production of microspheroids that are differentiated into callus organoids (Fig. 6a) [236]. The assembly of multiple callus organoids into an easy-to-handle scaffold-free implant resulted in the complete bridging of a critical-sized long bone defect via the formation of cortical-like bone tissue with a medullary cavity containing bone marrow with the absence of fibrous tissue. In addition, the structure of the regenerated defect was similar to that of the native tibia. The effectiveness of vascularized organoids in the treatment of ischemic conditions observed in peripheral artery disease was evaluated (Fig. 6b) [134]. The vascularized organoids formed by dissolvable alginate microwells, which enable the formation of highly reproducible, scalable, and easy-to-harvest organoids, improved rapid restoration of vascular perfusion and muscle fiber regeneration. Cyst-like skin organoids derived from human embryonic stem cells were used for reconstructing appendage-bearing skin tissue (Fig. 6c) [60]. The developed organoids had cranial epithelial cells and neural crest cells that enhanced the formation of planar hair-bearing skin, including hair follicles and sweat glands, when grafted onto nude mice. Intestinal organoids generated by encapsulation of hPSCs into a synthetic hydrogel matrix based on the four-armed maleimide-terminated PEG hydrogel were delivered to repair colonic mucosal intestine wounds (Fig. 6d) [228]. Intestinal organoids developed using the PEG hydrogel system allowed for differentiated intestinal epithelium, including an intestine with crypt–villus architecture and underlying lamina propria, muscularis mucosae, and submucosa with structured collagen fibers and differentiated goblet cells. The matured organoids exhibited the enhanced engraftment into host intestinal epithelial tissue and significantly improved the mucosal colonic wound closure. Jee et al. evaluated the ability of colon organoids to treat radiation proctitis. After induction of radiation proctitis by irradiating the recta, the colon organoids generated from colonic crypts were transplanted into mice [237]. The transplanted colon organoids were found to successfully engraft onto the damaged rectal mucosa of the irradiated mice, owing to which they reconstituted epithelial structure and integrity. The liver organoids derived from human iPSCs were transplanted for treatment of chronic liver damage [238]. The liver organoids promoted significant

Table 5
Stem cell-derived organoid-based therapies for regeneration of tissues and restoration of organ function.

Target organ	Stem cell type (co-culture cell)	Formation method	Seeding density	*In vivo* model (Functional evaluation)	Ref.
Bone	Human periosteum-derived stem cells	Agarose microwell	5×10^5 cells	Segmental bone defect (Bone regeneration: 83.3%/8 weeks)	[236]
Blood-vessel	Mouse MSCs (HUVEC)	Alginate microwell	0.5×10^6 cells	Ischemic hindlimb mouse model (Rapid restoration of vascular perfusion and muscle fiber regeneration)	[134]
Hair bearing skin	PSC	Defined protocol	3500 cells	2–5-mm hairs growing out, rete-ridge-like structures, vasculature, and sebaceous glands and bulge	[60]
IntestineIntestine	ESC and iPSC	Synthetic polymer hydrogel matrix	–	Improved engraftment and colonic wound healing	[228]
	Intestinal stem cell	Matrigel, non-adherent plates plate	–	Epithelial regeneration with crypt and villus structures; differentiation into various intestinal epithelial cells	[237]
Liver	iPSC	Microwell	900 cells	Efficient delivery and attachment of organoid into liver	[238]
Brain	ESC	Ultra-low attachment 96-well plate	1.35×10^4 cells	Enhanced neurogenesis, synaptic reconstruction, axonal regeneration, and angiogenesis, and decreased neural apoptosis	[239]

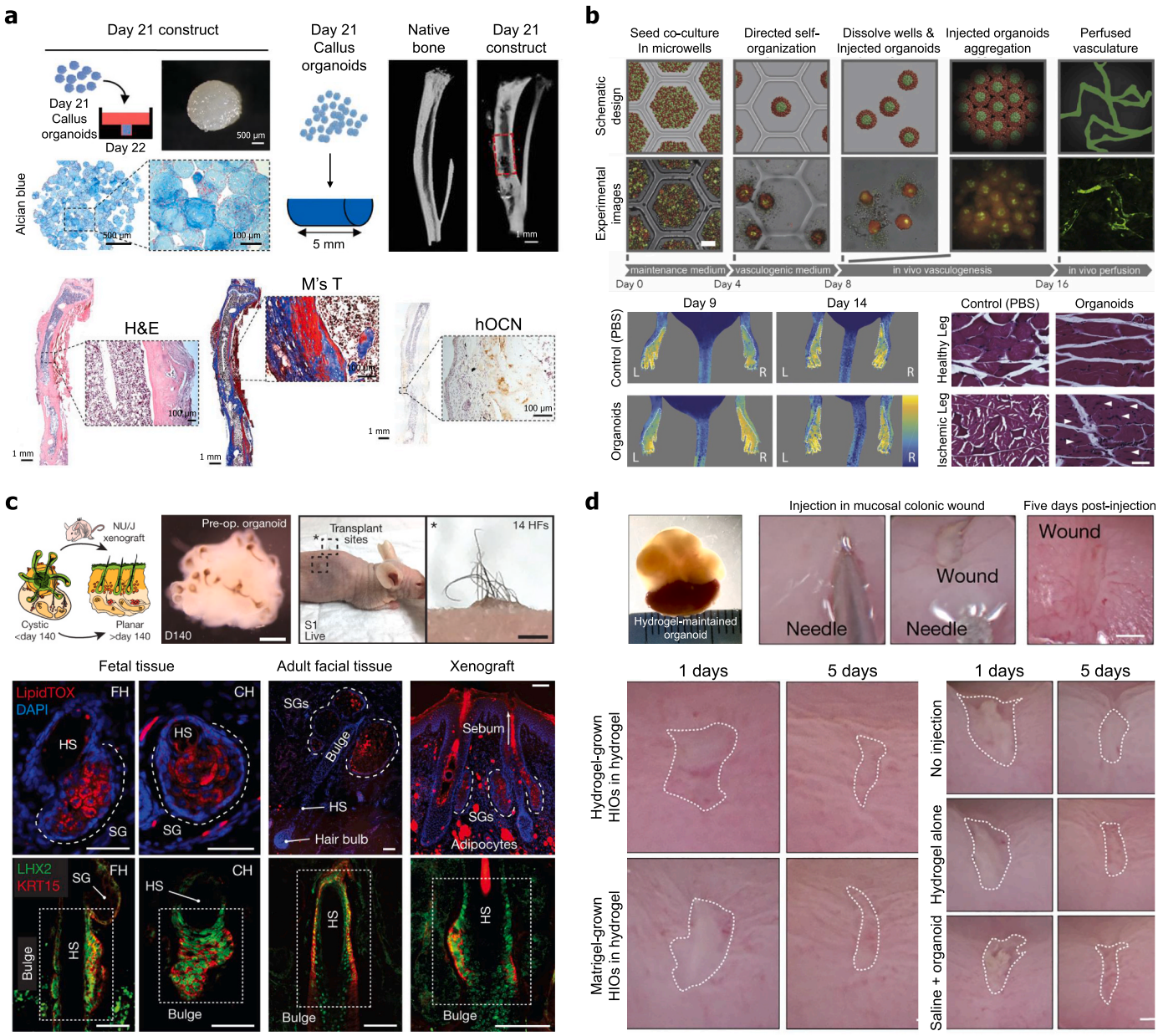

Fig. 6. Therapeutic applications of stem cell-induced organoids for regeneration of tissue and restoration of organ function. **(a)** Bone tissue repair and regeneration using human periosteum-derived stem cells-derived multiple callus organoids developed by agarose microwell. Reproduced with permission from Ref. [236]. Callus organoids were assembled to form larger constructs in agarose well. Assembled callus organoids enhanced regeneration of critical-sized long bone defects. **(b)** Blood vessel and muscle tissue repair and regeneration using vascularized organoids. Mouse MSCs and HUVECs-derived vascularized organoids developed by sacrificial alginate microwells. Prevascularized organoids regained perfusion of the ischemic limb, had more viable myofibers, and exhibited regenerating myofibers. Reproduced with permission from Ref. [134]. **(c)** Skin tissue repair and regeneration using cyst-like skin organoids. Cyst-like skin organoids derived from human ESCs were used for reconstructing appendage-bearing skin tissue. The developed organoids enhanced the formation of planar hair-bearing skin, including hair follicles and sweat glands. Reproduced with permission from Ref. [60]. **(d)** Intestinal tissue repair and regeneration using intestinal organoids. Intestinal organoids generated by encapsulation of hPSCs into a synthetic four-armed maleimide-terminated PEG hydrogel. The matured organoids improved the mucosal colonic wound closure. Reproduced with permission from Ref. [228].

reconstruction of hepatocytes and replacement of 70% of the damaged liver. Cerebral organoids induced from human embryonic stem cells were transplanted into model rats with middle cerebral artery occlusion to investigate stroke treatment capacity. The transplanted cerebral organoids significantly reduced brain infarct volume, improved neurological motor function, showed multilineage differentiation potential, supported motor cortex region-specific reconstruction, formed neurotransmitter-related neurons, and achieved synaptic connection [239].

9. Limitations, challenges, and prospects

9.1. Limitations

Since the emergence of tissue engineering and regenerative medicine field, the technologies and applications of stem cells have been actively studied to restore damaged tissues or organs. A variety of engineering (e.g., biomaterial, genetic modification, external stimuli, molecule, and ECM) have been proposed to control engraftment, viability, renewal, proliferation, differentiation, and paracrine effect of stem cells.

Nevertheless, the limitations for increasing treatment efficiency of the viability and loss of stem cells transplanted into tissues or organs still remain as the task to be researched. If the damaged area of tissues or organs is large or continuous treatment is required, transplantation of a sufficient amount of stem cells can maximize the treatment efficiency. Since the area, disease mechanisms, and biological properties of damaged human tissues are diverse, a guideline on the appropriate amount of stem cells for maximizing therapeutic efficacy is needed. Currently, in the European Union, spheroids composed of autologous chondrocytes are used for the treatment of focal human cartilage defects [181]. This spheroid therapy contains 1×10^5 or 2×10^5 cells, and in clinical application studies, less than 70 spheroids are implanted in the focal cartilage defect (>2 cm^2) of the patients. If it is expanded to spheroids using stem cells based on these clinical trials, it has the potential to provide information on stem cell density and spheroid size in clinical studies using stem cell-based spheroids. In addition, it is important to establish a standardized system that can secure a large number of stem cells for the treatment of the large area. Stem cell therapy consumes a lot of time and money. Therefore, technologies to maximize survival and to prevent cell loss for efficiently delivering a large number of stem cells should be actively developed to maximize treatment efficiency without complicated procedures. 3D cultured stem cells could be a good alternative to the transplantation of 2D cultured stem cells.

9.2. Challenges

3D stem cell culture systems, such as spheroids and organoids, are promising therapeutic strategies that can overcome the limitations of stem cell therapy. However, there are several limitations and challenges to be addressed. Spheroids and organoids have some limitations in mass production for high-throughput screening and therapeutic application. Commonly used cell culture plates with round bottom and low attachment are difficult to control the shape of spheroids and organoids and lacks flexibility and controllability in terms of size. In addition, there is a limit to mass production as expanding the culture surface area is not possible. These limitations can be overcome by the technological realization of automated high-throughput production and directed fusion into large tissues. Recently, the formation of 1500 spheroids/ml was achieved using a bioreactor-based 3D cell culture system [240]. Based on this point, the combination of general 3D culture methods and micro/nanoengineering can produce a large number of spheroids and organoids, and at the same time, it is possible to produce a suitable treatment. Micro-fluidic platforms and 3D bioprinting allow the mass production and fused organization of spheroids and organoids of uniformly sized spheroids. In addition, nanoscale biomaterials (e.g., nanoparticles, nanocapsules, and nanofibers) are promising as functional additive materials to modulate the spheroid formation process and therapeutic potential. Furthermore, functional nanomaterials facilitate improving the engraftment, viability, renewal, proliferation, differentiation, and paracrine effect of stem cell spheroids. Recently, with the advancement of 3D bioprinting, it has become possible to construct a tissue body maintained in a desired position by printing and fusing spheroids and organoids as bio-inks [241]. In addition, this approach improves the production and efficiency of various cellular structures [242]. However, Spheroids and organoids are required to be mass-produced in a standardized and uniform size and to construct tissues with similar biological properties to apply clinical trials. Therefore, functional biomaterials, physical/structural modulation, and tissue organization technology combined with the existing 3D cell culture system can lead to the development and production of spheroids and organoids suitable for clinical therapeutic applications.

9.3. Prospects

In the near future, organoids may serve as a promising strategy as regenerative therapies for the regeneration of damaged tissues and the replacement of parts in damaged organs. Despite considerable success in culturing physiologically relevant organoids, research on regenerative therapy through transplantation of organoids is still in its infancy and challenges to achieve practical applications remain. Since organoids have already differentiated with functions similar to those of organs, there is a problem of engraftment and fusion at the transplant organ site. In addition, organoids are limited in reproducing the function of a fully mature organ with current technology and cannot be expanded to the scale of native organs. In addition, the organoids currently developed have limitations in completely mimicking the functions of the vascular system, the immune system, and the nervous system. Therefore, it is a challenge to reach full maturity using organ on a chip, co-culture, and vascularization based on the current organoid culture methodologies through temporal, spatial, and chemical control. After the establishment of fully matured organoids, technology development for mass-producing organoids of uniform size should be actively conducted. Organoid technology can be extended to personalized cancer research and biotherapy using patients' autologous stem cells and the application of artificial organ transplantation. Recently, research for drug screening by developing organoids using patients' adult stem cells, ESCs, and iPSCs are emerging, enabling the realization of personalized medicine [243–245]. Personalized stem cell organoids can overcome the transplant immune rejection of artificial organ transplantation. The fusion of micro/nanoengineering-based maturation, biological factors, and mass production systems will enable regenerative therapy through personalized organoid transplantation.

10. Conclusion

Spheroids and organoids have been developed for advanced therapeutic applications. In this review, we summarized the therapeutic applications of functional 3D stem cell culture using state-of-the-art engineering technologies for tissue regeneration and repair. Compared with stem cell therapy, which is limited by low transplant efficiency, limited survival rate, and rapid loss of transplanted stem cells in the host tissue, the spheroids and organoids enable efficient tissue regeneration and repair, owing to their more *in vivo*-like structure. The use of advanced engineering technologies could improve the production, size, and maturity of spheroids and organoids to achieve the goals of efficient tissue repair and regeneration and enhanced organ function. Organoids have started to gain attention for their ability to promote tissue regeneration, especially considering the limitations of donor sources for organ transplantation. We envisage that spheroids and organoids generated using 3D stem cell culture systems will overcome the limitations of current stem cell therapy and will be used in the regeneration and repair of tissues or organs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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