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REVIEW

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Stem cells in organogenesis and regeneration

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Abstract

Stem cells are the basis of organogenesis and regeneration, providing cellular support during the development, maintenance, and repair of tissues. This review provides a brief overview of the major stem cell types and their sources, as well as the key stages of organogenesis that depend on stem cell activity. This review highlights critical signalling pathways, including Wnt, Notch, Hedgehog, and BMP. These pathways regulate the fate and lineage specification of stem cells. The review identifies the roles of embryonic stem cells and induced pluripotent stem cells in organ formation as well as the newly arising methods for directed differentiation. Mesenchymal stem cells play a crucial role in tissue regeneration and therapeutic repair. Organoids are potent experimental models for studying development and disease. The impact of stem cell niches and microenvironmental regulation is discussed, along with the cellular and molecular processes that underlie recovery after damage. The review encompasses the translational progress of stem-cell-based therapies, current clinical trials, and the challenges in safety and efficacy. Moreover, the review also explores the introduction of advanced technologies, such as CRISPR, 3D bioprinting, and synthetic biology, as well as theoretical considerations, including future directions and ethical issues. Together, these insights provide a comprehensive overview of stem cell biology and highlight their potential for clinical translation.

Keywords Stem cells, Organogenesis, Regeneration, Embryonic stem cells, CRISPR, 3D bioprinting, Synthetic biology

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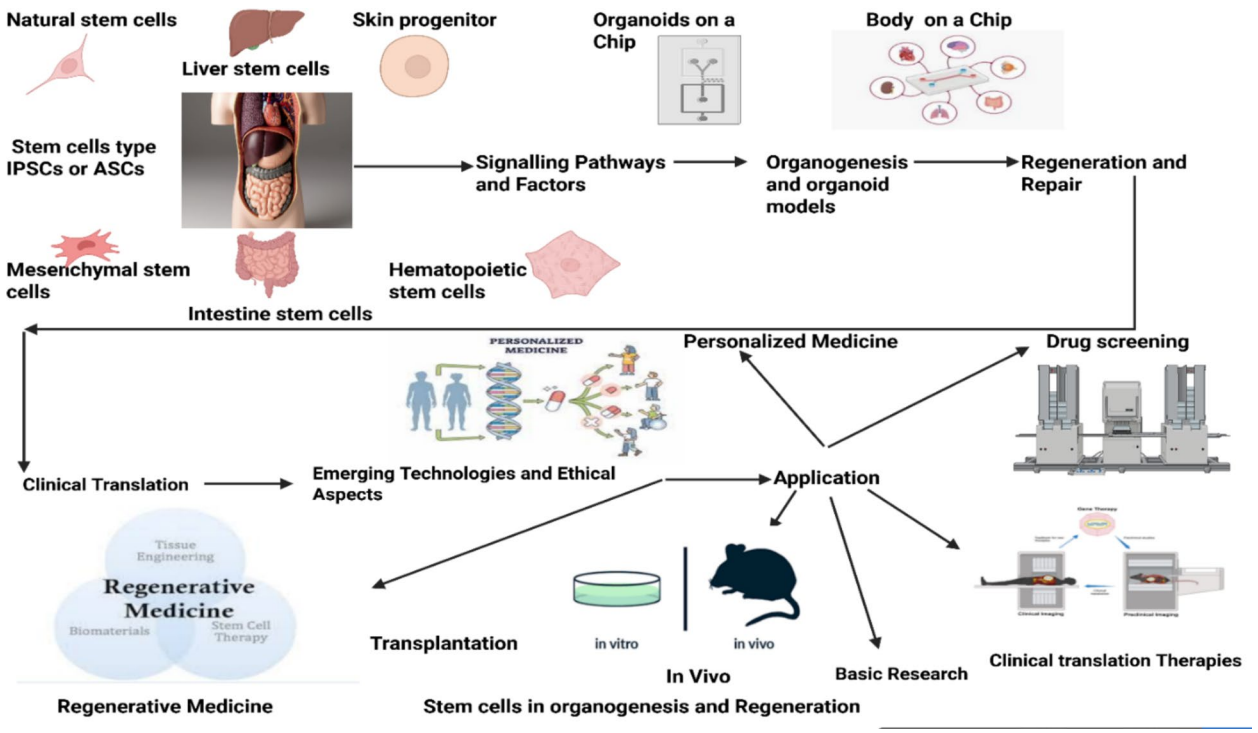
Graphical Abstract



Types and sources of stem cells involved in organ development

Stem cells are classified as totipotent, pluripotent, multipotent, or unipotent, based on their differentiation potential [1]. Totipotent stem cells can differentiate into all cell lineages present in an organism, with the zygote being the only totipotent stem cell. Pluripotent stem cells (PSCs) can differentiate into cells of the three germ layers. Unipotent and multipotent stem cells can differentiate into cells of either a single lineage or a specific tissue or organ [2]. Stem cells primarily occur as Embryonic Stem Cells (ESCs), Adult Stem Cells (ASCs), and Fetal Stem Cells (FSCs) [3]. During embryonic development, pluripotency-associated factors determine the plasticity of each type of cell [1]. Upregulation, by reprogramming, of pluripotency-associated transcription factors gives rise to induced pluripotent stem cells (iPSCs) [4].

Throughout life, adult stem cells or resident stem cells are found in the tissue of the body and are responsible for tissue repair [5]. These cells have both self-renewal ability and multipotency, giving them the ability to sustain and maintain the tissue-repairing process throughout the lifetime [6]. Some of the major adult stem cells include hematopoietic stem cells, mesenchymal stem cells, tissue-specific stem cells, skeletal muscle stem cells and so on [7].

In regenerative medicine, organoids are generated using stem cells, which include (i) PSCs, that is, ESCs or induced pluripotent stem cells (iPSCs) and (ii) organ-specific ASCs [8]. ESCs are found in an early-stage embryo and can be isolated from the blastocyst, a hollow sphere of cells formed during embryonic development [9]. Multiple transcription factors maintain the pluripotency of ESCs and are collectively called pluripotency factors, allowing ESCs to differentiate into nearly all somatic cell types [2]. Embryonic stem cells (ESCs) possess the ability to self-renew and hold great promise for therapeutic applications [10]. The types of stem cells, their roles and their sources are highlighted in Table 1.

Key stages of organogenesis driven by stem cells

The fusion of differentiated cells, sperm, and egg results in the formation of the zygote, which is totipotent in nature. Initially, the zygotic genome is not expressed because a process, the maternal-to-zygote transition (MZT), is necessary, which occurs when degradation of maternally derived transcripts is paired with zygotic genome activation (ZGA). Several transcription factors (TFs) play a significant role in the process, mainly the pluripotency factors, which maintain the undifferentiated state of the zygote [11]. The zygote undergoes symmetrical divisions, resulting in a compact, ball-like structure known as a morula. Further cell divisions and cavitation

Table 1 Stem cell types and their roles in different organs

Type	Plasticity	Source	Role	References
ESCs	Pluripotent	Inner cell mass of a blastocyst, an early-stage embryo	can differentiate into any cell type within the fetus	[3]
FSCs	Pluripotent	Fetus (primitive cell type)	Differentiate and develop into various organs of the fetus	[9]
Tissue-specific stem cells	Multipotent/unipotent	Skin and tissue of other organs	Regenerate the tissue of damaged organ(s)	[1]
Hematopoietic Stem cells (HSC)	Multipotent	Bone marrow, umbilical cord, placenta	Differentiate into various blood cells	[9]
Mesenchymal/stromal stem cells	Multipotent	Cord blood, bone marrow, adipose tissue. Placental tissue	Differentiate into bone, cartilage and fat cells help in tissue repair and regeneration	[1, 2]

form the blastocyst, which contains outer trophectoderm (TE) cells, inner cell mass (ICM) and a fluid-filled cavity called the blastocoel. The ICM contains embryonic stem cells (ESCs), which are pluripotent and capable of differentiating into any cell of the three germ layers, and possess self-renewal properties. TFs induced differentiation of these PSCs mediates gastrulation, turning the blastocyst into a gastrula, giving rise to the three germ layers: ectoderm, mesoderm and endoderm [12–14]. At this stage, the stem cells in the early embryo lack self-renewal properties, a condition that persists until the organ developmental stage [15].

The ectoderm differentiates into neuroectoderm and surface ectoderm. The neuroectoderm gives rise to the neural crest and neural tube, which ultimately develop into the central nervous system and peripheral nerves. The surface ectoderm develops into epidermis, appendages and oral epithelium, which include skin, nails, hair, sebaceous glands, sweat glands, mammary gland, oral mucosa and dental tissues [15, 16]. The next layer, the mesoderm, differentiates and eventually develops into various tissues, including blood cells, the heart, kidneys, gonads, bone, cartilage, adipose tissue, and muscle [16, 17]. The innermost layer, endoderm, initially folds to form the primitive gut tube, comprising the foregut, midgut and hindgut. The foregut ultimately develops into the esophagus, trachea, lungs, thyroid gland, parathyroid glands, thymus, stomach, liver, pancreas, and biliary system. On the other hand, the midgut and hindgut develop into the small intestine and the colon. The key stages of organogenesis are illustrated in Fig. 1.

Molecular signalling pathways in stem cell-mediated organ development

Differentiation of stem cells requires regulation through multiple signalling pathways, which are key to understanding the transition of stem cell phenotypes. These signals determine whether the stem cell will remain in its undifferentiated state or differentiate into a specific cell lineage. Such regulation can be mediated genetically or epigenetically [18]. In addition to regulating differentiation, the controlled self-renewal of stem cells is vital during organogenesis [19]. During human ESC differentiation, various signalling pathways come into play and determine the fate of the ESCs. For instance, transforming growth factor-β (TGF-β) and WNT signals promote the differentiation of ESCs into endoderm and mesendoderm. In contrast, bone morphogenic protein (BMP), endothelial growth factor, and FGF2 stimulate the ESCs to differentiate into mesoderm. Conversely, some regulators inhibit the differentiation of ESCs. Such suppression is exhibited by TGF-β, WNT or BMP signals during the differentiation of ESCs into ectoderm. Examining key signalling pathways provides valuable insights for stem cell research [18, 20]. Signalling pathways and their organ-specific roles are summarised in Table 2.

Critical pathways

Wnt signalling

The wnt signalling pathway plays a vital role in cellular processes, embryonic development, cell proliferation, differentiation, migration, and tissue homeostasis. The Wnt gene was first derived from integrase-1 in mouse breast cancer, and the wingless gene of *Drosophila* and the Wnt genes are homologous to these genes. The pathway, through which the signal is transduced, can be classified into canonical and non-canonical pathways, based on the involvement of β-catenin in transcriptional activation; canonical regulates cell proliferation while non-canonical controls cell polarity and migration [26, 27]. Canonical WNT3/β-catenin signalling pathway promotes cell

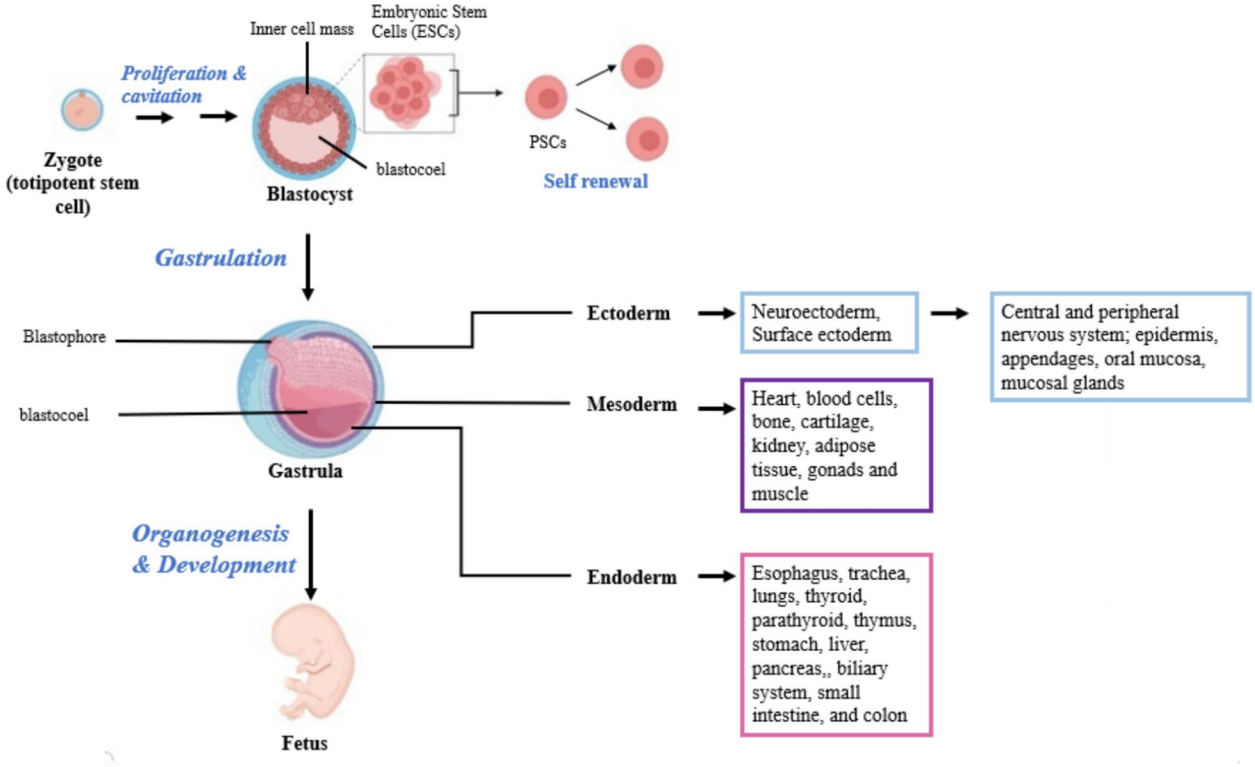


Fig. 1 Organogenesis stages highlighting stem cell activity: zygote, the totipotent stem cell, undergoes a series of proliferations to form the blastocyst, which contains pluripotent Embryonic stem cells (ESCs). ESCs differentiate into ectoderm, mesoderm and endoderm layers, and they give rise to different organs

proliferation and is activated by the binding of extracellular wnt ligands to cell membrane receptors. After activation, the wnt pathway induces the stability of β-catenin, which is translocated to the nucleus. Once inside the nucleus, the β-catenin up-regulates the expression of genes associated with cell proliferation, survival, differentiation and migration, via TCF/EF transcription factors [18, 26, 27].

Notch signaling

The Notch signalling pathway is considered to be an evolutionarily conserved pathway. In the 1910s, NOTCH was first identified in Drosophila melanogaster while studying its notched wings. Variants in humans are associated with the regulation of brain size. This pathway is associated with organ formation, tissue function and tissue repair; therefore, a defect in the pathway may cause pathological consequences [28]. The Notch receptor is a transmembrane protein that undergoes proteolytic cleavage upon ligand-receptor interaction, resulting in the release of the Notch intracellular domain (NICD). Freed NICD translocate to the nucleus and, along with DNA-binding proteins, upregulates transcription of target genes. During the early stage of embryonic development, this pathway is active, while in the mature stage, it remains low. However, the cases of stress or tissue injury, the signal is transduced rapidly through this pathway. This pathway also induces self-renewal and dedifferentiation of stem and progenitor cells, thus maintaining a pool of these cells [28, 29].

Hedgehog

Decades ago, Hedgehog (Hh) was originally found during genetic screens in *Drosophila melanogaster.* Then, the mammalian counterpart was identified, known as Sonic Hedgehog (SHH). Hh plays a vital role in organogenesis, primarily during embryonic limb and bone development, as well as the determination of cell fate. Defective signalling is observed in different types of cancer [18, 30, 31]. Sonic Hh signalling also regulates vascular differentiation. The Hh pathway mainly targets the Notch pathway, the epithelial-mesenchymal transition pathway, the TGF-β signalling pathway and the WNT signalling pathway. This pathway also regulates the cell cycle, cell adhesion, cell fate determination, and signalling related to stem cells [32].

BMP pathway

Bone morphogenetic proteins (BMPs) are part of the transforming growth factor-beta (TGF-β) superfamily of growth factors. BMP plays a vital role in osteoblast differentiation and ultimately in bone formation [33].

Table 2 Signalling pathways and their organ-specific effects

Pathway	Organ-specific effects	References
Wnt	Embryo: limb and neural development, cell fate determination, cell migration Liver: metabolism, regeneration Bone: regulates the maturity and activity of osteoblasts Intestine: maintain intestinal epithelial homeostasis Kidney: regulates homeostasis and repair Adipose tissue: inhibits adipogenesis	[21, 22]
Notch	Embryo: Cell fate determination. Boundary formation Vascular: regulates angiogenesis, vascular remodelling, cell metabolism Heart: epicardial and coronary vessels development, controls the proliferation in the myocardium, regulates stem cells Hematopoietic system: controls HSCs differentiation, promotes T cell lineage Nervous system: involved in neural development, maintains a slow turnover of cells in the brain Liver: involved in liver regeneration Skin: regulates skin renewal and tissue maintenance Intestine: upregulates enterocyte differentiation, involved in the self-renewal of stem cells in the crypts Lung: controls ciliary cell differentiation	[23]
Hedgehog	Nervous system: regulates both embryonic CNS development and adult neurogenesis Skin and hair: regulates skin regeneration and promotes hair growth Teeth: involved in tooth growth and morphogenesis Bone: helps in proliferation and differentiation during skeletal development, maintains bone structure Tissues: helps in tissue regeneration during injury	[24]
BMP	Involved in bone formation, the development of the tooth, the kidney, the heart and the hypothalamus	[25]

BMPs are produced as large precursor proteins, and once dimerisation and cleavage take place, they undergo maturation. After secretion, BMPs bind to a heterodimeric transmembrane receptor, type I and type II, which has serine-threonine kinase activity. When the ligand binds to the receptor, the type II receptor phosphorylates the type I receptor, activating the type I kinase. Subsequently, the type I kinase phosphorylates a transcription factor from the Smad family, which enters the nucleus and upregulates the expression of target genes. By detecting the expression pattern of various BMPs in different cells or tissues, it is possible to predict whether the corresponding BMP has any potential function. Based on the presence of transcripts, it has been concluded that BMPs are involved in the characterisation of the skeleton, tooth development, the development of lungs, kidneys and skin. These regulators are also expressed in the gonads, heart, hypothalamus, and nervous system, indicating that they play a significant role in these organs as well [25].

Regulation of stem cell fate and differentiation

Stem cells have the potential to self-regenerate or differentiate into specialised cells. To ensure these potentials are maintained, strict regulation is essential. Genetic and epigenetic regulations determine a cell's fate, with transcription factors and chromatin remodelling proteins playing crucial roles. The expression of lineage-specific TFs activates or represses particular sets of genes to govern cell identity, while chromatin remodelling proteins regulate changes in gene expression (34). A complex regulatory network exists to propagate stem cells to their distinct cell fates [35]. Key components of the complex network that regulates pluripotency include transcription factors such as NANOG, OCT3/4, Tbx3, Klf4/5, and SOX2. These TFs either downregulate the expression of genes responsible for lineage differentiation or promote the expression of each other. There are three classes of genes based on the cell lineage they determine: mesendoderm-class genes (e.g., Klf4/5, Nanog, Oct3/4, and Tbx3), neuroectoderm-class genes (e.g., Sox2), and extraembryonic-class genes (e.g., spalt-like transcription factor 4 (Sall4)) [36–38].

During the 4-cell stage of the blastomere, differential expression of epigenetic modifiers occurs, including the histone methyltransferase CARM1. Dimethylation of histone H3 arginine 26 (H3R26me2) regulates the DNA-binding ability of pluripotency transcription factors, SOX2 and OCT4, leading to increased expression of downstream genes. At the 4-cell stage, Sox21, a downstream gene of SOX2, downregulates the expression of caudal type homebox 2 (Cdx2), which specifies lineage for trophectoderm (TE) differentiation [39, 40]. Expression of Cdx2, which the BMP pathway can stimulate, leads to TE differentiation [36]. The ESCs within the blastocysts are of two categories: naïve and primed. Naïve ESCs reside in the ICM of a pre-implanted blastocyst and can differentiate into all three germ layers and primordial germ cells. Primed ESCs are present in the post-implanted blastocyst and can differentiate into all three germ layers. The BMP4-mediated signal is predominant in the early blastocyst stage, promoting self-renewal [41]. Commitment to mesendoderm and neuroectoderm requires extracellular signal mediators that transduce signals through pathways such as WNT, BMP, LIF/STAT3 and Notch. The canonical WNT/β-catenin pathway contributes to body axis patterning, the formation of cells of extraembryonic lineage, and differentiation into mesoderm [42]. LIF/STAT3 signalling pathway activates core

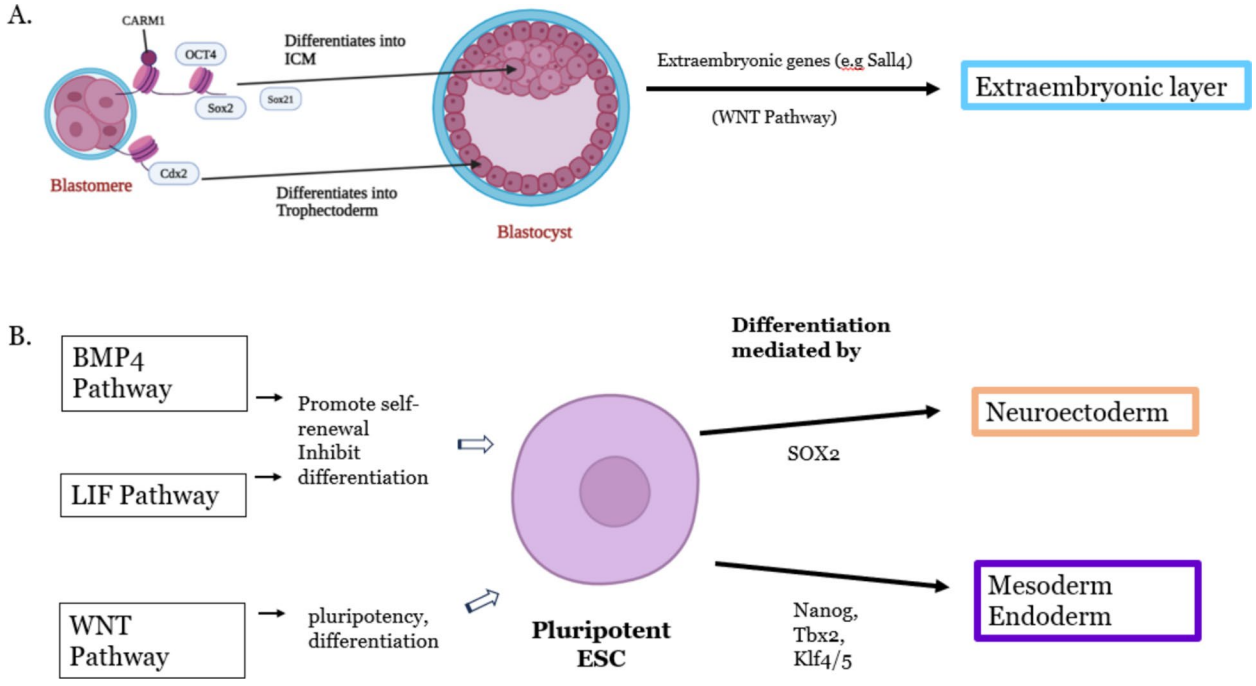


Fig. 2 Signalling networks regulating stem cell fate during organogenesis. **A** Blastomere to blastocyst transition. **B** Regulation of ESC fate via Wnt, Notch, BMP, and LIF/STAT3 pathways

transcription factors, including Octamer binding transcription factor 3/4 (Oct3/4), sex-determining region Y (SRY) box 2 (Sox2), and Nanog, which maintain the self-renewal state [43]. STAT3 can directly bind to OCT3/4, promoting self-renewal and inhibiting differentiation [44]. Therefore, in the absence of the LIF and BMP4 signalling, the ESCs differentiate into cells of mesodermal and endodermal lineages [36, 41]. Depending on the type of signal, Oct3/4 mediates self-renewal or promotes differentiation to determine cell fate [44]. The regulation of stem cell fate is summarised in Fig. 2.

Role of embryonic stem cells and induced pluripotent stem cells in organ formation

Comparison of ESCs and iPSCs in developmental potential

ESCs are derived from the inner cell mass of the blastocyst, which can differentiate into various cell lineages and have self-renewal capacity [45] Similarly, iPSCs reprogrammed from somatic cells by stem cell master regulatory genes (Oct4, Nanog, Sox2, c-Myc/Lin28, Klf4) exhibit similar characteristics to ESCs, including self-renewal ability, which can differentiate into the three major germ layers (ectoderm, endoderm and mesoderm). Pluripotent stem cells can be differentiated into many cell types, including neurons, microglia, cardiomyocytes, kidney cells, pancreatic β cells, skeletal muscle cells, lung cells, liver cells and hematopoietic cells. ESCs and iPSCs hold a promising role in disease modelling, regenerative medicine, and cell therapy [46].

To differentiate into various organs, such as the gastrointestinal tract, lungs, urinary tract, or pancreas, ESC must first differentiate into DE. The intermediate differentiation acts as a biomarker to target lineages of different types of cells. AFP is used as an endodermal marker to identify specific hepatic endoderm and hepatocyte progenitors. Activin A is a well-known inducer involved in nodal signalling for endoderm differentiation. The intermediate mesoderm leads to the formation of gonads and cardiomyocytes. In addition, mesodermal cells contribute to the development of the cardiovascular system, musculoskeletal system, urogenital system, connective tissues and notochord [47].

iPSCs are reprogrammed into pluripotent stem cells by the combination of different transcription factors, such as Oct3/4, Sox2, Klf4, and c-Myc, which support the pluripotency stage. iPSCs are created from a patient's own cells and can potentially differentiate into mature cells. They represent a useful source for cell transplants and are used for studying physiopathological mechanisms in the identification of new therapies. Induced pluripotent stem cell represents a versatile tool for therapeutic and regenerative development [48].

Techniques for directed differentiation into organ-specific lineages

Induced pluripotent stem cell (iPSC) and embryonic stem cell (ESC)-derived lineages are powerful tools to study both disease and development. These in vitro models are

used as a source of unlimited material for experimental manipulation. iPSCs/ESCs can generate any lineage of target cells through directed differentiation. The recapitulation of the lineage sequence was observed in vivo by the addition of growth factors or small molecules as signalling pathways for cell development [49].

Development of essential organs, such as the lungs, esophagus, liver, and stomach, is characterised by signalling pathways in endoderm and mesoderm cells in the fetal foregut. hPSCs differentiate into various types of organ-specific mesoderm. It is derived by signalling interactions and molecular biomarkers, guided by single-cell transcriptomics of foregut organogenesis. These are directed into target organ mesenchyme lineages by a combination of activating or inhibiting WNT, BMP, RA, or HH pathways. iPSCs are reprogrammed from adult somatic cells into a pluripotent state. This is typically achieved by inducing transcription factors, such as Oct4 (Octamer-binding transcription factor 4), Sox2 (SRY-box transcription factor 2), Klf4 (Kruppel-like factor 4), and c-Myc (cellular Myc proto-oncogene), which reprogram the epigenetic profile and gene expression.

Pluripotent stem cells are differentiated into target organ lineages using a direct differentiation method, which involves a combination of cell–cell interactions (co-culture with specific cell lines), exposure to diffusible factors (growth factors), and other positional cues (extracellular matrix) that target a specific cellular identity and function by altering gene expression. The chemical compound (−)-indolactam V activate protein kinase C (PKC) signalling, used to induce differentiation of human ES cells into pancreatic cells [50].

The intracellular mechanotransductive mechanisms have also been used in cellular responses to extracellular nanotopography. These events may combine with classical signal transduction pathways to control stem cell fate, lithographic patterning, pattern transfer, surface roughening, and material synthesis. Lithographic patterning and pattern transfer are other approaches that utilise predefined patterns to create nanotopographical features on two-dimensional planar surfaces. Surface roughening and material synthesis directly generate nanostructures on material surfaces from the bottom up using chemical or physical means. By combining these methods, a wide spectrum of fabrication tools is presented, capable of generating nanotopographical features of various sizes and geometries, as well as hierarchical (micro) nanotopographical surfaces. To successfully utilise stem cell-nanotopography interactions for stem cell applications [51]. Table 3 describes the differentiation protocols and efficiency Metrics.

Figure 3 describes the differentiation pathways of ESCs and iPSCs into organoids derived from ectoderm, mesoderm, and endoderm.

Table 3 Differentiation protocols and efficiency metrics

Target organ	Cell source	Key inducing factors	Culture method	Efficiency	References
Cardiomyocyte	ECs	BMP-4 + VEGF followed by M3534	3D culture	44.03%	[52]
Hepatocyte	iPSCs	Activin A, FGF2, CHIR99021	2D culture	97.97% double-positive cells	[52]
Lung cells	ESCs, iPSCs	CHIR99021 inhibitor	2D culture induction, 3D organoid	20%–30%	[53]
Pancreatic β cell	ESCs iPSCs	Activin A + FGF7, RA + EGF + Nicotinamide, ALK5iII + T3 + Gamma-secretase inhibitor, R428 + N acetyl cysteine	2D planar culture. 3D suspension culture	60%–82%	[54]
Hematopoietic stem/progenitor cells	ESCs	OP9 mouse stromal cell line	2D co-culture system	2.47%–10%	[55]

Mesenchymal stem cells in tissue regeneration and repair

Characteristics and regenerative mechanisms of MSCs

Mesenchymal stem cells (MSCs) are multipotent stromal cells with self-renewal capacity and multilineage differentiation. They predominantly reside in the mesenchyme and connective tissue. They have restricted differentiation capacity and give rise to certain types of lineages in specific cells, including bone, cartilage and fat cells. MSCs are characterised by immunomodulatory effects, cytokine secretion and an extracellular reservoir with regenerative properties. A well-established consensus driven by multipotent efficiency under standard culture conditions, expression of characteristic biomarkers CD90, CD73 and CD105, and absence of hematopoietic surface markers including CD14, CD19, CD34, CD79 and HLA-DR. These biomarkers help to induce the MSC population [56].

Under in vitro cultural conditions, MSCs can be directionally differentiated into multiple mesodermal lineages: adipocytes, osteoblasts, chondrocytes and myocytes [57]. MSCs in bone marrow can differentiate into adipose tissue, cartilage, bone, and skeletal muscle, regulated by certain regulatory factors (MRFs): Myf5, MyoD, myogenin, and MRF4. The process of differentiation can be induced by transcription factors, which are responsible

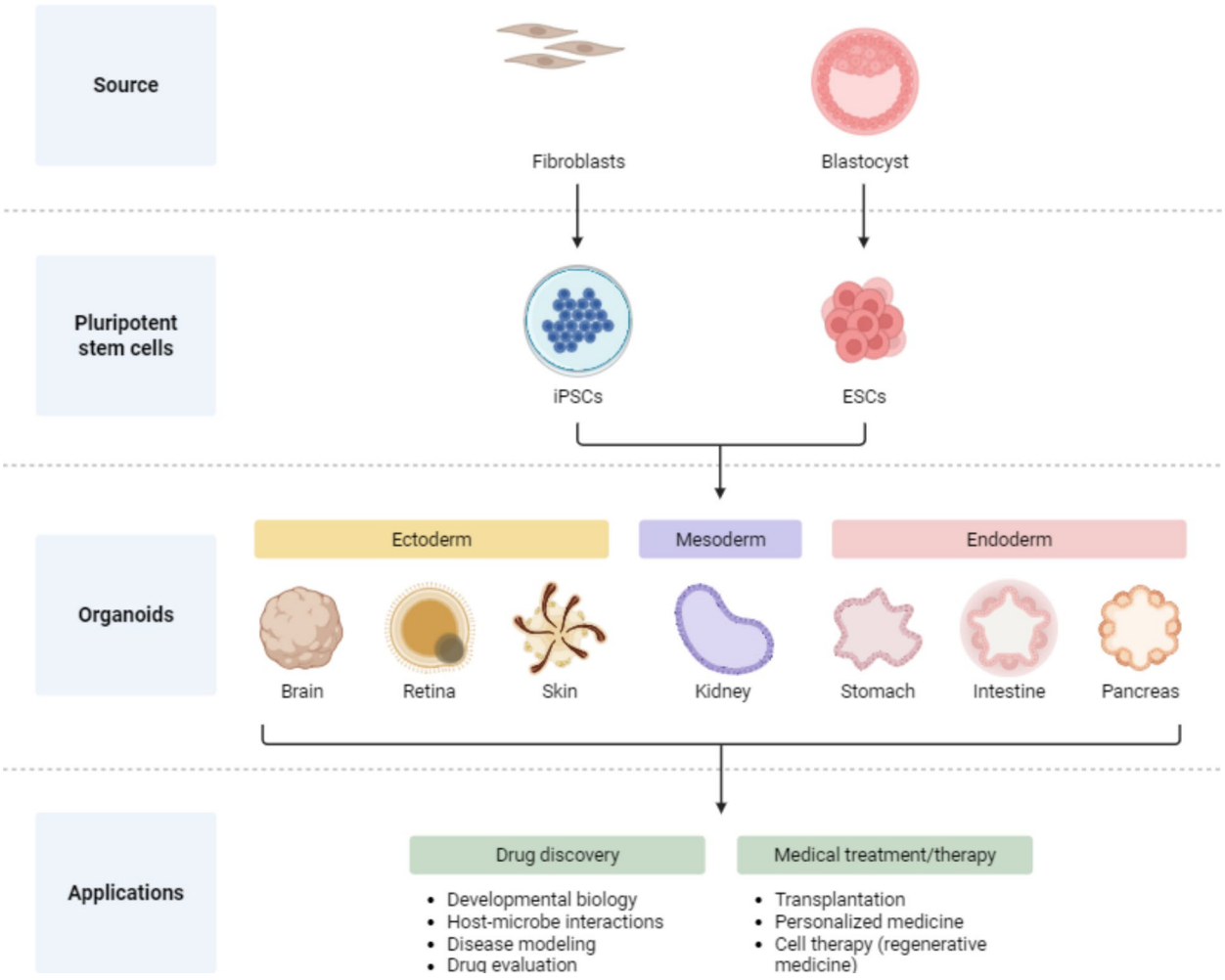


Fig. 3 Flowchart of ESC and iPSC differentiation pathways

for the multiple molecular mechanisms, signalling pathways, and epigenetic regulators [58].

MSCs are highly secretory cells, much of their regenerative potential attributed to paracrine activity. They release certain growth factors such as (NGF), (BDNF), (HGF), and (VEGF), which can activate the neuroprotection, angiogenesis and tissue repair mechanisms. The secretome induces strong immunomodulatory effects by inhibiting T cell proliferation, releasing cytokines and promoting pro pro-regenerative immune environment. The adaptive immunity induced by T and B cells inhibits Th1/Th17 phenotypes and supports cytokines such as TGF-β and IL-10. Moreover, they release extracellular vesicles and enzymes such as indoleamine 2,3-dioxygenase (IDO), which mediate T cell suppression and immune tolerance. The secretion of prostaglandins through cell-to-cell interaction by soluble factors enhances their immunosuppressive effects [59].

Therapeutic applications in organ repair

Regenerative medicines have unprecedented properties to repair organ function through targeted therapeutic approaches. MSCs have the main therapeutic application in accelerating to repair of damaged tissues, modulating immune response and autoimmune disease. In dermatological repair, MSCs have therapeutic applications in skin regeneration in damaged tissues by inhibiting microfibroblast activation or fibrosis, which promotes vascular stability and angiogenesis. MSCs communicate through cell–cell interaction, paracrine signals, cytokines, growth factors and chemokines. It demonstrates therapeutic applications in neural regeneration, liver and cardiac repair.

MSCs have a potential contribution to the neuron repair mechanism to suppress central nervous system inflammation. They promote remyelination and paracrine functions by secreting growth factors, or promote neuronal survival by neurogenesis and neurotrophic factors. In case of Immunomodulation, they transfer

Table 4 MSC sources and comparative regenerative potential

Source of MSCs	Yield	Proliferation capacity	Differentiation potential	Immunomodulatory properties	Regenerative applications	References
Bone marrow	Low	Moderate	Chondrogenic, Adipogenic	strong against T-cell alloreactivity and proliferation	Orthopaedic and Osteogenic capacity	[63]
Adipose tissues	High	Moderate to high	Higher osteogenic capacity	strong	Soft tissue repair and wound healing,	[64]
Dental pulp	High	High	Osteogenic differentiation capacity	Very strong	Hepatic pulmonary regeneration	[63]
Umbilical cord	High	Moderate	Multipotent	Very strong	Neurological regeneration	[65]

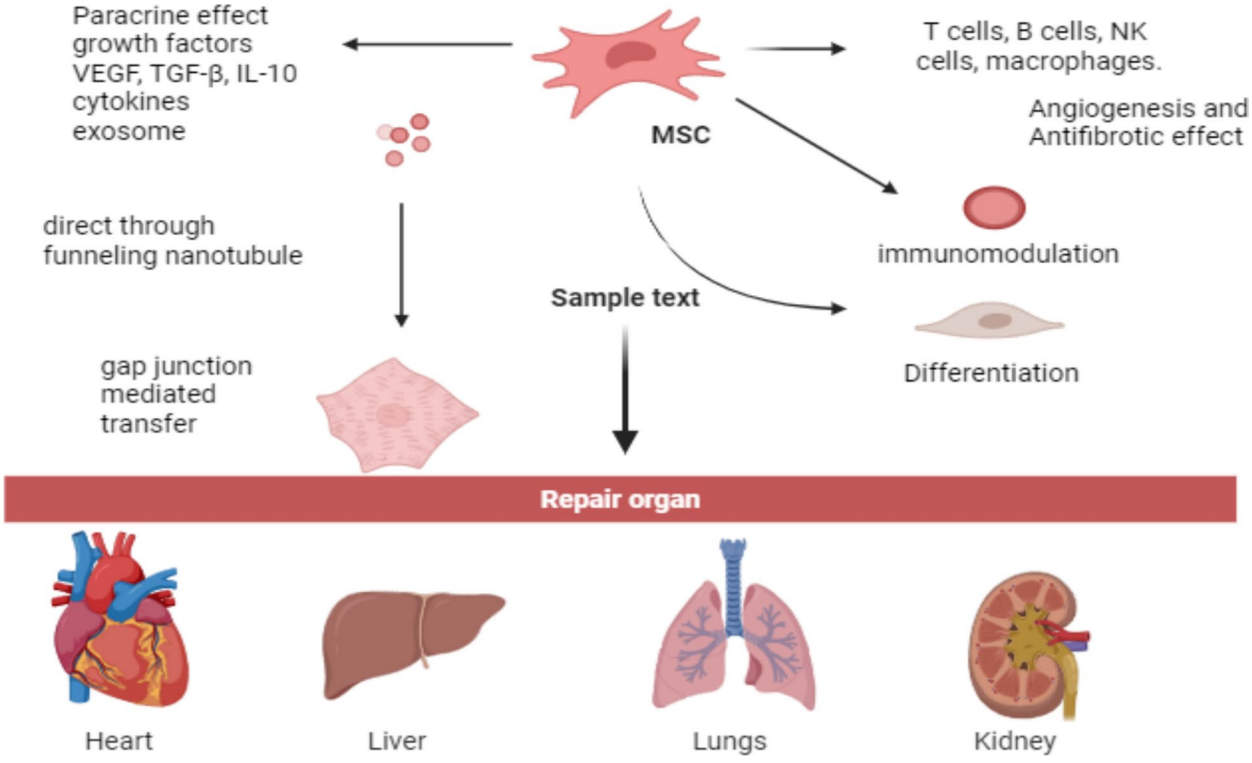


Fig. 4 Illustrates how mesenchymal stem cells (MSCs) repair organs through paracrine signalling, direct cell transfer, immunomodulation, angiogenesis, antifibrotic effects, and differentiation, ultimately facilitating the regeneration of the heart, liver, lungs, and kidneys

mitochondria to target cells for organ repair and inhibit ferroptosis by reducing oxidative stress [59]. In cardiac repair mechanisms, therapeutic application in myocardial function approaches Anti-inflammatory and Chemokine modulation. Cardiac repair function can be supported by targeting anti-inflammatory pathways to improve cardiac function and its regeneration. Inhibition of cytokine IL-1β and IL-6 reduces cardiac remodelling, targeting chemokine pathways such as CXCL12/SDF-1 support recruitment of reparative cells. Supplementation of growth factors like PDGF-AB improves tissue regeneration in cardiac repair function. Cell-based immunomodulation therapies can target specific cells to induce positive outcomes by B-cell depletion or regulatory T-cell amplification [60].

Mesenchymal stem cells (MSCs) show considerable therapeutic potential in kidney repair mechanisms due to their regenerative and immunomodulatory properties. In acute kidney injury, it reduces oxidative stress, inhibits cytokines, and accelerates tissue regeneration. In kidney disease, it may slow down disease progression through its anti-fibrotic effect and anti-inflammatory properties. In kidney repair and transplantation, it reduces graft rejection by immunosuppression and improves its function [61].

In liver repair mechanisms, it promotes the regeneration of liver cells through direct differentiation and paracrine mechanisms. In addition to reducing oxidative stress to support hepatocyte vitality, their immunomodulatory property transfers macrophages towards anti-inflammatory phenotypes, modulates T cell activity and reduces extracellular trap formation and neutrophil infiltration. Moreover, it also exerts an anti-fibrotic effect by inhibiting the activation of hepatic cells, as well as the

biomarkers α-SMA and collagen type, thereby alleviating lipid accumulation and improving hepatocyte damage. In pulmonary regeneration, it promotes epithelial repair, reduces inflammation and alleviates fibrosis. It protects the lungs through angiogenesis and restores function by transferring mitochondria to the target cell. These actions suggest a promising candidate for treating chronic lung injury and respiratory diseases. Meanwhile, MSCs validate the potential role in organ repair mechanisms; particularly, well-designed clinical trials are needed [62]. Table 4 represents the MSC Sources and Comparative Regenerative Potential (Fig. 4).

Organoids as models to study organogenesis

Overview of organoids

Organoids are 3D (three-dimensional) in vitro cell cultures that recapitulate the structure, function, and physiology of human organs [66]. Unlike 2D monolayer cultures, organoids are grown as 3D structures. Remarkably, organoids can self-organise, differentiate, and form such complex structures that they are reminiscent of tissues in vivo [67]. They originate from two major cell sources, namely pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and adult stem cells (adSCs), and are acquired by isolating tissue-specific niches [68]. Upon culturing in permissive extracellular matrix scaffolds, such as Matrigel, and in the presence of defined signaling factors, these stem cells give rise to lineage-specific assemblies and form miniature organ-like structures [69].

In this context, one significant breakthrough was achieved by Sat and colleagues, who in 2009 demonstrated that Lgr5 + adult stem cells could be used to generate intestinal organoids. [70, 71]. This important discovery laid the foundation for organoid culture systems and stimulated the fast development of the field. Since then, various types of organoids have been successfully developed, including cerebral organoids, hepatic organoids, retinal organoids, gastric organoids, and kidney organoids [72]. By utilising new technologies such as CRISPR-Cas9-based genome editing, microfluidics, and bioengineered scaffolds, researchers have further increased the physiological relevance of organoids, enabling their use in studying human development and pathology [73].

The fact that organoids can be used to overcome the drawbacks of animal models is one of the greatest strengths. Meanwhile, animal systems have played a crucial role in developmental biology; however, they are usually unable to reflect species-specific elements of human physiology. Organoids also offer benefits over immortalised cell lines, which, although commonly used, lack the tissue architecture or microenvironmental cues to recapitulate organogenesis. Consequently, organoids have become dynamic systems for tracking lineage specification, tissue morphogenesis, and spatial signalling interactions during development.

Despite these advantages, organoids also have several intrinsic shortcomings that need to be recognised. Many organoid systems maintain a predominantly fetal identity and never fully mature; this fundamentally limits their ability to model physiology in adult tissues [74]. In addition, the absence of vascularisation limits nutrient and oxygen diffusion, thereby constraining their long-term growth and structural complexity [75]. Organoids also do not receive complete immune, stromal, and mechanical microenvironmental signals, another prerequisite for faithfully recapitulating in vivo organogenesis [76]. Together, these factors contribute to variability, impair reproducibility, and create barriers to high-throughput drug screening and disease modelling. Identifying these limitations is essential to the interpretation of organoid-based findings and informs future technological development.

Applications in development biology and biological modelling

Developmental biology

Organoids are unique systems that can be used to study the cellular and molecular mechanisms that regulate organogenesis [72]. Lancaster and others have used cerebral organoids to investigate the fundamental mechanisms of cortical layer development, neuronal migration and synaptic formation [77]. These models have provided a greater understanding than ever before about the development of the human brain, such as the folding of the neocortex, the functionality of radial glial cells that cannot be studied in the living brain because of ethical and technical reasons [78].

PSC-derived kidney organoids are, just as well, transformative. They revert towards elements of nephrogenesis, such as nephron differentiation and branching morphogenesis [79]. It is hoped that this renders them invaluable in the dissection of the cellular and molecular basis of renal development and of congenital kidney disorders. Retinal organoids that differentiate into layered retinal structures with photoreceptors, bipolar cells, and ganglion cells have been commonly utilised to study eye development and congenital retinal diseases [80]. Both the PSCs and AdSCs give rise to hepatocyte- and cholangiocyte-like populations in hepatocyte-like organoids of the liver, allowing the study of bile duct morphogenesis and liver tissue development in detail [81].

These heterogeneous organoid platforms share a fundamental feature; they can recapitulate the interaction between major signalling pathways, including Wnt, Notch, Hedgehog, and BMP [82]. Modulating these pathways enables researchers to investigate lineage

specification and tissue patterning with exceptional precision. Accordingly, organoids are convenient but biologically relevant models to investigate the role of the self-organisation of stem cells into functioning organs. [83].

Disease modelling

Besides development, organoids have changed disease modelling. A well-known example is the use of intestinal organoids obtained from a healthy patient to model cystic fibrosis (CF) [84]. The forskolin-induced swelling (FIS) assay in such systems has become a useful functional readout of CFTR channel activity that researchers can use to test patient-specific responses to drugs [85]. Similarly, kidney organoids have been used to re-create polycystic kidney disease (PKD), in which case, the cyst-like structures spontaneously occur in culture, similar to the pathology of the disease [86].

Cerebral organoids can be useful in modeling neurological diseases. They have, in turn, been used to examine microcephaly, and it has been found that mutations that affect neurogenesis cause loss of cortical expansion [87, 88]. They have also been used as models of Zika virus infection, Rett syndrome, and Down syndrome effects to provide valuable information on mechanisms of impaired brain development [89]. Organoids resembling degeneration diseases such as retinitis pigmentosa have been modelled in retinal organoids, and diseases such as 1-antitrypsin deficiency and viral hepatitis B and C have been modelled in hepatic organoids [90].

Another way in which organoid technology has revolutionised cancer research is in the field of cancer research. Tumoroids or tumour-derived organoids maintain the genetic, histological, and phenotypic characteristics of the parental tumour. They are also being applied to screen drugs in the field of precision oncology, where tumoroids are used to test therapies on patient-derived material to guide clinical decision-making. Translational applications have also been accelerated by the formation of large-scale organoid biobanks for cancers such as colorectal, pancreatic, and breast tumors [91].

Furthermore, apical-out intestinal organoids represent a specialised form of 3D cell culture in which the apical surface of the intestinal epithelial cells is exposed outwards, making the lumen-facing interface directly accessible. The unique orientation enables the researchers to investigate nutrient uptake, drug delivery, and pathogen interactions at a better level compared to traditional basal-out organoids [92]. These are normally produced by centrifuging conventional organoids either through

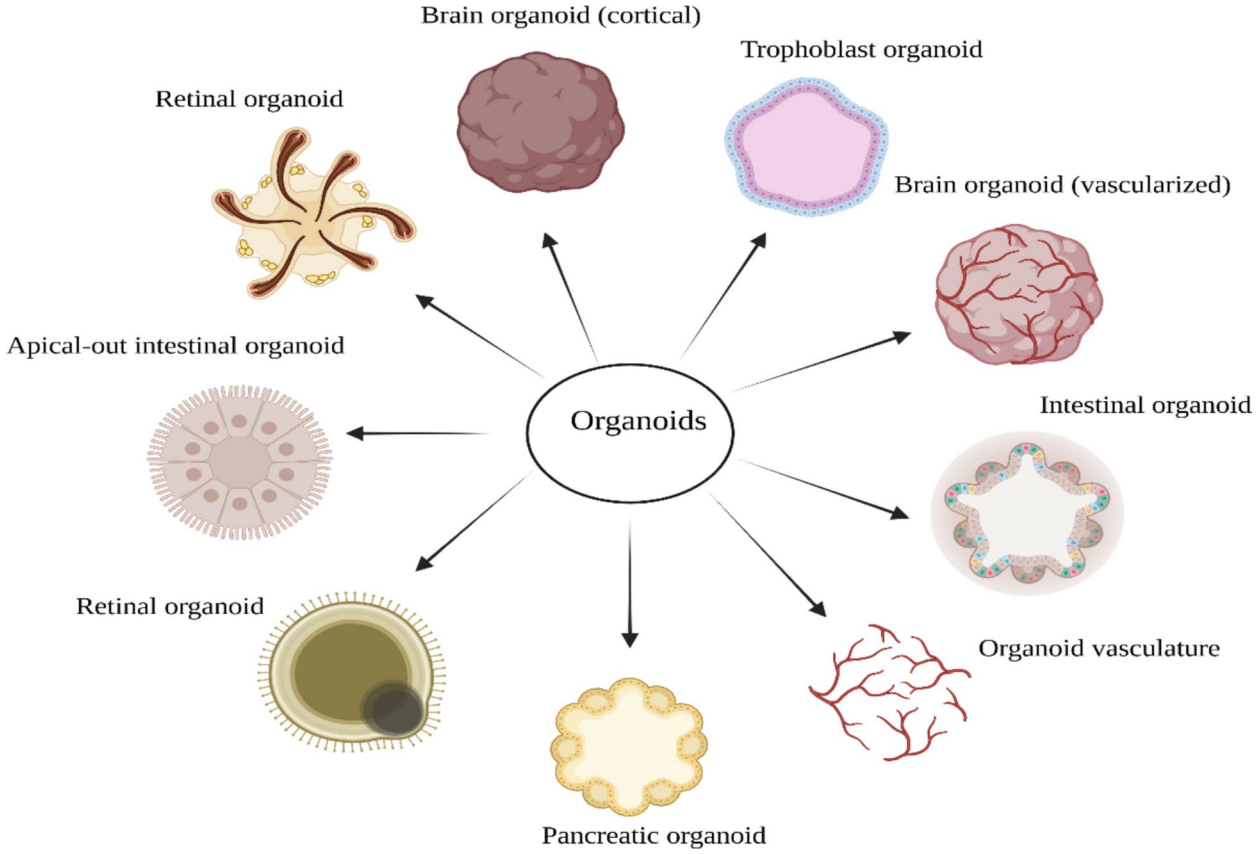


Fig. 5 Representative 3D organoids, including cerebral, intestinal, retinal, and hepatic models, showing structural resemblance to in vivo tissues

mechanical destruction or perturbation of the extracellular matrix. Apical-out organoids are highly reminiscent of the physiological roles of the native intestine, and serve as an ideal model system with which to study barrier functionality, the interactions between hosts and their microbes, and epithelial transport. Due to their accessibility and functional relevance, these organoids are increasingly being utilised in personalised medicine, infection research and preclinical drug resourcing [93].

Individualised medicine is also a thrilling field. Since organoids can maintain patient-specific genetic and epigenetic characteristics, individualised drug testing and optimisation of treatment can be performed [94]. One such example in CF is that patient-derived organoids can predict clinical effects of CFTR modulators in advance, and such prediction has direct therapeutic implications [95]. Biomaterials engineering, combined with new technologies like single-cell transcriptomics, CRISPR screening, and organoids, will enable even better representations of disease and therapeutic discovery platforms [95]. Figure 5 demonstrates the organoids.

Table 5 lists the key classes of organoids, their sources of stem cells, and the organs or tissues they recapitulate. PSCs or AdSCs derived intestinal organoids replicate the colon and the small intestine. The PSC-derived cerebral organoids recapitulate forebrain and cortical maturation. Hepatic organoids recapitulate hepatocytes and bile ducts, and retinal organoids develop a layered retina with photosensors. Kidney organoids produce nephron-like structures, and patient biopsies produce tumoroids, which are cancer tissues. Lung organoids recapitulate alveoli and airways, skin/hair organoids recapitulate epidermis and sweat glands, and eye organoids recapitulate lens and retina.

Figure 5 illustrates the various types of organoids derived from stem cells, including brain (cortical and vascularized), trophoblast, intestinal, apical-out intestinal, retinal, pancreatic, and vascular organoids. Each is a three-dimensional model that replicates the structure and functionality of the related human tissues.

Table 5 Types of organoids and derived organs or tissues

Organoid types	Stem cell source	Derived organs / tissues	References
Intestinal	PSCs, AdSCs	Small Intestine, colon	[96]
Cerebral (brain)	PSCs	Forebrain and cortex structures	[97]
Hepatic (liver)	PSCs, AdSCs	Hepatocytes and biliary duct structures	[98]
Retinal	PSCs	Retinal layers, photoreceptors and ganglion cells	[99]
Kidney	PSCs	Nephrons and branching tubules	[100]
Tumoroids (cancer organoids)	Tumour biopsy samples	Colorectal, pancreatic, breast and prostate tumours	[101, 102]
Lung	PSCs	Alveolar tissue and airways	[103]
Skin and hair	hiPSCs	Epidermis and sweat glands	[104]
Eye	PSCs	Lens and retina	[105]

Stem cell niches and microenvironmental regulation in organ development and regeneration

Components and influence of the stem cell niche

Stem cells are a distinct population of undifferentiated cells that have both self-renewal and the ability to differentiate into progeny of specialised lineages [106]. However, these intrinsic capabilities are not achieved singly. Instead, they are strongly influenced by a highly specialised microenvironment, the stem cell niche [107]. In addition to providing structural support for the tissue, the niche also contains biochemical, mechanical, and systemic cues that regulate cell fate during organogenesis and tissue regeneration [108].

At the most basic level, the niche is composed of several cellular and acellular structures. The endothelial cells, differentiated progeny, immune cells, and stromal fibroblasts are among the cellular elements with important roles [106]. Stromal fibroblasts secrete growth factors, including fibroblast growth factor (FGF) and hepatocyte growth factor (HGF), along with cytokines that support stem cell proliferation and regulate differentiation [109]. Angiocrine signalling from the endothelial cells of the vascular niche, with secretion of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and angiopoietins [106]. Besides promoting stem cell expansion, these signals also ensure there is appropriate delivery of nutrients and oxygen—a particular need in highly metabolic tissues such as the bone marrow or liver [110].

Immune cells, in particular macrophages and regulatory T cells, exert a major role in defining the inflammatory tone of the niche and thus impinge on stem cell performance [111]. The balance between pro-inflammatory and anti-inflammatory cytokines dictates whether stem cell function is enhanced or compromised [112]. M2 macrophages produce interleukin-10 (IL-10) that promotes tissue regeneration, while chronic pro-inflammatory signaling by M1 macrophages promotes stem cell exhaustion and fibrosis. Hence, immune regulation introduces a highly dynamic and context-dependent layer to niche behaviour [113–115].

Another important dimension is through feedback from differentiated progeny. Paneth cells in the intestinal crypt are the primary sources of Wnt ligands and EGF, and Notch ligands, which directly interact with Lgr5 + intestinal stem cells [116]. The replenishment of the precise number of cells required to turn over epithelial cells is provided by this feedback mechanism, which regulates the number of stem cells [117]. Similarly,

mature osteoblasts in the hematopoietic niche release angiopoietin-1 and osteopontin. These molecules keep hematopoietic stem cells (HSC) inactive. They also stop early cell changes [118].

Outside the local area, systemic regulators have a large impact. In addition to the local niche, systemic regulators also have significant roles. Hormonal messages, including glucocorticoids, the insulin-like growth factor (IGF), and thyroid hormones, transmit the state of metabolism, the state of circadian rhythms, and the state of development to the stem cell niche [119]. Sympathetic inputs within the marrow and neural innervation control HSC mobilisation and homing. Systemic stress messages, including hypoxia and changes in the state of metabolism, alter the rate of stem cell proliferation and lineage fate [120].

At the embryo stage, stem cell niches represent instructive microenvironments that actively guide morphogenesis. Embryonic cortex neural stem cell niches, for example, organise subtype specification of neurons, radial migration, and cortical layering [121]. By contrast, at the adult stage, niches broadly maintaining stem cell quiescence induce the activation of regenerative programs after tissue injury [122]. This is particularly evident in the skeletal muscle case, in which satellite cell niches lie quiescent before the activation by trauma of fibro-adipogenic progenitors and inflammatory cells that release activation signals [123]. Accordingly, the niche cannot be regarded as a structural compartmental housing stem cells; it represents an adaptive regulatory system at the heart of both organ development and tissue repair [124].

Extracellular matrix and paracrine signalling

One of the defining features of the stem cell niche is the extracellular matrix (ECM). Previously considered a passive scaffold, the ECM is now recognised as an active and highly complex structure that governs virtually all aspects of stem cell biology [125]. It provides not only structural support but also acts as a reservoir for soluble growth factors, regulates the mechanical properties of tissues, and transmits biochemical and biomechanical signals to resident stem cells [126]. These signals are conveyed largely by receptors such as integrins and cadherins and by focal adhesion complexes, which convert extracellular stimuli into intracellular cascades of signals that specify cell fate and lineage decisions [127].

The ECM protein composition is heterogeneous and niche-dependent, with proteins present in most of the niches. The main structural and functional constituents are laminins, collagens, fibronectin, and glucosaminoglycans [128]. Basal laminae containing laminin play a crucial role in maintaining progenitors of neural and epithelial lineages polarised and functionally separated [129]. Fibronectin, however, plays a major role in wound-healing microenvironments, where it acts as an adhesive surface and guides progenitor cell migration [130]. Glycosaminoglycans, such as heparan sulfate, possess unique biochemical properties, including the capacity to bind and stabilise morphogens like Wnts and FGFs, thereby regulating local signaling gradients [131]. Collagens, particularly type IV, provide structural rigidity but influence the balance between quiescence and differentiation within stem cell populations [132].

One of the most striking influences of the ECM is in its mechanical property, which regulates stem cell function through a process known as mechanotransduction [133]. A landmark study demonstrated that mesenchymal stem cells (MSCs) modify their differentiation lineage according to substrate stiffness [134, 135]. MSCs on soft substrates resembling brain tissue (0.1–1 kPa) are tending towards adopting neuronal characteristics. Intermediate stiffness substrates (~10 kPa), akin to muscle, stimulated adipogenic differentiation, while rigid substrates (~40 kPa), akin to bone, induced osteogenesis [136, 137]. These findings exhibited that physical signals alone are sufficient to dictate lineage specification in the absence of any exogenous growth factors. Mechanistically, these effects are mediated via integrin clustering, activation of focal adhesion kinase (FAK), reorganisation of the actin cytoskeleton, and nuclear translocation of mechanosensitive regulators such as YAP and TAZ. ECM thereby directly links the physical environment with transcriptional programs, regulatory stemness, and differentiation [138].

During organogenesis, ECM remodelling is essential for morphogenesis to arise. In the intestine, laminin isoforms in the crypt microenvironment help preserve stem cell identity and proliferation. In the liver, collagen IV preserves hepatoblast polarity and their differentiation into mature hepatocytes [139]. Similarly, in neural stem cell niches, the laminin-rich ECM of the ventricular zone provides radial glial progenitors with positional and polarity cues, orchestrating their controlled proliferation and differentiation into neurons and glia [140]. These examples illustrate how ECM composition is carefully adapted to the needs of each organ, producing permissive environments for maintenance and development.

In total, the ECM and paracrine signalling constitute a tightly coupled regulative network at the core of every stem cell niche. The ECM delivers structural support and dynamic biochemical and mechanical cues, while paracrine signals generate context-dependent gradients that influence stem cell decisions [141]. Their convergence produces an adaptive environment capable of guiding organ formation, maintaining tissue homeostasis, and initiating repair upon injury. In balance, this system allows for maintenance of stem cell function and tissue integrity. In breakdown, it causes degenerative disease

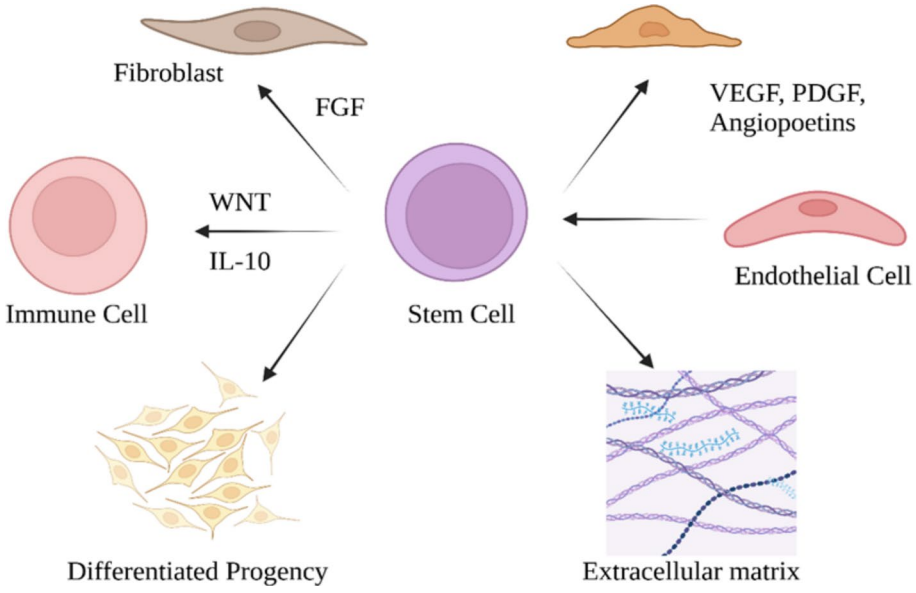


Fig. 6 Stem cell niche interactions, highlighting roles of fibroblasts, endothelial cells, immune cells, and ECM in regulating self-renewal and differentiation

Table 6 Key factors across different organs

Organ system	Key cellular components	ECM components	Dominant signalling pathways	Functional role in niche	References
Intestine	Paneth cells, stromal fibroblasts	Laminin, collagen IV	Wnt, Notch	Maintain Lgr5 + intestinal stem cells, regulate crypt-villus axis	[116]
Bone Marrow	Osteoblasts, endothelial cells, and macrophages	Osteopontin, fibronectin	Notch, CXCL12/SDF-1	Maintain HSC quiescence, regulate mobilisation	[143]
Brain	Radial glia, astrocytes, endothelial cells	Laminin-rich basal lamina	Notch, BMP, Shh	Regulate neurogenesis and cortical layering	[140]
Liver	Hepatic stellate cells, endothelial cells	Collagen IV, laminin	Wnt, HGF, TGF-β	Support hepatocyte and cholangiocyte progenitors during regeneration	[144]
Skeletal Muscle	Satellite cells, fibro-adipogenic progenitors	Fibronectin, collagen VI	Notch, Wnt	Activate regenerative programs after injury	[145]

and malignancy. Additional clarification of how ECM mechanics and paracrine pathways intersect will not only enhance our understanding of developmental biology but also inform the development of regenerative therapies aimed at restoring normal niche function in diseased or injured tissues [142]. Figure 6 mentioned stem cell signalling interactions.

Table 6 highlights the key constituents of stem cell niches in various organ systems. It describes the most important supporting cells, extracellular matrix (ECM) components, and the most important signalling pathways that control stem cell activity. Crypt stem cells are maintained in the intestine by Paneth cells and Wnt/Notch signals. The osteoblasts and CXCL12 used in maintaining hematopoietic stem cells are found in bone marrow niches. Radial glia and BMP/Shh signalling are used to form brain niches neurogenically. The WNT and TGF-β pathways recruit hepatocyte and cholangiocyte progenitors to the liver microenvironment, whereas skeletal muscle niches initiate regenerative programs through satellite cells and the Notch/Wnt pathway.

Figure 6 illustrates the intricate interactions in the stem cell niche. Niche components, including fibroblasts, endothelial cells, immune cells, and the extracellular matrix, influence the behaviour of stem cells. Many important signalling molecules, including fibroblast growth factors (FGF) secreted by fibroblasts, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and angiopoietins secreted by endothelial cells, promote stem cell survival and vascularisation. Stem cell behaviour and differentiation are regulated by immune cells through a series of pathways, including the WNT and IL-10 pathways. Taken together, these signalling pathways or cues coordinate self-renewal and differentiation into specialised lineages, which is adequate to promote tissue development and regeneration.

Mechanisms of stem cell-mediated organ regeneration

Organ regeneration, defined as the reconstruction of an organ's mass and functional capacity following injury, has been observed in many lower vertebrates and invertebrates [146–148]. The adult human body usually has a limited ability to regenerate complex organs (Brain, Heart muscles) [149–151]In most cases, tissue repair results in scar tissue rather than complete restoration of the original structure and function of the organ [152]. This limitation is a main reason for the field of regenerative medicine. It aims to create external treatments for various degenerative diseases and traumatic injuries [153, 154]. Stem cells are defined by their unique ability to renew themselves and differentiate into various cell types. They have become a key part of this new treatment approach [155, 156]. Stem cell-based therapies offer a promising approach that extends beyond alleviating symptoms. They help improve the basic cellular and functional problems of a damaged organ [157].

Stem cell-mediated regeneration happens through two main, non-exclusive mechanisms. The first process is direct differentiation and cell replacement [158]. Adult multipotent stem cells turn into damaged or lost cell types. This helps keep tissues balanced and regenerates specific lineages after an injury [159]. For example, the formation of myocytes from cardiac stem cells, neurons and glial cells from neural stem cells, and MSCs developing into hepatocytes or neurons [160]. However, a more recent and significant shift has focused on the second mechanism, the paracrine effect. Stem cells have functions beyond direct cellular replacement and engraftment. This involves the transient release of various factors (cytokines, chemokines, and extracellular vesicles) which send signals to the adjacent cells in the damaged tissues to promote regenerative mechanisms, including cell regeneration, growth, specialisation, neovascularisation, and modulation of immune responses [161–163]. The fact that transplanted cells may not last long but still cause lasting regenerative effects strongly supports this mechanism. From this perspective, transplanted stem cells function as "drug factories," releasing a combination of therapeutic proteins in response to real-time signals from their environment.

Mammals have a paracrine-based repair mechanism, an evolutionary strategy from an all-out direct regenerative response. A localised, signal-based repair mechanism is both effective and sufficient for most minor injuries in complex, long-lived organisms. Tumorigenesis risk is increased when a large, full-organ regenerative response which consumes enormous energy. Therefore, stem cell therapy, by leveraging and amplifying this intrinsic paracrine mechanism, does not introduce a new biological process but rather supercharges an existing one. This shifts the goal from simply replacing lost cells to enhancing signals for the body to heal itself.

Post-injury cellular and molecular responses to organ damage

The process of regeneration is initiated by the body's response to an injurious agent, which can be a physical, chemical, or biological damage [164]. During injury, to restore homeostasis and protect the site from further damage, the body adopts an initial mechanism called the Cellular Response [165]. But when the injury is severe and prolonged, these intrinsic protective mechanisms become overbearing, resulting in cellular dysfunction and death [166].

Cell death occurs mainly by two mechanisms: programmed cell death (apoptosis) and unprogrammed cell death (necrosis) [167]. Ischemia–reperfusion injury (IRI) is the best example in this context, where initially the lack of oxygen during the phase of ischemia is followed by an instant re-establishment of blood supply [168]. Surprisingly, the reperfusion phase can make the initial damage much worse. This is mediated by a series of molecular events, including a rapid depletion of adenosine triphosphate (ATP), leading to the failure of energy-dependent ion pumps and a subsequent ionic imbalance, notably an overload of intracellular calcium (Ca2 +). When the blood flow (oxygen) is instantly reestablished, it triggers a burst of Reactive Oxygen Species (ROS), resulting in oxidative stress and damage to intracellular structures [169]. This overproduction of ROS activates transcription factors like nuclear factor-kappaB (NF – κB), which further escalates the inflammatory response and leads to additional cellular damage [170].

After this initial phase of injury (damage and inflammation), a complex network of signalling pathways is activated to start the repair process. It involves complex cellular dynamics during the repair process, as shown in Fig. 7. This regenerative response re-expresses key developmental pathways, such as WNT, Hedgehog (Hh), BMP, Notch, and IGF. All these pathways play a significant role in tissue proliferation and differentiation [171]. Activation of all these pathways is not random; rather, it is a direct response to specific molecular signals produced at the site of injury. For example, in zebrafish kidney regeneration, acute injury prompts renal interstitial cells to significantly increase Cox2a enzyme [172, 173]. This leads to the production of prostaglandin E2 (PGE2). Now, this lipid molecule starts the proliferation of renal progenitor cells by stabilising the key effector of the Wnt pathway. Similarly, Notch signaling has shown peripheral nerve regeneration by restoring Schwann cell phenotypic changes [174]. The Hh-GLI2-CKS1B axis was identified as a specific target for improving cardiac tissue engineering and regenerative therapies, which proves

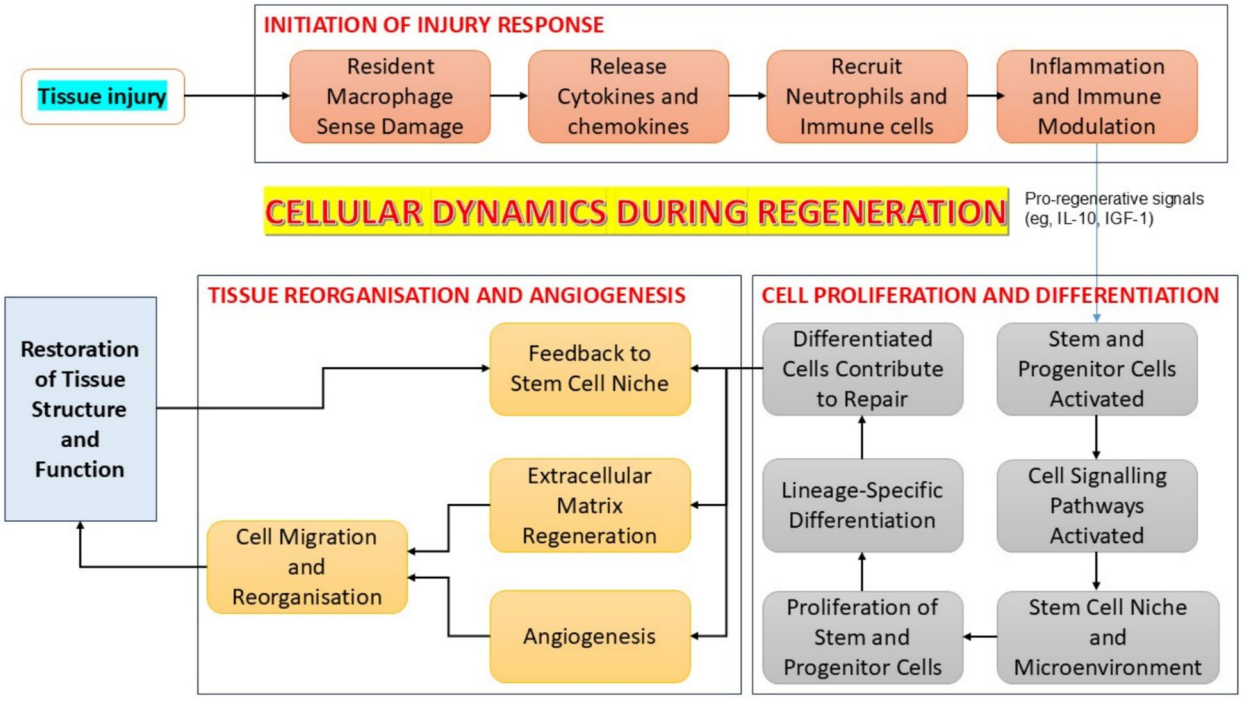


Fig. 7 Cellular dynamics during regeneration. **IL-1* Interleukin 1, *IGF-1* Insulin Like Growth Factor

a connection between post-injury molecular events and the activation of specific regenerative signals [175].

Role of inflammation and immune modulation

Inflammation is an important process for tissue repair and regeneration. The inflammatory response is not simply a negative outcome of injury, but a controlled series of events that starts the healing process. This process has two phases: the pro-inflammatory phase and the pro-reparative phase [176, 177].

Macrophages play a key role in this response. When an injury occurs, monocyte-derived macrophages migrate to the site and take on a pro-inflammatory (M1) phenotype. In this state, they efficiently clear cellular debris and pathogens through phagocytosis and release pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-12 (IL-12), and tumour necrosis factor-alpha (TNF – α) [178, 179]. This phase is important for getting tissue ready for repair. Once the inflammatory signals decrease, these macrophages change to a pro-reparative (M2) phenotype. M2 macrophages secrete a variety of pro-proliferative and anti-inflammatory factors, including transforming growth factor-beta (TGF-β), prostaglandin E2 (PGE2), and interleukin-10 (IL-10), which accelerate tissue regeneration, promote angiogenesis (new blood vessel formation), and facilitate the remodelling of the extracellular matrix (ECM) [180]. Any alterations or disruptions in this M1-M2 transition result in chronic inflammation and the development of fibrosis.

The microenvironment of an injured organ is significantly influenced by inflammation and other factors, like DNA damage. Ongoing DNA damage can lead to a lasting state of cell cycle arrest known as senescence [181]. This condition is marked by the release of senescence-associated secretory phenotype (SASP) factors. These SASP factors are strong immune modulators. They can either support regeneration or, with long-term exposure, cause paracrine senescence in nearby cells [182]. This process can reduce regenerative signals. This indicates that a careful balance is necessary. A controlled macrophage-induced inflammatory response under guided therapy can promote tissue regeneration. However, a long-lasting, uncontrolled response worsened by ongoing DNA damage and SASP is degenerative.

Stem cells, such as mesenchymal stem cells (MSCs), have immunomodulatory properties that enable them to interact and modulate this complex microenvironment. MSCs at the sites of injury suppress immune responses by reducing the pro-inflammatory cytokines and increasing the secretion of anti-inflammatory mediators [182, 183]. This process of restoring homeostasis of the immune response is how stem cells create a favourable condition for healing. Stem cells provide an effective therapeutic advantage for the treatment of conditions caused by immune dysfunction, such as autoimmune diseases and transplant rejections, by altering the tissue microenvironment from a chronic to an acute, pro-reparative inflammatory state. A few studies on regenerative

Table 7 Regenerative outcomes in various organ models

Organ/disease	Stem cell type(s)	Regenerative outcomes (expected/observed)	References
Heart (ischemic cardiomyopathy)	Autologous Bone Marrow MSCs and c-kit + Cardiac Progenitor Cells	Reduced occurrence of major heart problems, improved quality of life, and increased systolic motion	NCT02501811 [184, 185]
Spinal cord injury (SCI)	Neural Stem Cells, MSCs, Neural Progenitor Cells	Improvements in locomotor and sensory function; enhanced mobility; improved bladder and bowel control	NCT03935724 [186]
Parkinson's disease	hESC-derived dopamine-producing neurons	Visible reductions in tremors; continued neuron cell engraftment	NCT04802733 [187]
Liver (Cirrhosis)	Mesenchymal Stem Cells (MSCs)	Improved quality of life; reduced MELD score; slowed fibrosis progression; significant effect on ascites	NCT05080465 NCT03626090
Kidney (CKD)	Mesenchymal Stem Cells (MSCs)	Delayed disease progression; improved quality of life; reduced need for dialysis	NCT05362786

outcomes in various organ and disease models are listed in Table 7.

Translation of stem cell-based therapies in organ

The shift from promising preclinical models to approved clinical treatments involves several steps and can take years or even decades. While many regenerative therapies are undergoing clinical trials, some products have already received regulatory approval. This shows the current situation in clinical practice. This process starts with the pre-clinical stage, as shown in Fig. 8.

Regeneration current clinical trials and outcomes

Clinical trials for organ regeneration and repair are ongoing worldwide. While some results are promising, they often face limitations due to small sample sizes, brief follow-up periods, and differences among trials. For example, in cardiac regeneration, trials such as BAMI and C-CURE have suggested that stem cell therapies may improve left ventricular ejection fraction and reduce infarct size. In liver cirrhosis, mesenchymal stem cell (MSC) therapy has shown promising results in clinical trials. Up to 60% of patients have experienced better liver function. Studies have also reported a significant decrease in the MELD (Model for End-Stage Liver Disease) score and a reduction in complications like ascites. Beyond traditional cell therapies, organoid-based treatments are also entering the clinic. There are approved studies for intestinal Behçet's disease and radiation proctitis, as well as trials for salivary gland and liver

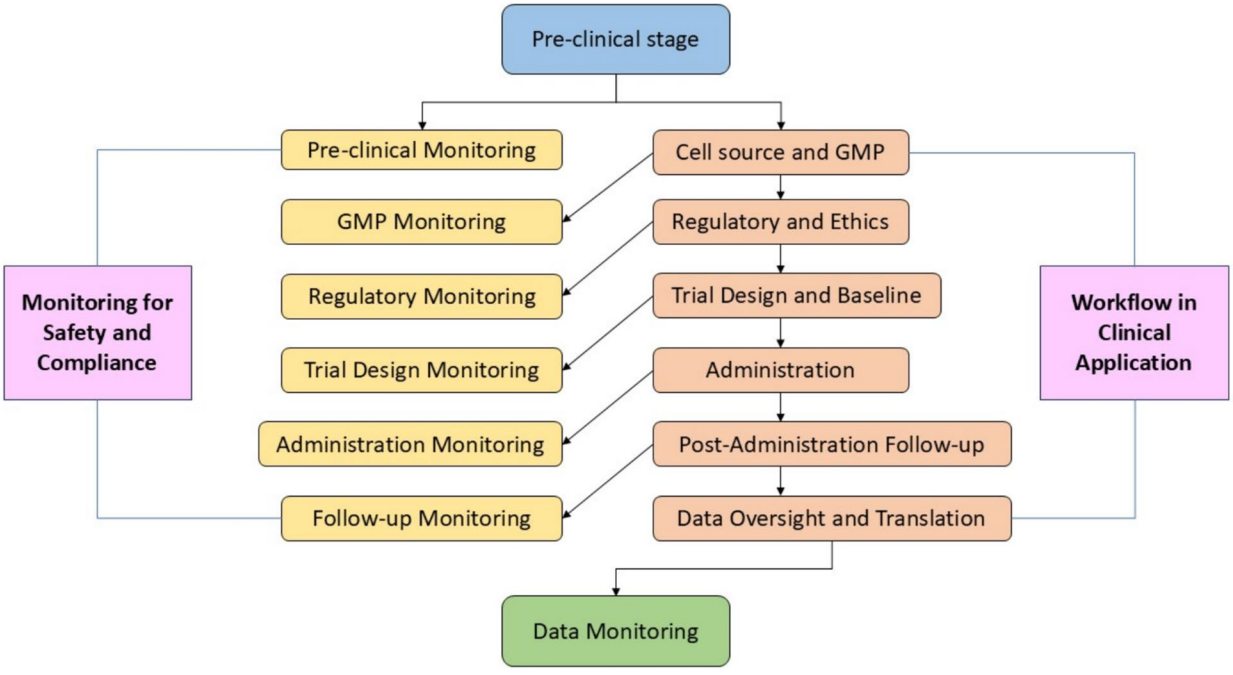


Fig. 8 Clinical application workflow and monitoring

Table 8 Clinical trials for organ regeneration

Stem cell and organ/disease	Trial	References
Small mobile stem cells (chronic obstructive lung disease)	The primary objective of this study is to determine the safety of SORT-COPD (SMS cells) at three different doses in individuals with chronic obstructive pulmonary disease	NCT06986070
Autologous hematopoietic stem cell (autoimmune diseases)	Phase I/II Open-Label Monocentric Clinical Trial for Induction of Tolerance with CD34-Enriched Autologous Hematopoietic Stem Cell Transplantation After High-Dose Chemotherapy with Cyclophosphamide and Rabbit-Antithymocyte Globulin for Refractory Autoimmune Diseases	NCT00742300 [188–191]
Human umbilical cord-derived mesenchymal stem cells (COVID-19)	A Phase II, Multicenter, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Efficacy and Safety of Human Umbilical Cord-derived Mesenchymal Stem Cells in the Treatment of Severe COVID-19 Patients	NCT04288102 [192–195]
Mesenchymal stem cell (non-viral acute on chronic liver failure (ACLF))	National Collaborative Centre for Hepatic Regenerative Medicine (NC-CHRM): To Study the Safety and Efficacy of Mesenchymal Stem-Cell (MSC) Therapy in the Management of Non-viral ACLF Patients: Phase-II Clinical Study	NCT07131306
Autologous bone marrow cells (type 1 diabetes mellitus)	The objective is to achieve a significant increase in C-peptide levels, indicating a regeneration of beta islet cells and a decrease in exogenous insulin use in at least 70% of the patients	NCT00971503

conditions. A list of completed and current clinical trials is summarised in Table 8.

Challenges in safety, efficacy, and delivery systems

Despite these successes, several challenges still exist in translating stem cell therapies into clinical practice. This suggests that a treatment that works in a simple animal model may not consider the chronic nature of human disease, differences among patients, or the complexities of the human immune system.

As in many studies, safety is the most important concern. For example, the risk of rejection due to an immune response, survival of a limited number of transplanted cells, and potential risk of cancer (tumorigenesis), particularly when pluripotent stem cells are involved. Ensuring the long-term safety of cells that may remain in a patient's body for years is paramount and requires rigorous, long-term monitoring and follow-up.

Efficacy remains a significant hurdle. Even when some trials show improvements, the long-term benefits, such as better survival rates and fewer hospital re-admissions, are often unclear. The logistical and financial challenges of manufacturing living cells for therapeutic use are significant as well. Unlike traditional pharmaceutical drugs, stem cell products are living entities. They require specialised and consistent quality control measures. The manufacturing model for these therapies is complex and probably won't be "one-size-fits-all." Different approaches are necessary for universal (allogeneic) therapies and personalised (autologous) therapies.

Another major challenge is the transplantation or delivery of stem cells to the injury site and ensuring their survival. To address these problems, researchers are developing new biomaterials that will serve as smart delivery systems. Materials such as hydrogels and porous scaffolds can be designed to mimic the local extracellular matrix, which offers structural support and controlled release of therapeutic molecules. These biomaterials can be designed with important properties such as biocompatibility, porosity, and surface chemistry. These properties are crucial for regulating stem cell behaviour and directing their differentiation.

Emerging technologies enhancing stem cell organogenesis and regeneration

The dream of fabricating functional, living tissue as a substitute for damaged or incapacitated organs has been an integral part of regenerative medicine from the very beginning, that is, when the field was primarily identified with tissue engineering [196] For many years, scientists have focused on stem cells, remarkable cells that can give rise to any other type of cell in our organs. A key limitation in stem cell biology is the difficulty in controlling their differentiation. But what's more, we cannot control them in a way that allows us to produce the complex 3D structures that our native organs have. Earlier, cells were seeded passively onto biodegradable scaffolds. However, there was limited control of the final tissue structure and functions. We are now entering a space where more potent tools are providing impact, registration, and control over the biological and physical processes of tissue formation. The combination of genome editing, 3D bioprinting and synthetic biology at the target is making it possible to shift the manufacture of generic cell therapies to programmable functional tissue constructs. The evolution of our technical capabilities, which have enabled the reliable regeneration of tissues, is not a revolution but a gradual advancement in technology.

Innovations advancing tissue regeneration

The key to advancement lies in the ability to control the universal elements of biological systems with increasing

precision: the genetic code, the individual cell, and the cell's microenvironment. Every technology will address a different, but complementary, aspect of the tissue engineering problem.

The CRISPR-Cas9 system allows for genome editing [197]. It offers a way to alter a cell's DNA precisely. It is helpful for regenerative medicine in two ways. First, it offers a straightforward method for repairing harmful mutations in induced pluripotent stem cells (iPSCs) derived from patients. This application achieves a significant objective of therapeutic genome editing by going beyond symptom management to produce a genetically sound cell source for autologous transplantation [198]. Second, CRISPR enables proactive cell engineering with enhanced therapeutic properties. For instance, by deleting essential genes involved in immune recognition, stem cells can be modified to become hypoimmunogenic, potentially yielding universal cell lines. In addition, they can be engineered to overexpress specific growth factors that support tissue integration and post-transplant survival, or equipped with safety switches that enable their removal in the event of complications.

3D bioprinting addresses the complex issue of tissue architecture, whereas genome editing enhances cells. Traditional 2D cell culture is recognised as physiologically inadequate for modelling in vivo settings. This is addressed by 3D bioprinting, which enables the precise deposition of bio-ink hydrogel materials loaded with living cells layer by layer to create complex, heterogeneous tissue scaffolds from the bottom up [199]. To replicate the intricate cellular neighbourhoods in natural organs, researchers can utilise this technology to place various cell types in precise locations. It is not just a way to create shape. Creating integrated vascular networks is arguably its most important use, as it is an essential step in ensuring that engineered tissues receive the nutrients and oxygen they need to function. This problem has long plagued the field [200].

Synthetic biology enables the integration of new, programmable cellular properties through a dynamic layer of cellular regulation. This entails creating artificial gene circuits and incorporating them into stem cells to mediate their behaviour upon recognition of specific stimuli [201]. These circuits enable cells to act as independent agents by using logical operators (such as IF–THEN). One could, for example, design cells with an algorithm that detects inflammatory cues (the "IF") and reacts by releasing an anti-inflammatory medication (the "THEN"). One important use of synthetic biology in next-generation cell therapies is the potential for these "smart cells" to act as living diagnostics and treatments, changing their actions in real-time to the patient's physiological condition [202].

Stem cell integration for restoring organ function

The full value of these technologies is realised when they are brought together into a single therapeutic platform. In other words, a single therapeutic workflow can bridge a patient's cellular material into a functional tissue construct suitable for implantation. This process hinges on robust delivery technologies for genetic payloads [203] and involves several meticulous stages.

The power of these technologies is unlocked when they come together as a one-stop therapeutic platform. This type of process can trace a route from patient cells to a usable, implantable tissue construct. This procedure, which entails multiple painstaking steps, depends on reliable delivery technologies for genetic payloads [204].

Therapeutic gene correction is the next step for patients with monogenic disorders. By entering the cell nucleus and correcting the specific typo that causes the disease with CRISPR, researchers can fix the disease. This has been demonstrated effectively in studies of the significant disease Duchenne muscular dystrophy, where a healthy, genetically repaired cell line was produced by correcting the underlying mutation in patient-derived iPSCs [198]. Before proceeding, this step requires rigorous quality control, including whole-genome sequencing, to verify the absence of unwanted off-target mutations [205].

After validating a pool of authentic, personalised stem cells, the next step is to construct something from them. The first step is to instruct the induced pluripotent stem cells to change into the appropriate cell types for a target organ, such as cardiomyocytes, neurons, or hepatocytes. Next, a 3D bioprinter places and supports cells with bio-ink, and simultaneously a high-resolution computer model is generated from a patient's MRI or CT scan to serve as a reference. This approach constructs the tissue layer by layer, precisely and accurately placing cells and materials. Research milestones, such as the printing of a minor, vascularized human heart patch that beats in unison, demonstrate that this method is feasible. However, making these structures larger to accommodate a whole organ remains a significant challenge [206].

Finally, a newly printed tissue construct cannot be implanted immediately. It must be matured in a bioreactor, a machine that can replicate the stresses a body experiences by supplying nutrients, electrical stimulation, or mechanical strain. Once placed in a bioreactor, the cells will organise, connect, and respond as a group. After it has grown, this living structure can be moved. Theoretically, there is no longer a need for lifelong immunosuppressive drugs, as they are made from the patient's own corrected cells. (Fig. 9) This integrated approach sets the stage for a future in which a specific, individualised regeneration process could replace the wait for a donor organ. CRISPR editing, stem cell organogenesis, and 3D bioprinting are emerging technologies that, together,

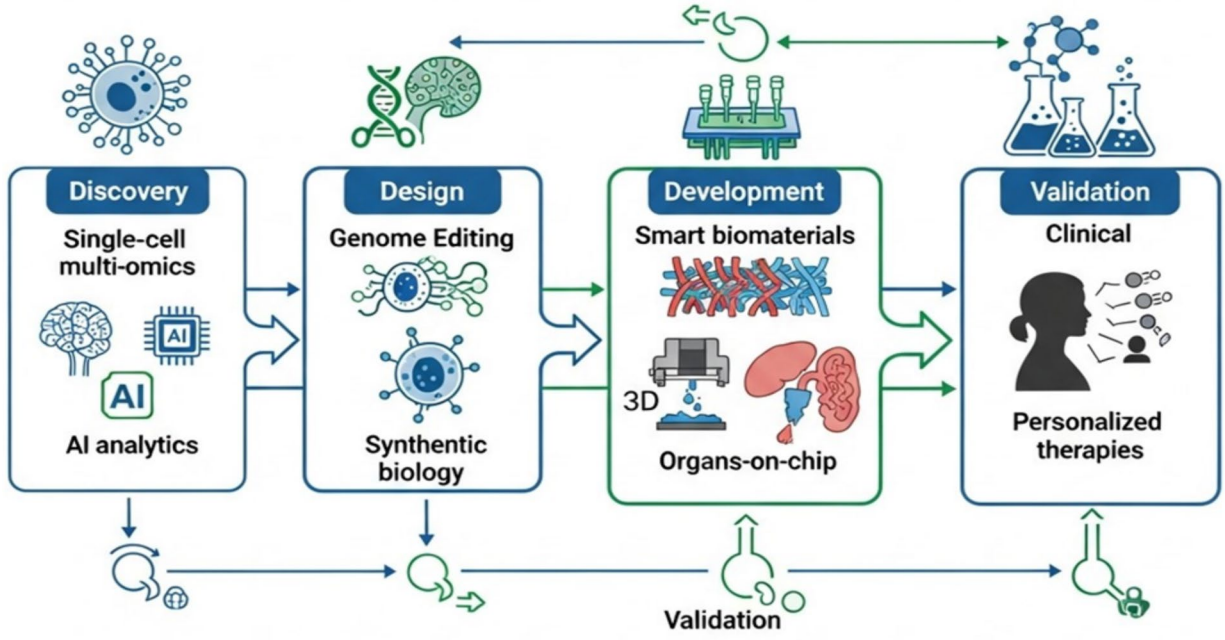


Fig. 9 Conceptual diagram of the advanced regenerative platforms

Table 9 Emerging tools and their impact on regenerative medicine

Emerging tool	Core function	Impact on regenerative medicine	References
Genome EditingCRISPR-Cas9)	To make specific, targeted changes to the genomic DNA of a cell	It provides a direct method for treating gene-based diseases in a patient's own cells, allowing scientists to create safer or more effective cells	[197]
3D bioprinting	To create 3D tissue models by applying "bio-inks" that are packed with cells	Helps solve the crucial issue of building blood vessel networks to sustain them while assembling various cell types into intricate three-dimensional tissues	[199]
Synthetic biology	To use gene circuits to program new, controllable behaviours within cells	More responsive, self-sufficient treatments could result from programming smart cells that can sense their surroundings and release a medication when called upon	[208]
Organ-on-a-chip (microfluidics)	To develop tiny gadgets that replicate the way human organ units work	Offers a reliable platform for simulating the progression of complex diseases and assessing drug safety in tissues that closely resemble human tissues in vivo	[209]
Single-cell multi-omics	To examine a single cell's genome, transcriptome, or epigenome	Helps identify the main cell types responsible for tissue repair by revealing the precise process by which stem cells determine their fate and by monitoring individual cells	[210]
Advanced smart biomaterials	To create materials that actively control the behaviour of cells	Serves as both a time-release drug depot and an intelligent scaffold that provides cells with the proper signals to proliferate and organise	[211]
AI & machine learning	To analyse and forecast large biological datasets using algorithms	Aids in the interpretation of large datasets to forecast the behaviour of cells and is employed to optimise intricate procedures such as bioprinting	[212]

move regenerative medicine closer to the goal of building functional tissues and organs [207].

Table 9 summarizes essential emerging tools in regenerative medicine, including genome editing, 3D bioprinting of organs, synthetic biology, organs-on-chip modeling, single-cell multi-omics, smart biomaterials, and AI analytics. Collectively, these will enable us to make particular changes to a genome, create functional tissues, improve disease modeling, and develop data-driven approaches to optimize and facilitate clinical translation.

Figure 9 illustrates emerging technologies that promote regeneration. CRISPR-Cas9 genome editing enables precise DNA modifications, correcting genetic defects. Stem cell organogenesis refers to the process of using stem cells and the extracellular matrix to generate specialised cells, including neurons and cardiomyocytes. Three-dimensional bioprinting enables the creation of tissue constructs from bio-inks containing living cells, thereby enabling the development of more complex and functional tissues.

Table 10 Global guidelines shaping stem cell research

Issuing body/framework	Core principles	Key directives	References
ISSCR Guidelines	Public involvement, scientific rigour, transparency, responsible research, and protection of research subjects	Prohibits the cloning of human reproductive organs; establishes specialised control committees; and offers particular guidelines for the study of embryos, chimerism, organoids, and clinical translation	[216]
U.S NIH Guidelines	Strong control of research involving human pluripotent stem cells; federal funding is limited to certain, ethically derived human embryonic stem cell lines	Creates the NIH Stem Cell Registry, demands institutional review board (IRB) approval, and prohibits funding for studies that use human embryos for development or destruction	[217]
European medicines agency (EMA)	Focuses on the efficacy, safety, and quality of advanced therapy medicinal products (ATMPs), including cell-based therapies	Requires phased clinical trials (Phase I–III), thorough preclinical data, and long-term patient follow-up for safety monitoring. It also requires Good Manufacturing Practice (GMP)	[218]
U.S.FDA regulations	Promises the efficacy and safety of stem cell products meant for use by humans	Bans unproven treatments and requires the submission of an Investigational New Drug (IND) application for clinical trials. It defines stem cell products as medications, devices, or biological products	[219]

Future perspectives and ethical considerations

Stem cell science is rapidly evolving from an observational domain into a biological field with very real ethical dilemmas, as well as unanticipated therapeutic promise. Scientists are not only building sophisticated biological systems. Still, they are also exploring cell reprogramming in situ to create multi-component tissues in a dish, while we have moved beyond simple discoveries. To ensure that advancements align with societal values, this leap into biological engineering requires a corresponding evolution in our ethical frameworks.

Assembloids, which are created by fusing different organoids to simulate intricate interregional relationships, provide researchers with a new perspective on neuropsychiatric disorders by allowing them to recreate developmental processes, such as neural migration, remarkably accurately [213]. Even more radical is the idea of in vivo reprogramming, which completely avoids transplantation and aims to regenerate tissues from within by transforming resident cells into the necessary therapeutic types [214]. However, this approach is associated with significant risks of tumorigenesis and functional immaturity that need to be addressed. Synthetic biology provides instruments such as synthetic Notch (synNotch) receptors, which function as programmable logic gates to control cellular behaviour with engineered specificity, to attain the required precision [215]. Interspecies blastocyst complementation, the bold plan to grow human organs in animal hosts to solve the present organ shortage, is at the very end of this frontier. However, the vast evolutionary distance between species severely hinders this endeavour, leading to low chimerism efficiency [216].

Deep ethical issues must be addressed as these scientific capabilities grow. The most direct harm is caused by stem cell tourism. In this predatory international industry, clinics take advantage of patients' desperation by offering risky and unproven treatments, resulting in serious physical harm and lowering public confidence in reputable science [217]. Emerging technologies give rise to more complex issues than this obvious fraud. The terrifying potential of emergent awareness in a dish is amplified by the development of more complex brain organoids that exhibit coordinated electrical activity, sparking debate over their moral standing and our responsibilities to them [216].

Germline modification is arguably the most significant ethical concern. Germline editing modifies reproductive cells, making changes that are transmissible to all subsequent generations. In contrast, somatic gene editing, which affects only the individual patient, is generally acknowledged as a therapeutic advancement. Fears of irreversible mistakes, the inability to obtain consent from future generations, and a downward trend towards new eugenics of human development have all contributed to the demands for prohibition around the world [217].

Strong and coordinated governance efforts are essential to address this complex system. International organisations, such as the International Society for Stem Cell Research (ISSCR), and organisations engaged in these processes, have established important ethical practices and provide a global standard for rigour and transparency [218]. In the United States, the National Institutes of Health (NIH) maintains a registry of cell lines that have been ethically sourced and prevents federally funded research for producing or destroying embryos [219]. (Table 10) Regulatory bodies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), which categorize stem cell therapies as biologics or Advanced Therapy Medicinal Products (ATMPs) and along with need for rigorous evidence of safety and efficacy before market approval, ultimately

leads to clinical translation and the principles and working flow of these governing bodies is summarised briefly (Table 10). A potential process of action for organ regeneration, from the start of scientific research to clinical implementation, that integrates advances in science, debates on ethics, and regulatory actions necessary for the resulting treatments to be both safe and efficacious, is the need of the hour [207].

Table 10 outlines several key ethical and regulatory frameworks that govern stem cell research and therapies. The ISSCR encourages responsible and transparent research; the U.S. National Institutes of Health (NIH) only funds ethically derived stem cell lines; the European Medicines Agency (EMA) enforces safety and good manufacturing practices for new advanced therapies; and the Food and Drug Administration (FDA) regulates clinical use by prohibiting unproven treatments and requiring trial approvals.

Conclusion

Stem cells play a central role in organogenesis and regeneration, driving the formation, maintenance, and repair of tissues through tightly regulated molecular and cellular mechanisms. Advances in understanding signalling pathways, stem cell niches, and regenerative processes have significantly enhanced our ability to model organ development and design targeted therapies. Technologies such as organoids, CRISPR-based editing, and 3D bioprinting are bridging the gap between experimental research and clinical translation, offering new hope for the functional replacement of organs. However, challenges related to safety, scalability, and ethical considerations must be addressed to fully realise the therapeutic potential of stem cell–based organ regeneration. These insights offer a comprehensive overview of stem cell biology, highlight current clinical progress, and outline future challenges in the safe and effective translation of therapeutic applications.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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