REVIEW

Stem cell niches functionalized strategies for organ regeneration and manufacturing

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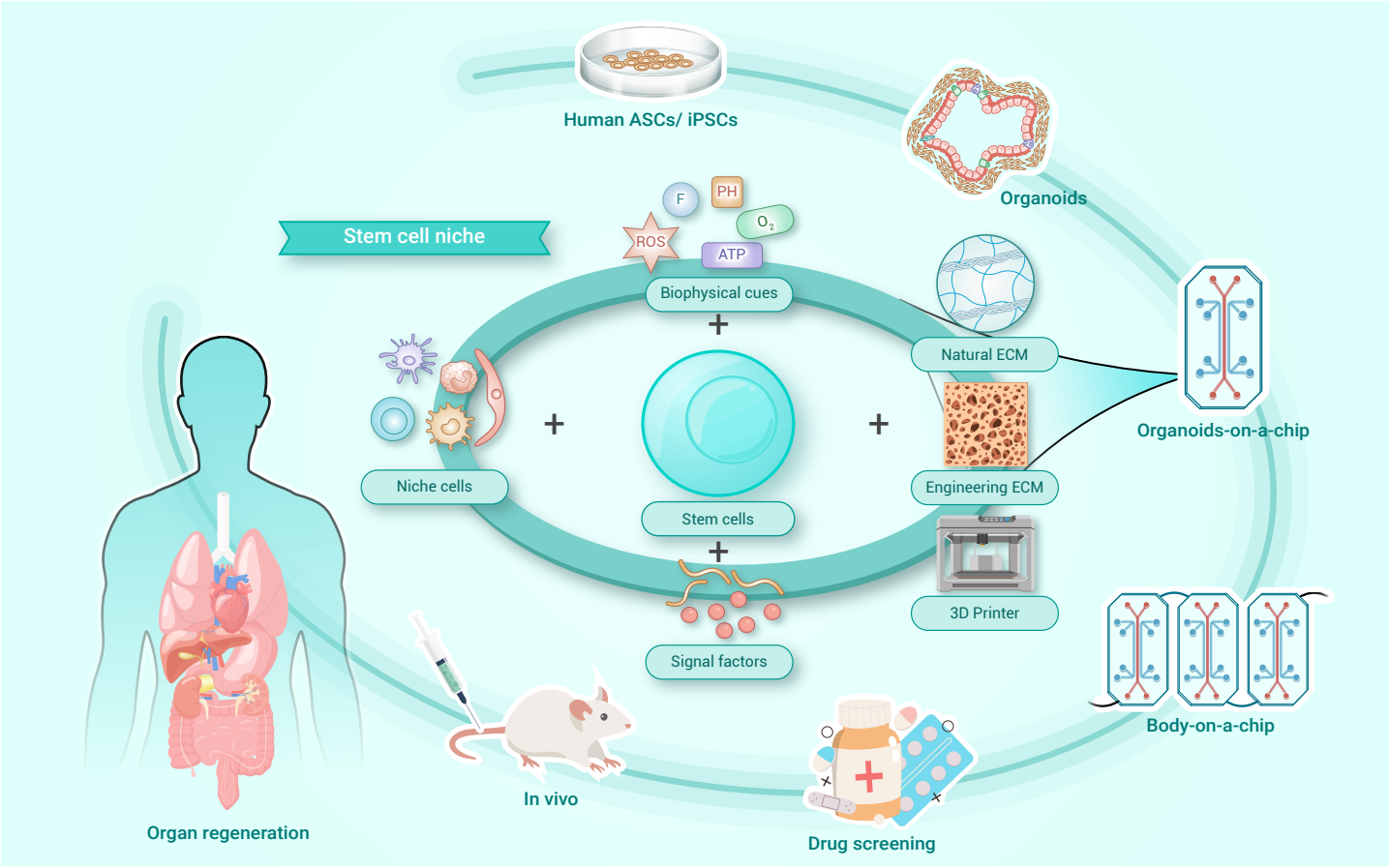
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Stem cell niches determine stem cells fate and homeostasis.
- Stem cell niches provide opportunities for organ regeneration.
- Stem cell niches-functionalized strategies have been emerging.

REVIEW

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Stem cell niches functionalized strategies for organ regeneration and manufacturing

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Organ regeneration and manufacturing are promising new research directions in the life sciences. Stem cells and their niches, which exist in most adult organs, play a central role in organ development, homeostasis, and regeneration. Although considerable advances have been made in stem cell-mediated organ regeneration and manufacturing in recent decades, their clinical effectiveness remains unsatisfactory. Stem cell niches comprise a dynamic microenvironment that supports stem cells throughout their lifetime and are critical to stem cell fate. Based on recent research on stem cell niches, tremendous progress has been made in organ regeneration and manufacturing *in vivo* and *in vitro*. In this review, we discuss recent advances in the composition and function of stem cell niches during regeneration. We also discuss stem cell niche remodeling using cell-cell interaction, extracellular matrix (ECM) reconstruction, cell-ECM interaction, and key signaling-based niche strategies to promote endogenous tooth, gastrointestinal tract, and liver regeneration and its application in organoids and organoid-on-chip construction.

INTRODUCTION

Organ regeneration and manufacturing aims to repair, regrow, or replace damaged tissue with a manufactured organ to restore normal organ functions. This technique holds great potential in treating patient symptoms and reversing disease or dysfunction. Stem cells play a vital role in tissue homeostasis and are maintained and regulated by their microenvironment (their "niche"), which forms a regenerative unit or region.[1] These regenerative regions can drastically respond to environmental stimuli, such as injury or aging, thus maintaining the homeostasis of organs. Based on their self-renewal and multiple differentiation functions, stem cells have been widely used in regenerative medicine,[2] either through the transplantation of stem cells or their differentiated derivatives.[3] Stem cell-based regenerative medicine has advanced dramatically in recent decades. Previous preclinical studies have shown enhanced regeneration of multiple organs following stem cell therapy. However, the results of clinical trials are unsatisfactory, with several clinical trials failing on primary endpoints due to the lack of an ideal microenvironment after transplantation, which results in low survival, reduced regenerative capacity, and immune rejection of stem cells.[4] Even if the organoids subsequently developed can provide a suitable niche for stem cells, ensuring their reproducibility, reliability, and maturity are key challenges and limitations in regenerative medicine. Several studies have analyzed the heterogeneity of various organoids and revealed differences between stem cell lines, protocols, and trial lots.[5] Organoid construction relies on animal-derived Matrigel, and uncertainty about its composition and differences in physicochemical properties between batches can lead to organoid variability and poor reproducibility. Additionally, organoids poorly replicate the physiological characteristics of actual organs, lack functional vascular, nervous, or immune systems, and cannot fully simulate human stem cell niches.[6,7] Therefore, a key problem that must be addressed is that the microenvironments of stem cells *in vivo* cannot maintain stem cell function. Bionics and regulation of stem cell niches are the most common strategies applied to address these issues in organ regeneration.[8]

In stem cell niches, the specific microenvironment of stem cells plays an essential role in promoting tissue regeneration through precisely controlled stem cell self-stabilization, renewal, survival, differentiation, and migration/homing.[9,10] The stem cell niche was first verified by investigating the germline stem cells in the Drosophila germarium that require direct contact with cap cells to maintain stemness.[11] To date, stem cell niches have been found in most organs, such as bone marrow, the gastrointestinal tract, the liver, hair follicles, and skeletal muscle.[12-17] Important components of stem cell niches are neighboring niche cells, which interact with stem cells through direct cell-cell contacts and by releasing soluble factors, as well as factors such as low oxygen tension, extracellular matrix (ECM) composition, physical factors, and metabolic cues. Given the critical role of stem cell niches in stem cell and organ homeostasis, deciphering the composition of niches and identifying factors that can regulate stem cell properties are crucial in regenerative medicine. As such, various stem cell niche functionalization strategies have been developed for endogenous organ regeneration and organoid construction.

In this review, we discuss the composition and function of stem cell niches during organ regeneration and strategies for remodeling stem cell niches by cell-cell interactions, ECM reconstruction, cell-ECM interactions, and critical signaling-based niche remodeling. Using these strategies, the *in situ* organ regeneration of hard organs (such as teeth), hollow organs (such as the gastrointestinal tract), and solid organs (such as the liver) has been achieved. Furthermore, strategies for functionalizing the stem cell niche, which plays a critical role in organ manufacturing, such as organoid or organoid on-chip construction, will be discussed.[18]

COMPARISON OF STEM CELL NICHES

Zebrafish and amphibians exhibit a high degree of genetic and functional homology to mammals, including humans; their conservation of cell biology and developmental processes renders them valuable tools for basic and translational research.[19,20] However, zebrafish and amphibians have a more powerful regenerative capacity, which can be attributed to the presence of numerous stem cell niches in their tissues and organs. This is in contrast to humans, where adult mammalian tissues and organs occupy a smaller proportion of stem cell niches and have limited regenerative potential. In addition, the inflammatory process after injury in zebrafish often activates the regenerative process, whereas inflammatory cell proliferation and scar tissue formation prevent the regenerative process in mammals. The activation of pro-inflammatory molecules and consequent tissue inflammation, typically deemed detrimental in mammals, may represent the key process driving zebrafish regeneration when occurring within the appropriate molecular milieu. In addition, the mammalian tissue environment does not provide the necessary cues to support the survival and integration of neonatal daughter cells generated through stem cell regeneration after injury, resulting in the demise of more than 80% through apoptosis, with minimal migration to the site of injury.[21-23] This difference between zebrafish and mammals is the most pronounced in neurogenic capacity. In the zebrafish brain and spinal cord,

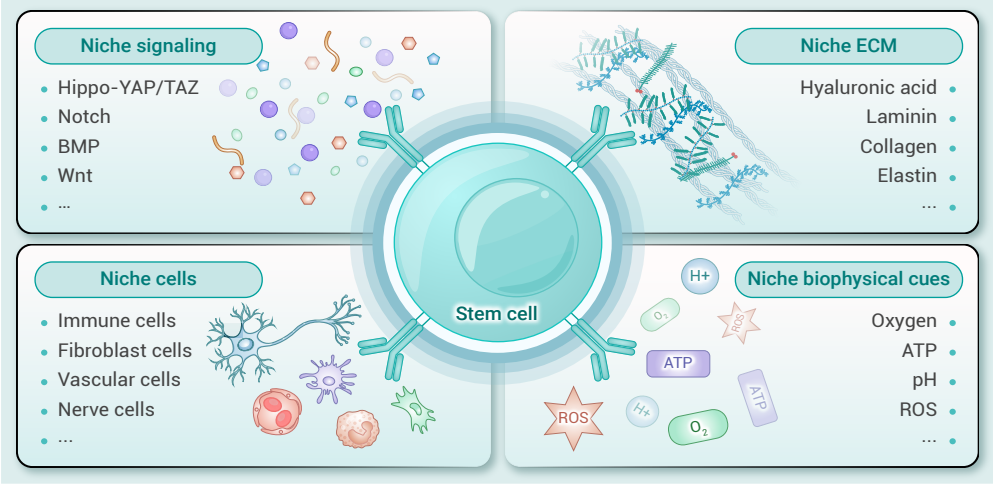

Figure 1. Composition of stem cell niches.

stem cells can reactivate the molecular programs required to regenerate the central nervous system; effective nerve regeneration occurs, and function is successfully restored. In mammals, traumatic brain injury is followed by decreased neurogenesis, axon regeneration, and severe functional impairment. Furthermore, spinal cord injuries in humans have limited capacity for self-repair.[24,25] Therefore, characterizing the composition of stem cell niches is crucial to understanding differences in the regenerative mechanisms between zebrafish, amphibians, and mammals.[26]

COMPOSITION AND FUNCTION OF STEM CELL NICHES

Stem cell niches contain various physiological and biochemical factors. Niche heterogeneity exists in different organs; however, common elements include stem cells, multiple heterologous cell types, niche-specific ECM, and soluble factors, which are closely coordinated interactively to influence stem cell fate (Figure 1).

Niche cells

In a regenerative microenvironment, stem and niche cells (including progeny and other supporting cells) can interact closely through gaps or adherent junctions, secretion of soluble factors, or direct contact. Cell-to-cell communication is essential for maintaining tissue homeostasis. Niche cells not only maintain the function of resident stem cell populations but also play a regulatory role in tissue and organ development. $Msx1^+$ $Sox9^+$ niche cells were found to migrate to the dental papilla, promote the development of the dental papilla, and participate in the control of tooth morphogenesis.[27] In the intestinal crypts, Paneth cells can enhance the amplification and differentiation of $LGR5^+$ intestinal stem cells (ISCs).[28] Simultaneously, Paneth cells produce $LGR5^+$ ISCs after radiation damage as a cell source for regeneration after stem cell loss.[9]

Niche cells contain multiple heterologous cell types, including immune, fibroblast, vascular, and nerve cells. During tissue damage, immune cells are recruited to wounds and secrete various factors that affect stem cell function. For example, in the regeneration of skeletal muscle, macrophages provide a transient but specific niche for stem cell proliferation through the secretion of nicotinamide phosphoribosyl transferase (NAMPT)[29] and promote proliferation and differentiation through the binding of the very late antigen 4 (VLA4) to the satellite cell (SC) cell-surface molecule vascular cellular adhesion molecule-1 (VCAM-1). Other stem cell niche-resident immune cells, including regulatory T cells (Tregs), dendritic (DC) cells, and natural killer (NK) cells, also play a role in regulating stem cell homeostasis and tissue regeneration.[30] Maintaining the regenerative function of stem cells requires new blood vessels to supply oxygen and nutrients and carry away metabolites. Vascularization of niche endothelial cells (ECs) is a necessary stage in this process. ECs can directly contact stem cells, receive secreted vascular endothelial growth factor (VEGF), and respond to mechanical and chemical stimuli, promoting self-proliferation and the recruitment of nearby blood vessels. Niche fibroblasts are responsible for the production of type I or III collagen fibronectin. They also regulate niche vascularization through various pathways, including direct differentiation into ECs and indirect promotion of angiogenesis through their interactions.

Niche ECM

The ECM is a complex, dynamic, and tissue-specific niche environment of noncellular components that can provide mechanical support for stem cells through macromolecular networks. This network isolates internal stem cells from activation-associated growth factors and signaling molecules, providing a stable microenvironment for stem cells.[31] The key characteristics of the niche ECM are biochemical composition, microstructure, and mechanical properties.[32] ECM components mainly include collagen, laminin, hyaluronic acid (HA), and elastin. The main component of the vertebrate ECM is collagen, which maintains the structure and shape of the tissue and plays an important role in stem cell proliferation and differentiation. For example, type VI collagen-mediated changes in muscle stiffness can affect the self-renewal and maintenance of SCs. Deletion of type III collagen affects osteogenic differentiation, whereas denaturation of type I collagen promotes osteogenic differentiation. Laminin is another important component. *In vitro* experiments showed that the regenerative capacity of SCs was significantly enhanced when cultured on hydrogels cross-linked with laminin, and static stretching of neural stem cells (NSCs) in a laminin-coated matrix promotes their differentiation into the oligodendrocyte lineage.[33] Laminin α5 deficiency results in 60% proliferation of NSC niche cells. Furthermore, HA, a major space-filling component of the neural ECM of the brain, provides a niche for the survival and migration of NSCs.[34] Microcompartmentalization of HA is important for maintaining homeostasis in the brain tissue. Brain tissues lacking HA synthase exhibit frequent epileptic phenotypes.[35] Elastin also promotes the adhesion and migration of mesenchymal stem cells (MSCs) and interacts with integrins to encourage the proliferation of MSCs and hematopoietic stem cells (HSCs).[36] The mechanical properties (stiffness, viscosity, and porosity) of the ECM are influenced by macromolecules such as fibers and polysaccharides, which have distinct tissue-specific properties. This difference between soft and hard matrices is the main physical regulatory factor that determines the fate of stem cells. For example, human MSCs (hMSCs) differentiate into a wide and flat morphology on hard substrates and exhibit osteoblast-specific gene expression.

The properties and composition of the niche ECM are not constant. The ECM is remodeled according to stem cell fate requirements by regulating the production and degradation of its components. Niche cells can secrete matrix metalloproteinases (MMPs), which cleave the ECM to regulate its stiffness in response to tissue remodeling.[37] When tissue is damaged, MMPs degrade the ECM in the migration pathway to remove mechanical resistance and accelerate stem cell migration to the site of injury to complete repair. In contrast, inhibition of MMPs blocks the migration of MSCs to the injury site, affecting their proliferation and osteogenic differentiation, as demonstrated in muscle tissue.[38]

Niche signaling

Niche signaling, which involves the Wnt, Notch, bone morphogenetic protein (BMP), and Hippo-YAP/TAZ pathways, is a key regulator of stem cell maintenance, determination of cell fate, and dedifferentiation, as well as organ development and tissue repair processes.

In the quiescent state, stem cells can maintain their undifferentiated state through autocrine Wnt signaling. Niche cells secrete key Wnt ligands such as Wnt3a and Wnt4, which are essential for stem cell self-renewal.[39]

Notch signaling is critical to maintaining stem cell quiescence, migration, and proliferation. For example, Notch signaling can induce the expression of V-type collagen and promote the quiescent state of stem cells by regulating calcitonin and drive miR-708 expression to counteract stem cell migration.[40]

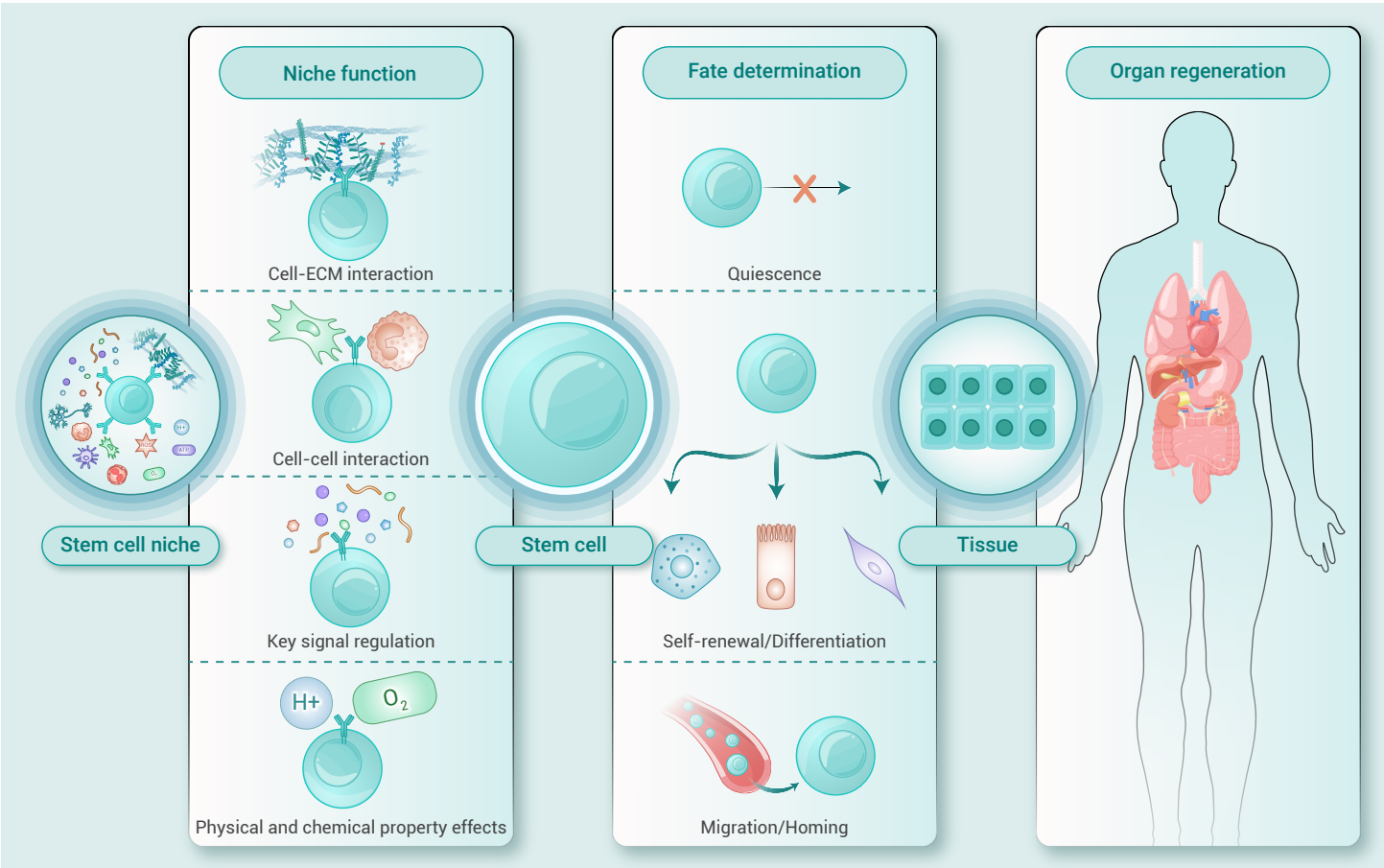

Figure 2. Function of stem cell niches.

Furthermore, progeny stem cells express delta-like 1 (DLL1) during proliferation and differentiation, which can trigger Notch signals associated with self-renewal.[40] In the intestinal niche, Notch signaling regulates local Wnt activity to promote the proliferation of LGR5$^+$ ISCs and inhibit their differentiation toward secretory cell lineages.

BMP, a subclass of the transforming growth factor-β (TGF-β) superfamily, is widely distributed in various tissues and cell populations of the body and plays an important regulatory role in growth, development, and regenerative repair.[41] Its role as an osteogenic regulator has been extensively studied, of which BMP-2 is one of the most widely used growth factors in bone tissue engineering due to its strong osteogenic induction capacity.[42] BMP also plays an essential role in regulating tooth morphogenesis, cell proliferation and differentiation, and tooth eruption in the dental niche. BMP interacts with the sonic hedgehog (SHH) signaling pathway to determine the fate of tooth epithelial stem cells. Furthermore, BMP-2 negatively regulates liver regeneration, and BMP-4 is involved in the formation of nonparenchymal hepatic cords and the shaping of the liver during liver regeneration. BMP can also promote ISC differentiation into intestinal epithelial cells, and this effect can be attenuated by inhibitors of mesenchymal cell secretion in niches, reducing the occurrence of cell trans-differentiation in crypts.[38,43] For example, Follistatin-mediated regulation of niche cell fate plays an important role in stem cell regeneration. Follistatin is an extracellular protein found in various tissues and regulates homeostasis, immunity, and organ regeneration. As a highly effective inhibitor of the TGF-β signaling pathway, it recognizes and binds activin (ACT) and BMPs in an autocrine and paracrine manner and functions by inhibiting the function of the downstream signaling protein Smad.[44] In tooth development, Follistatin/ACT participates in the regulation of enamel and dentin formation through the phosphorylation of Smad2/3 and maintains the homeostasis of odontogenic stem cells through Smad4.[45] *In vitro*, Follistatin promotes the migration of MSCs and ECs and the formation and expansion of the vasculature, enhancing bone tissue repair.[46]

The Hippo-YAP/TAZ signaling pathway has been identified as a mechanical stem cell sensor, which can respond to changes in cell polarity, substrate stiffness, and topology in niches.[47] In stem cell niches, the regulatory role of YAP/TAZ is mainly controlled by actin-myosin contractility in the cytoskeleton. Actin cleavage proteins can affect YAP/TAZ nuclear localization, whereas damage to the actin cap proteins LINC or Nesprins leads to YAP/TAZ retention in the cytoplasm.[48,49] Furthermore, when cell density increases in niches, the cell surface actin backbone can facilitate the translocation of YAP to the cytoplasm, reducing cell proliferation and restoring homeostasis. The interaction of YAP/TAZ with cytoskeletal proteins makes it a powerful tool for responding to mechanical forces and controlling stem cell behavior, organ size, and volume.[50,51]

Biophysical cues

Oxygen, ATP, pH, and reactive oxygen species (ROS) levels in stem cell niches are crucial in maintaining stem cell status. Maintaining a specific oxygen level is particularly important, as the relatively closed spatial structure in stem cell niches leads to a limited oxygen supply and a hypoxic environment. Most studies have confirmed that a hypoxic environment promotes stem cell proliferation, survival, protection against adverse microenvironments, and gene stability.[52,53] Additionally, hypoxic pre-activated stem cells have improved paracrine function and therapeutic potential,[54,55] as a hypoxic environment satisfies the metabolic needs of stem cells,[56,57] inhibits mitochondrial function, and reduces the excessive production of harmful ROS, oxidative damage, and the inflammatory response.[58] The benefit of a hypoxic environment is confirmed by the heavy reliance of stem cells on glycolysis rather than mitochondrial respiration for their bioenergy needs.[53] Furthermore, *in vitro* studies revealed that air exposure during stem cell collection severely limits their production and state, a phenomenon known as the "extra physiological oxygen shock/stress" effect.[52] Stem cells tightly regulate hypoxia levels by expressing HIF-1α, which plays an essential role in promoting the proliferation of stem cells in a hypoxic environment.[59] In the hypoxic environment, various other survival-related genes, such as anti-apoptotic and metabolic adaptation genes, improve the viability of stem cells and maintain

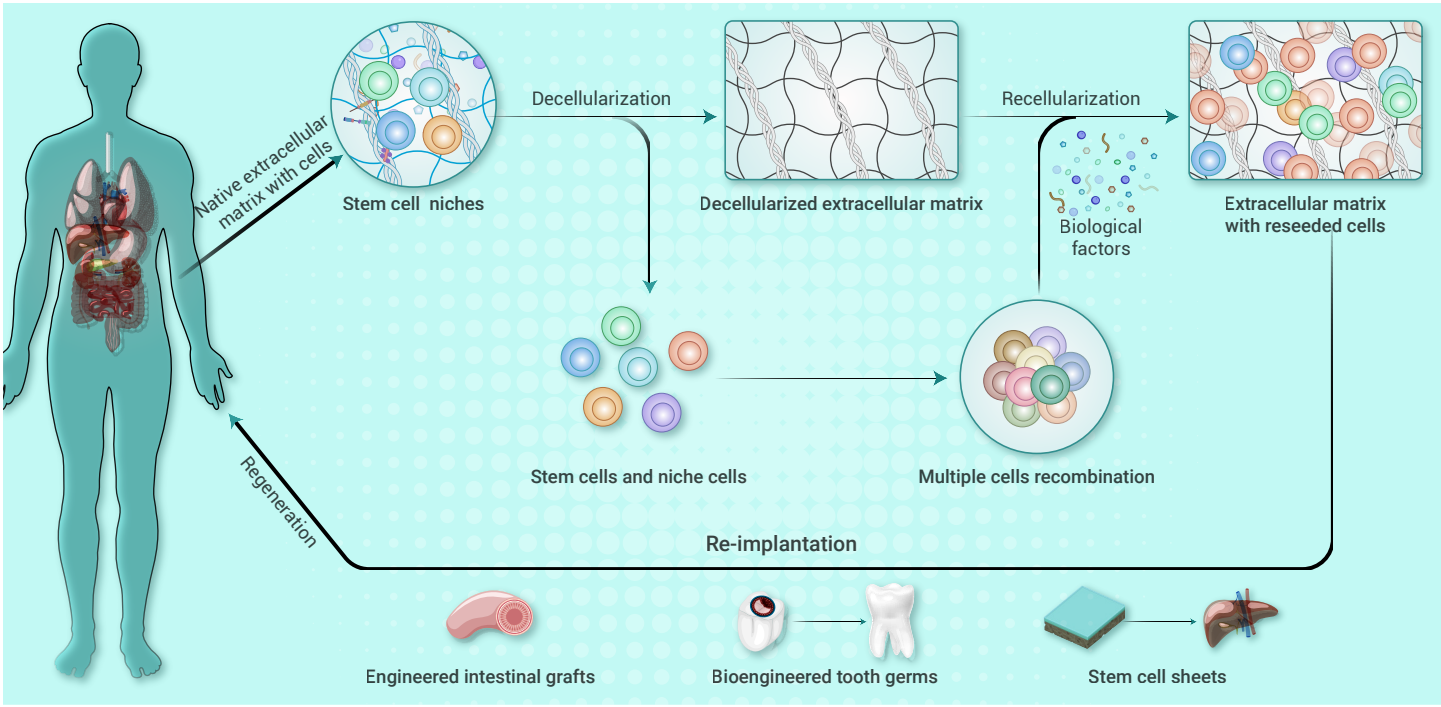

Figure 3. Stem cell niche-functionalized strategies for gastrointestinal tract, tooth and liver regeneration.

their stability.[60,61]

STEM CELL NICHE-FUNCTIONALIZED STRATEGIES FOR ORGAN REGENERATION

Stem cells are the key seed cells in various fields of regenerative medicine. However, organ regeneration depends on the activation and regeneration response.[62] In recent years, strategies to enhance organ regeneration by manipulating endogenous stem cell niches have made substantial progress with advances in basic science and the addition of new tools and techniques.[63] Numerous strategies, such as cell-cell interaction, ECM reconstruction, cell-ECM interaction, and key signaling-based niche remodeling strategies, have been widely used as niche functionalization strategies for organ regeneration. Due to these advances, the *in situ* organ regeneration of hard organs (such as teeth), hollow organs (such as the gastrointestinal tract), and solid organs (such as the liver) has been achieved (Figure 2).

Functionalized strategies of stem cell niches

Cell-cell interaction strategy. Most organ development requires epithelium-mesenchyme interactions during the early stages of morphogenesis.[64,65] These interactions are sequential, coordinated, and reciprocal. The mesenchyme specifies the epithelial morphology and spatial organization, and epithelial cells coordinate mesenchymal cell behavior. During the postnatal stage, niche cells play an essential role in the coordination of organ regeneration. Therefore, epithelium-mesenchyme reconstitutions[66] and multiple cells assembled by bioprinting or cell adhesion molecules have been widely used for organ regeneration.[67,68]

ECM reconstruction strategy. The native ECM is compositionally and topographically complex and organ-specific. Therefore, decellularized organs, which retain organ-specific ECM, are a promising biomaterial for organ regeneration.[69,70] In recent years, this technology has been widely used and various decellularized organs have been generated, such as kidneys,[71] lungs,[72] hearts,[73] livers,[74] and teeth.[75] Furthermore, organ-derived ECM is widely used as a bioink for organ bioprinting of the heart, liver, vasculature, and skin.[76] ECM proteins are commonly mixed with other biomaterials to mimic natural ECM and are used for organ regeneration.[77]

Cell-ECM interaction strategy. The ECM mediates complex communication between stem and niche cells. These cell-ECM interactions affect various physiological and pathological processes. Any changes in fluid flow, partial pressure of gases, or adjacent units can lead to tensile, compressive, and shear forces inside or outside the niche around the ECM.[78,79] These mechanical stimuli are transduced into biological signals and play an indispensable role in defining stem cell behavior through cell-ECM interactions. The use of automatic force-sensing mechanisms to promote organ regeneration has been widely reported.[80] Devices that use mechanical force to stretch the intestine, such as intracavitary telescopic hydraulics, intraluminal coil springs, and extraluminal expanding polymers, have emerged in intestinal regeneration. Mechanical forces loaded from the outside can promote jaw regeneration by targeting various cells, including $Gli1^+$, $Axin2^+$, $Prrx1^+$, sutured mesenchymal stem cells, ECs, and fibroblasts.[42]

Key signaling-based niche remodeling strategy. Stem cells can receive and respond to a variety of signaling factors from the niche, including autocrine (stem cells) and paracrine (niche cells) factors, organ interactions, and other forms of signals, such as contact-dependent Notch, ECM-mediated adhesion receptor, or physical and mechanical signals. Critical signaling factors are crucial to maintain stem cell function and provide targets for clinical remodeling of the regeneration niche of stem cells.

Stem cell niche-functionalized strategies for tooth regeneration

Teeth are essential for chewing, swallowing, and facial aesthetics. Their structural complexities include highly calcified tooth enamel, dentin, and soft tissues (pulp and periodontal ligaments). Tooth development in mammalian embryos depends on the interaction between the ectoderm-derived epithelium and neural crest-derived mesenchymal cells. Based on the cell-cell interaction strategy, dental epithelial and mesenchymal tissues isolated from mouse embryonic tooth germs can be reconstituted into bioengineered tooth germs, which develop into fully-fledged functional teeth.[75,81,82] In a miniature swine model, the molecular level of epithelium-mesenchyme interactions,[83-86] cell-ECM interactions,[87] biomechanical stress,[88] and tooth development niches have been well identified. A miniature swine bioengineered tooth bud was generated based on the epithelium-mesenchyme interaction strategy verified after transplantation in mouse subrenal capsules and jawbone,[89] in addition to infusion with bone marrow stem cells and local aspirin administration to remodel immune cells. A miniature swine bioengineered tooth bud has also been used to develop a critical size tooth in the jawbone of miniature swine.[90] The tooth root provides a stable anchor for a natural or post-supported crown. Through multiple cell interactions (periodontal ligament stem cells and stem cells from the apical papilla or dental pulp stem cells), a root/periodontal complex can be formed, and a bioroot was verified in minia-

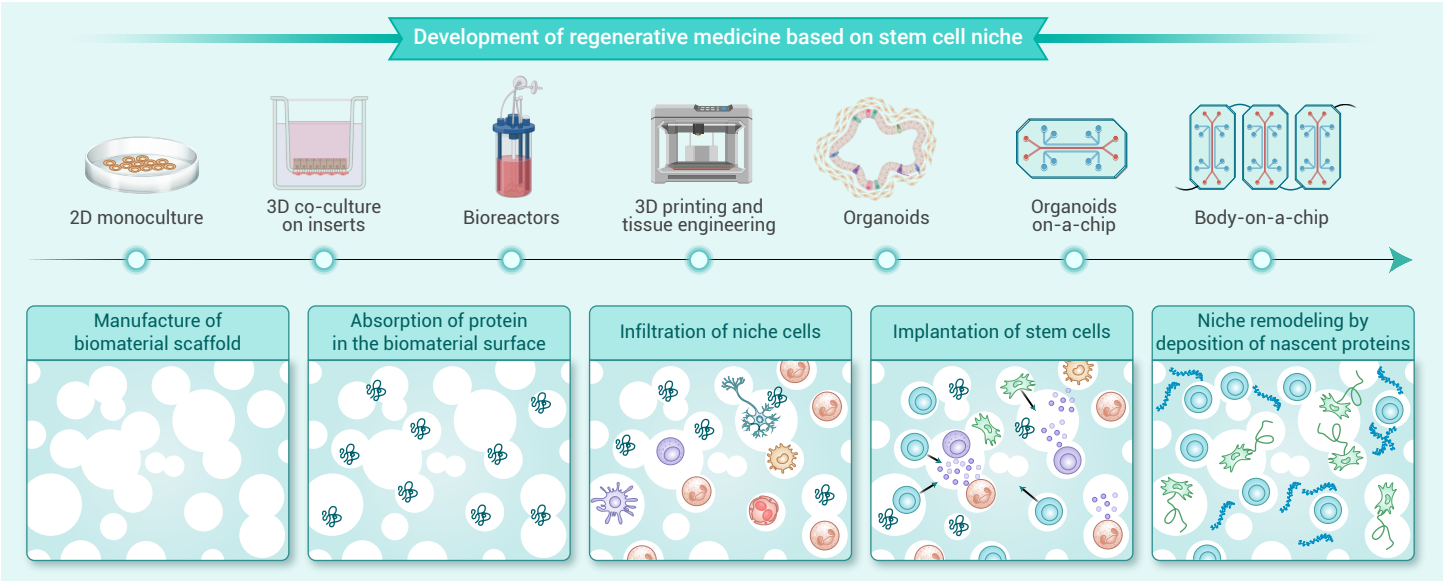

Figure 4. Development of regenerative medicine based on stem cell niche.

ture pigs.[91-93] Furthermore, a treated dentin matrix containing the tooth root ECM and critical signaling can enhance tooth root construction.[94,95] Finally, mesenchymal condensation spatiotemporally can assemble from dental stem cells and sequentially mediate functional and physiologically relevant tooth regeneration after implantation in animals and humans.[96]

Stem cell niche-functionalized strategies for gastrointestinal tract regeneration

The gastrointestinal tract is a hollow organ system with complex pronounced structures such as concentric rings of longitudinal and annular muscle fibers, invaginated crypts, and raised villi that can contain and digest food to extract and absorb energy and nutrients and expel food debris. The excellent self-renewal capacity of this organ depends on $Lgr5^+$ ISCs residing in the intestinal crypt. Paneth cells and the mesenchymal framework in the lamina propria comprising a network of fibrous and structural ECM proteins and cells, including fibroblasts and myofibroblasts, constitute the stem cell niche to support ISC self-renewal and differentiation.[97] Through interactions between ISCs and stromal cells, scaffold support, vascularization, and enteric nerve involvement, engineered intestinal grafts can be generated.[98] Smooth sphincter muscles and progenitor enteric neuronal cell interactions were used to fully bioengineer a gastrointestinal tract that was successfully transplanted into mice and regenerate a functional gastrointestinal tract *in vivo*.[99] Decellularized gastrointestinal tract acellular ECM scaffolds have been widely applied in gastroenterology.[100,101] Wnt and BMP signaling-based niche remodeling strategies have been widely used for intestinal regeneration. Inhibition of Notum activity restores Wnt-mediated Paneth cell and stem cell functions.[102] Other key signaling-based niche remodeling strategies that involve the Hippo-YAP pathway, Wnt signaling, JAK/STAT signaling, GTPase, and IGF have been reported.[103-105]

Stem cell niche-functionalized strategies for liver regeneration

The liver is a solid organ with various metabolic functions. Liver regeneration is a complex process that relies on hepatic stem/progenitor cells with associated niches comprising other resident cellular components, including hepatic stellate cells/myofibroblasts, macrophages, ECs, and the ECM, which maintain and regulate liver regeneration.[106] Decades ago, evidence showed that stem cells could regulate liver regeneration,[107] but their clinical efficacy still needs to be enhanced. By mediating stem cell niches, regeneration efficacy has dramatically increased.[16] Using a cell-cell interaction strategy, functional liver regeneration was achieved after ECs, hepatocytes, and fibroblasts were structurally organized in a degradable hydrogel and implanted in mice with liver injury.[108] Using an ECM reconstruction strategy, the liver tissue of different species was decellularized to produce a natural liver scaffold with preserved ECM, then recellularized with various cells types (primary hepatocytes, hepatoblasts, MSCs, induced pluripotent stem cells [iPSCs]), which show promising liver regeneration.[109] Furthermore, innovative hepatocytes or stem cell sheet technology introduced for liver regeneration have exhibited very promising outcomes.[110] These sheets preserve cell-ECM contacts and can be transplanted into ectopic sites or directly into the liver, leading to effective engraftment of adjacent cells necessary for maintaining liver function (Figure 3).[111,112]

STEM CELL NICHE-FUNCTIONALIZED STRATEGIES FOR ORGAN MANUFACTURING

Functionalized stem cell niche strategies can enhance the function of endogenous organs *in vivo* and promote organ manufacturing *in vitro*. Organoids and organoid-on-chip technologies have been developed based on these strategies. Over the past decade of concentrated development, various mini-organs with physiological structures and functions have been successfully constructed, including kidneys, livers, lungs, intestines, brains, prostates, pancreases, and retinas.[113-116]

Stem cell niche-functionalized strategies for organoid construction

With a deeper understanding of stem cell niches and organogenesis, three-dimensional (3D) organoids have emerged. The basic definition of an organoid is a 3D multicellular construct of cells with unlimited expansion ability and differentiation into functional cell types formed by self-assembly of *in vitro* biomimetic 3D structures. Organoids are a promising research tool in the life sciences. Various organoid models have been generated using biomimetics to simulate stem cell niches of different tissues *in vitro*, including ECM reconstruction, cell-ECM interactions, cell-cell interactions, and mechanical and biophysical factors (Figure 4).[117]

ECM reconstruction strategy. Organoids are typically cultured with biological or synthetic hydrogel scaffolds similar to native ECMs. Among these scaffolds, the most widely used is the Matrigel matrix gel, a mixture of heterogeneous glial proteins secreted by Engelbreth-Holm-Swarm mouse sarcoma cells. However, Matrigel does not have tissue-specific ECM signals and cannot provide a tissue-specific environment.[118] Subsequently, native tissue-derived decellularized ECM (dECM) hydrogels have been developed for organoid culture. The dECM provides organoids with the ECM and cell niche of the original tissue with excellent tissue specificity. They provide structural support, a unique combination of ECM biochemical factors and good manufacturing practices compliance, which are essential for clinical regeneration. In an experiment on ECM hydrogels obtained from decellularized porcine small intestinal mucosa, intestinal ECM-derived gels exhibited physiological characteristics and mechanical properties similar to those of synthetic gels. By adjusting the ECM parameters, including geometry, stiffness, stress relaxation, degradation rate, mechanical conduction, and various other compo-

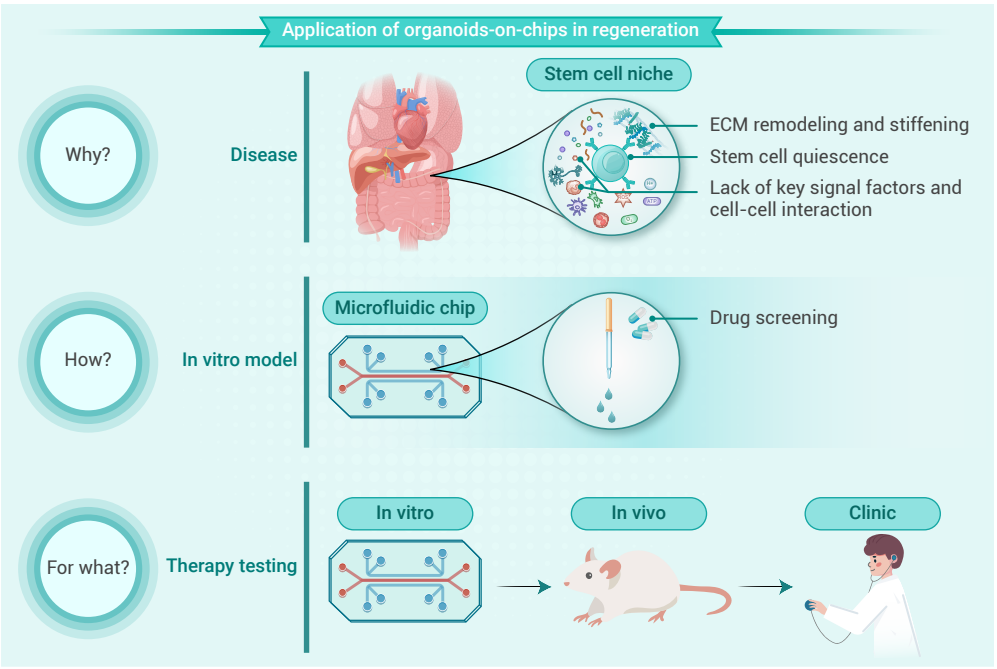

Figure 5. Application of organoids-on-chips in regeneration.

nents, the efficiency of organoid construction can be significantly increased.[118]

Key signaling-based niche remodeling strategy. The heterogeneity of stem cell niches in different tissues indicates that organoids require different niche signals. Therefore, additional growth factors or inhibitors must be provided in organoid culture systems based on the signaling or hormonal needs of the source tissue. In the intestinal tract of adults, in the presence of the Wnt agonist R-spondin, EGF, and the BMP inhibitor Noggin, $Lgr5^+$ stem cells form organoids in Matrigel.[119] Embryonic stem cells or pluripotent stem cell-derived organoids often require a gradual differentiation protocol. For example, the structural differentiation of the three germ layers from pluripotent stem cells depends on TGF-β-Nodal signal level. Low-level Nodal signaling induces mesodermal differentiation, high-level Nodal signaling induces endodermal differentiation, and inhibition of Nodal signaling leads to neuroectodermal differentiation. Furthermore, retinoic acid and BMP signaling antagonists can induce the development of gastric organoids, whereas FGF and SHH signaling lead to respiratory epithelial development. Subtle changes in each signaling factor in the culture system can yield different results. Therefore, the prerequisite for successfully establishing a mature organoid is a complete understanding of the factors and signaling pathways that regulate stem cells in tissue-specific stem cell niches *in vivo*.

Microbiome-cell interaction strategy. The importance of microbiome-stem cell interactions in organoid construction has become apparent in recent years, furthering exploration of the microbiome-stem cell niche. The micobiome-stem cell interaction strategy involves recovering organoids from the ECM, fragmenting them, mixing them with microorganisms and their products, and reseeding them in the ECM. Bacteria can be introduced into organoid structures through the self-assembly of organoids, and with the polarization of human intestinal organoids, direct contact with bacteria can be achieved. The organoid 3D structure is maintained while ensuring the apical-side contact of bacteria with crypt epithelial physiology. Hypoxia-tolerant bacteria survive, but bacterial overgrowth and organoid damage is prevented.[120] For example, following the microinjection of *E. coli* into pluripotent stem cell-derived intestinal organoids, their barrier function and antimicrobial properties are significantly enhanced.[121]

Cell-ECM interaction strategy. Organoid construction strategies based on mechanistic perception may have great potential in improving the regenerative capacity of stem cells.[122] Meng etc. developed an organoid culture system that can perform dynamic mechanical induction by combining traditional static culture models with cyclic stretching. Organoids generated in this culture system exhibited larger size and many more crypts than those generated under conventional static culture conditions, significantly promoting the growth of intestinal organoids.[123] Furthermore, vigorous proliferation of stem cells was observed in stretched organoids and the Wnt pathway was an essential signaling pathway in response to mechanical stretching and maintenance of stem cell stemness. These findings accelerated the application of stretch-induced organoid cultures in regenerative medicine. Moreover, the application of physical cues, such as mechanical movement, has also helped to develop organoids. For example, rotating bioreactors can increase the level of nutrients and oxygen perfusion of organoids, extend the time of organoid culture, and increase the size of organoids.[124,125]

Stem cell niche-functionalized strategies for organoids-on-chips construction

In recent years, the combination of organoids with the latest bioengineering tool, microfluidic organoids-on-chips, has overcome several limitations. This technology uses multiple separate flow-through microchambers to cultivate various cell types and simultaneously controls the culture environment in a cell-type-dependent manner.[126] By stimulating the mechanical and biochemical composition of target organs using chemical, mechanical, or electromagnetic methods, precise control of the organoid microenvironment can be realized, such as controlling the biochemical gradient of soluble factors in the local microenvironment, supporting the self-assembly of angiogenic ECs into 3D networks that can perfuse blood vessels, the real-time monitoring and control of oxygen concentration in 3D cell cultures,[127] and mechanical force control through external equipment.[128-130] More importantly, the modeling of tissue-tissue and multi-organ interactions is achieved through multi-compartment microdevices, which is essential for simulating the comprehensive behavior of complex physiological systems *in vitro*. Furthermore, a high degree of controllability reduces randomness and variability, significantly reduces organoid variability, and improves clinical reproducibility.[131,132]

Organoids-on-chips have advantages in terms of controllability and standardization of modeling, and more complex models can be constructed using co-culture technology. Therefore, the central role of the organoid-on-chip in regenerative medicine is to develop regenerative medicine through high-throughput analysis and screening of organoids under optimized conditions *in vitro*. Novel and available regenerative therapies increasingly rely on information obtained from organoids and organoid-on-chip systems, which provide essential validation data before early-stage clinical trials.[133,134] Through the separation and assembly of the constituent elements, the key participants in the decision-making process are determined and, ultimately, the mechanism of regenerative therapy is understood through organoids. Furthermore, organoid-on-chip systems can be used to test the safety of regenerative drugs, accelerate clinical trials, and reduce risks and costs, exhibiting excellent potential for development in regenerative tissue engineering (Figure 5).[135,136]

FUNCTIONALIZED STRATEGIES OF EMERGING NICHES

New applications that use developmental principles to generate emerging niches from human pluripotent stem cells (hPSCs) bring more possibilities to the field of organ regeneration.[137,138] Just as adult stem cells are dynamically regulated by their niches, the embryonic niche formed during development also controls the fate of cells from early precursor or progenitor cells to adult quiescent stem cells. Its role involves supporting the progenitor cells that build organ systems during development and mature through different stages to form adult stem cell niches. Unlike adult stem cell niches, initial development niches share common characteristics that regulate activation during maturation, self-renewal, and early precursor or progenitor cell regeneration.[139] Therefore, hPSCs, as a potent regenerative cell system, can theo-

retically directly generate fully functional adult stem cell niches, which has important theoretical significance in organ regeneration.

CONCLUSIONS AND PERSPECTIVES

Organ regeneration and manufacturing is an interdisciplinary field that combines stem cell biology, materials science, and tissue engineering techniques and adds complexity to personalized treatments designed for specific patient needs. The analysis, remodeling, and bionics of stem cell niches are key to functionalization strategies. Traditional tissue engineering tends to ignore the heterogeneity between organs. The single combination mode, materials, and technology significantly limits the accurate simulation and regeneration of organs. The functionalized strategies of stem cell niches aim to improve our understanding of the tissue- and organ-specificity of stem cell niches, help to analyze niche structure and function, and facilitate the design and construction of a microenvironment more suitable for stem cell growth and differentiation through remodeling and bionics. The ECM and niche cells cultivated from specific stem cell niches can be recombined with corresponding adult stem cells, and critical niche factors can be sufficiently controlled, thus improving organ regeneration efficiency. Developing various niche engineering technologies, including novel biomaterials and micromachining systems, to improve the similarity between the bionic platform and stem cell niche will aid in the manufacturing of high-fidelity organs for basic scientific applications, drug screening, and personalized medical applications. In addition, the functional strategy of generating emerging niches from hPSCs based on developmental principles to form functional adult stem cell niches has excellent potential in organ regeneration and manufacturing.

Although stem cell niche functionalization strategies for organ regeneration and manufacturing are promising and exciting, certain limitations remain. The heterogeneity and complex interactions of stem cells and their niches *in vivo* remain unclear and require further studies. With the progress of organ development and organogenesis research, critical stem cells and their niches can be identified and precise stem cell niche-functionalized strategies can be constructed. With artificial intelligence-assisted learning and design, the exact components of organs can be confirmed, and with advances in materials science and bio-fabrication, whole organ regeneration and manufacturing can be realized *in vitro* and *in vivo*.

REFERENCES

1. Yamanaka, S. (2020). Pluripotent stem cell-based cell therapy-promise and challenges. Cell Stem Cell **27**: 523–531. DOI: 10.1016/j.stem.2020.09.014.
2. Watt, F.M., and Driskell, R.R. (2010). The therapeutic potential of stem cells. Philos. Trans. R. Soc. Lond. B Biol. Sci. **365**: 155–163. DOI: 10.1098/rstb.2009.0149.
3. Daley, G.Q. (2012). The promise and perils of stem cell therapeutics. Cell Stem Cell **10**: 740–749. DOI: 10.1016/j.stem.2012.05.010.
4. Galipeau, J., and Sensébé, L. (2018). Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell **22**: 824–833. DOI: 10.1016/j.stem.2018.05.004.
5. Hofer, M., and Lutolf, M.P. (2021). Engineering organoids. Nat. Rev. Mater. **6**: 402–420. DOI: 10.1038/s41578-021-00279-y.
6. Rossi, G., Manfrin, A., and Lutolf, M.P. (2018). Progress and potential in organoid research. Nat. Rev. Genet. **19**: 671–687. DOI: 10.1038/s41576-018-0051-9.
7. Kim, W., Gwon, Y., Park, S., et al. (2023). Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. Bioact. Mater. **19**: 50–74. DOI: 10.1016/j.bioactmat.2022.03.039.
8. Lane, S.W., Williams, D.A., and Watt, F.M. (2014). Modulating the stem cell niche for tissue regeneration. Nat. Biotechnol. **32**: 795–803. DOI: 10.1038/nbt.2978.
9. Chacón-Martínez, C.A., Koester, J., and Wickström, S.A. (2018). Signaling in the stem cell niche: regulating cell fate, function and plasticity. Development **145**: dev165399. DOI: 10.1242/dev.165399.
10. Hicks, M.R., and Pyle, A.D. (2023). The emergence of the stem cell niche. Trends Cell Biol. **33**: 112–123. DOI: 10.1016/j.tcb.2022.07.003.
11. Xie, T., and Spradling, A.C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. Science **290**: 328–330. DOI: 10.1126/science.290.5490.328.
12. Morrison, S.J., and Spradling, A.C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell **132**: 598–611. DOI: 10.1016/j.cell.2008.01.038.
13. Schmidt, M., Schüler, S.C., Hüttner, S.S., et al. (2019). Adult stem cells at work: regenerating skeletal muscle. Cell. Mol. Life Sci. **76**: 2559–2570. DOI: 10.1007/s00018-019-03093-6.
14. Brittan, M., and Wright, N.A. (2002). Gastrointestinal stem cells. J. Pathol. **197**: 492–509. DOI: 10.1002/path.1155.
15. Shaker, A., and Rubin, D.C. (2010). Intestinal stem cells and epithelial-mesenchymal interactions in the crypt and stem cell niche. Transl. Res. **156**: 180–187. DOI: 10.1016/j.trsl.2010.06.003.
16. Zhang, L., Theise, N., Chua, M., et al. (2008). The stem cell niche of human livers: Symmetry between development and regeneration. Hepatology **48**: 1598–1607. DOI: 10.1002/hep.22516.
17. Gonzales, K.A.U., and Fuchs, E. (2017). Skin and its regenerative powers: An alliance between stem cells and their niche. Dev. Cell **43**: 387–401. DOI: 10.1016/j.devcel.2017.10.001.
18. Ort, C., Dayekh, K., Xing, M., et al. (2018). Emerging strategies for stem cell lineage commitment in tissue engineering and regenerative medicine. ACS Biomater. Sci. Eng. **4**: 3644–3657. DOI: 10.1021/acsbiomaterials.8b00532.
19. Wattrus, S.J., and Zon, L.I. (2018). Stem cell safe harbor: The hematopoietic stem cell niche in zebrafish. Blood Adv. **2**: 3063–3069. DOI: 10.1182/bloodadvances.2018021725.
20. Diotel, N., Lübke, L., Strähle, U., et al. (2020). Common and distinct features of adult neurogenesis and regeneration in the telencephalon of zebrafish and mammals. Front. Neurosci. **14**: 568930. DOI: 10.3389/fnins.2020.568930.
21. Schwarzer, S., Rekhade, D.R., Machate, A., et al. (2022). Reactivation of the neurogenic niche in the adult zebrafish statoacoustic ganglion following a mechanical lesion. Front. Cell Dev. Biol. **10**: 850624. DOI: 10.3389/fcell.2022.850624.
22. Rajan, V., Melong, N., Hing Wong, W., et al. (2020). Humanized zebrafish enhance human hematopoietic stem cell survival and promote acute myeloid leukemia clonal diversity. Haematologica **105**: 2391–2399. DOI: 10.3324/haematol.2019.223040.
23. Ishizuya-Oka, A., and Hasebe, T. (2013). Establishment of intestinal stem cell niche during amphibian metamorphosis. Curr. Top. Dev. Biol. **103**: 305–327. DOI: 10.1016/B978-0-12-385979-2.00011-3.
24. Ghaddar, B., Lübke, L., Couret, D., et al. (2021). Cellular mechanisms participating in brain repair of adult zebrafish and mammals after injury. Cell **10**: 391. DOI: 10.3390/cells10020391.
25. Ceci, M., Mariano, V., and Romano, N. (2018). Zebrafish as a translational regeneration model to study the activation of neural stem cells and role of their environment. Rev. Neurosci. **30**: 45–66. DOI: 10.1515/revneuro-2018-0020.
26. Xia, J., Kang, Z., Xue, Y., et al. (2021). A single-cell resolution developmental atlas of hematopoietic stem and progenitor cell expansion in zebrafish. Proc. Natl. Acad. Sci. U. S. A. **118**: e2015748118. DOI: 10.1073/pnas.2015748118.
27. Hu, H., Duan, Y., Wang, K., et al. (2022). Dental niche cells directly contribute to tooth reconstitution and morphogenesis. Cell Rep. **41**: 111737. DOI: 10.1016/j.celrep.2022.111737.
28. Santos, A.J.M., Lo, Y.H., Mah, A.T., et al. (2018). The intestinal stem cell niche: Homeostasis and adaptations. Trends Cell Biol. **28**: 1062–1078. DOI: 10.1016/j.tcb.2018.08.001.
29. Ratnayake, D., Nguyen, P.D., Rossello, F.J., et al. (2021). Macrophages provide a transient muscle stem cell niche via NAMPT secretion. Nature **591**: 281–287. DOI: 10.1038/s41586-021-03199-7.
30. Li, J., Tan, J., Martino, M.M., et al. (2018). Regulatory T-cells: Potential regulator of tissue repair and regeneration. Front. Immunol. **9**: 585. DOI: 10.3389/fimmu.2018.00585.
31. Schüler, S.C., Liu, Y., Dumontier, S., et al. (2022). Extracellular matrix: Brick and mortar in the skeletal muscle stem cell niche. Front. Cell Dev. Biol. **10**: 1056523. DOI: 10.3389/fcell.2022.1056523.
32. Lee-Thedieck, C., Schertl, P., and Klein, G. (2022). The extracellular matrix of hematopoietic stem cell niches. Adv. Drug Deliv. Rev. **181**: 114069. DOI: 10.1016/j.addr.2021.114069.
33. Arulmoli, J., Pathak, M.M., McDonnell, L.P., et al. (2015). Static stretch affects neural stem cell differentiation in an extracellular matrix-dependent manner. Sci. Rep. **5**: 8499. DOI: 10.1038/srep08499.
34. Sun, Y., Xu, S., Jiang, M., et al. (2021). Role of the extracellular matrix in Alzheimer's disease. Front. Aging Neurosci. **13**: 707466. DOI: 10.3389/fnagi.2021.707466.
35. Arranz, A.M., Perkins, K.L., Irie, F., et al. (2014). Hyaluronan deficiency due to Has3 knock-out causes altered neuronal activity and seizures via reduction in brain extracellular space. J. Neurosci. **34**: 6164–6176. DOI: 10.1523/JNEUROSCI.3458-13.2014.
36. Yeo, G.C., and Weiss, A.S. (2019). Soluble matrix protein is a potent modulator of mesenchymal stem cell performance. Proc. Natl. Acad. Sci. U. S. A. **116**: 2042–2051. DOI: 10.1073/pnas.1812951116.
37. Saw, S., Weiss, A., Khokha, R., et al. (2019). Metalloproteases: On the watch in the hematopoietic niche. Trends Immunol. **40**: 1053–1070. DOI: 10.1016/j.it.2019.09.006.
38. Martín-Alonso, M., Iqbal, S., Vornewald, P.M., et al. (2021). Smooth muscle-specific MMP17 (MT4-MMP) regulates the intestinal stem cell niche and regeneration after damage. Nat. Commun. **12**: 6741. DOI: 10.1038/s41467-021-26904-6.
39. Nusse, R., and Clevers, H. (2017). Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities. Cell **169**: 985–999. DOI: 10.1016/j.cell.2017.05.016.
40. Chakrabarti, R., Celià-Terrassa, T., Kumar, S., et al. (2018). Notch ligand Dll1 mediates cross-talk between mammary stem cells and the macrophageal niche. Science **360**: eaan4153. DOI: 10.1126/science.aan4153.
41. Chung, M.I., Bujnis, M., Barkauskas, C.E., et al. (2018). Niche-mediated BMP/SMAD signaling regulates lung alveolar stem cell proliferation and differentiation. Development **145**: dev163014. DOI: 10.1242/dev.163014.
42. Chen, G., Xu, H., Yao, Y., et al. (2020). BMP signaling in the development and

regeneration of cranium bones and maintenance of calvarial stem cells. Front. Cell Dev. Biol. **8**: 135. DOI: 10.3389/fcell.2020.00135.
43. Wang, Y., Kim, R., Hinman, S.S., et al. (2018). Bioengineered systems and designer matrices that recapitulate the intestinal stem cell niche. Cell. Mol. Gastroenterol. Hepatol. **5**: 440–453.e441. DOI: 10.1016/j.jcmgh.2018.01.008.
44. Parfenova, O.K., Kukes, V.G., and Grishin, D.V. (2021). Follistatin-like proteins: Structure, functions and biomedical importance. Biomedicines **9**: 999. DOI: 10.3390/biomedicines9080999.
45. Nakamura, T., Jimenez-Rojo, L., Koyama, E., et al. (2017). Epiprofin regulates enamel formation and tooth morphogenesis by controlling epithelial-mesenchymal interactions during tooth development. J. Bone Miner. Res. **32**: 601–610. DOI: 10.1002/jbmr.3024.
46. Fahmy-Garcia, S., Farrell, E., Witte-Bouma, J., et al. (2019). Follistatin effects in migration, vascularization, and osteogenesis in vitro and bone repair in vivo. Front. Bioeng. Biotechnol. **7**: 38. DOI: 10.3389/fbioe.2019.00038.
47. Elbediwy, A., Vincent-Mistiaen, Z.I., and Thompson, B.J. (2016). YAP and TAZ in epithelial stem cells: A sensor for cell polarity, mechanical forces and tissue damage. Bioessays **38**: 644–653. DOI: 10.1002/bies.201600037.
48. van Soldt, B.J., and Cardoso, W.V. (2019). Hippo-Yap/Taz signaling: Complex network interactions and impact in epithelial cell behavior. Wiley Interdiscip. Rev. Dev. Biol. **9**: e371. DOI: 10.1002/wdev.371.
49. Piccolo, S., Dupont, S., and Cordenonsi, M. (2014). The biology of YAP/TAZ: Hippo signaling and beyond. Physiol. Rev. **94**: 1287–1312. DOI: 10.1152/physrev.00005.2014.
50. Moya, I.M., and Halder, G. (2019). Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. Nat. Rev. Mol. Cell Biol. **20**: 211–226. DOI: 10.1038/s41580-018-0086-y.
51. Park, H.W., Kim, Y.C., Yu, B., et al. (2015). Alternative Wnt signaling activates YAP/TAZ. Cell **162**: 780–794. DOI: 10.1016/j.cell.2015.07.013.
52. Adebayo, A.K., and Nakshatri, H. (2022). Modeling preclinical cancer studies under physioxia to enhance clinical translation. Cancer Res. **82**: 4313–4321. DOI: 10.1158/0008-5472.CAN-22-2311.
53. Li, C., Wu, B., Li, Y., et al. (2022). Loss of sphingosine kinase 2 promotes the expansion of hematopoietic stem cells by improving their metabolic fitness. Blood **140**: 1686–1701. DOI: 10.1182/blood.2022016112.
54. Liu, J., He, J., Ge, L., et al. (2021). Hypoxic preconditioning rejuvenates mesenchymal stem cells and enhances neuroprotection following intracerebral hemorrhage via the miR-326-mediated autophagy. Stem Cell. Res. Ther. **12**: 413. DOI: 10.1186/s13287-021-02480-w.
55. Mu, J., Li, L., Wu, J., et al. (2022). Hypoxia-stimulated mesenchymal stem cell-derived exosomes loaded by adhesive hydrogel for effective angiogenic treatment of spinal cord injury. Biomater Sci **10**: 1803–1811. DOI: 10.1039/D1BM01722E.
56. Hu, C., Fan, L., Cen, P., et al. (2016). Energy metabolism plays a critical role in stem cell maintenance and differentiation. Int. J. Mol. Sci. **17**: 253. DOI: 10.3390/ijms17020253.
57. Folmes, C.D., Dzeja, P.P., Nelson, T.J., et al. (2012). Metabolic plasticity in stem cell homeostasis and differentiation. Cell Stem Cell **11**: 596–606. DOI: 10.1016/j.stem.2012.10.002.
58. Morimoto, H., Yamamoto, T., Miyazaki, T., et al. (2021). An interplay of NOX1-derived ROS and oxygen determines the spermatogonial stem cell self-renewal efficiency under hypoxia. Genes Dev. **35**: 250–260. DOI: 10.1101/gad.339903.120.
59. He, R., Wang, Z., Cui, M., et al. (2021). HIF1A Alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. Autophagy **17**: 3338–3360. DOI: 10.1080/15548627.2021.1872227.
60. Choi, W., Kwon, S.J., Jin, H.J., et al. (2017). Optimization of culture conditions for rapid clinical-scale expansion of human umbilical cord blood-derived mesenchymal stem cells. Clin. Transl. Med. **6**: 38. DOI: 10.1186/s40169-017-0168-z.
61. Nakamura, N., Shi, X., Darabi, R., et al. (2021). Hypoxia in cell reprogramming and the epigenetic regulations. Front. Cell Dev. Biol. **9**: 609984. DOI: 10.3389/fcell.2021.609984.
62. Wang, W., Hu, C.K., Zeng, A., et al. (2020). Changes in regeneration-responsive enhancers shape regenerative capacities in vertebrates. Science **369**: eaaz3090. DOI: 10.1126/science.aaz3090.
63. Lumelsky, N., O'Hayre, M., Chander, P., et al. (2018). Autotherapies: Enhancing endogenous healing and regeneration. Trends Mol. Med. **24**: 919–930. DOI: 10.1016/j.molmed.2018.08.004.
64. Dong, J., Hu, Y., Fan, X., et al. (2018). Single-cell RNA-seq analysis unveils a prevalent epithelial/mesenchymal hybrid state during mouse organogenesis. Genome Biol. **19**: 31. DOI: 10.1186/s13059-018-1416-2.
65. Ribatti, D., and Santoiemma, M. (2014). Epithelial-mesenchymal interactions: A fundamental Developmental Biology mechanism. Int. J. Dev. Biol. **58**: 303–306. DOI: 10.1387/ijdb.140143dr.
66. Nakao, K., Morita, R., Saji, Y., et al. (2007). The development of a bioengineered organ germ method. Nat. Methods **4**: 227–230. DOI: 10.1038/nmeth1012.
67. Wang, Z., Kapadia, W., Li, C., et al. (2021). Tissue-specific engineering: 3D bioprinting in regenerative medicine. J. Control. Release **329**: 237–256. DOI: 10.1016/j.jconrel.2020.11.044.
68. Stevens, A.J., Harris, A.R., Gerdts, J., et al. (2023). Programming multicellular assembly with synthetic cell adhesion molecules. Nature **614**: 144–152. DOI: 10.1038/s41586-022-05622-z.
69. Song, J.J., and Ott, H.C. (2011). Organ engineering based on decellularized matrix scaffolds. Trends Mol. Med. **17**: 424–432. DOI: 10.1016/j.molmed.2011.03.005.
70. Rajab, T.K., O'Malley, T.J., and Tchantchaleishvili, V. (2020). Decellularized scaffolds for tissue engineering: Current status and future perspective. Artif. Organs **44**: 1031–1043. DOI: 10.1111/aor.13701.
71. Orlando, G., Booth, C., Wang, Z., et al. (2013). Discarded human kidneys as a source of ECM scaffold for kidney regeneration technologies. Biomaterials **34**: 5915–5925. DOI: 10.1016/j.biomaterials.2013.04.033.
72. Nichols, J.E., Niles, J., Riddle, M., et al. (2013). Production and assessment of decellularized pig and human lung scaffolds. Tissue Eng. Part A **19**: 2045–2062. DOI: 10.1089/ten.tea.2012.0250.
73. Sánchez, P.L., Fernández-Santos, M.E., Costanza, S., et al. (2015). Acellular human heart matrix: A critical step toward whole heart grafts. Biomaterials **61**: 279–289. DOI: 10.1016/j.biomaterials.2015.04.056.
74. Mazza, G., Rombouts, K., Rennie Hall, A., et al. (2015). Decellularized human liver as a natural 3D-scaffold for liver bioengineering and transplantation. Sci. Rep. **5**: 13079. DOI: 10.1038/srep13079.
75. Zhang, W., Vazquez, B., Oreadi, D., et al. (2017). Decellularized tooth bud scaffolds for tooth regeneration. J. Dent. Res. **96**: 516–523. DOI: 10.1177/0022034516689082.
76. Choudhury, D., Tun, H.W., Wang, T., et al. (2018). Organ-derived decellularized extracellular matrix: A game changer for bioink manufacturing. Trends Biotechnol. **36**: 787–805. DOI: 10.1016/j.tibtech.2018.03.003.
77. Xing, H., Lee, H., Luo, L., et al. (2020). Extracellular matrix-derived biomaterials in engineering cell function. Biotechnol. Adv. **42**: 107421. DOI: 10.1016/j.biotechadv.2019.107421.
78. Xing, Y., and Naik, S. (2020). Under pressure: Stem cell-niche interactions coordinate tissue adaptation to inflammation. Curr. Opin. Cell Biol. **67**: 64–70. DOI: 10.1016/j.ceb.2020.08.009.
79. Chaudhuri, O., Cooper-White, J., Janmey, P.A., et al. (2020). Effects of extracellular matrix viscoelasticity on cellular behaviour. Nature **584**: 535–546. DOI: 10.1038/s41586-020-2612-2.
80. Xie, W., Wei, X., Kang, H., et al. (2023). Static and dynamic: Evolving biomaterial mechanical properties to control cellular mechanotransduction. Adv. Sci. **10**: e2204594. DOI: 10.1002/advs.202204594.
81. Ikeda, E., Morita, R., Nakao, K., et al. (2009). Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc. Natl. Acad. Sci. U. S. A. **106**: 13475–13480. DOI: 10.1073/pnas.0902944106.
82. Zhang, W., and Yelick, P.C. (2021). Tooth repair and regeneration: potential of dental stem cells. Trends Mol. Med. **27**: 501–511. DOI: 10.1016/j.molmed.2021.02.005.
83. Wang, F., Xiao, J., Cong, W., et al. (2014). Morphology and chronology of diphyodont dentition in miniature pigs, Sus Scrofa. Oral Dis. **20**: 367–379. DOI: 10.1111/odi.12126.
84. Li, Y., Li, G., Wang, F., et al. (2019). Integrated analysis of LncRNA-mRNA coexpression in the extracellular matrix of developing deciduous teeth in miniature pigs. Biomed. Res. Int. **2019**: 6159490. DOI: 10.1155/2019/6159490.
85. Li, Y., Gong, Y., Wu, X., et al. (2018). Quantitative proteomic analysis of deciduous molars during cap to bell transition in miniature pig. J. Proteomics **172**: 57–67. DOI: 10.1016/j.jprot.2017.10.013.
86. Wang, F., Li, Y., Wu, X., et al. (2017). Transcriptome analysis of coding and long non-coding RNAs highlights the regulatory network of cascade initiation of permanent molars in miniature pigs. BMC Genomics **18**: 148. DOI: 10.1186/s12864-017-3546-4.
87. Li, Y., Wang, X., Ren, J., et al. (2018). Mandible exosomal ssc-mir-133b regulates tooth development in miniature swine via endogenous apoptosis. Bone Res. **6**: 28. DOI: 10.1038/s41413-018-0028-5.
88. Wu, X., Hu, J., Li, G., et al. (2020). Biomechanical stress regulates mammalian tooth replacement via the integrin β1-RUNX2-Wnt pathway. EMBO J. **39**: e102374. DOI: 10.15252/embj.2019102374.
89. Wang, F., Wu, Z., Fan, Z., et al. (2018). The cell re-association-based whole-tooth regeneration strategies in large animal, Sus scrofa. Cell Prolif. **51**: e12479. DOI: 10.1111/cpr.12479.
90. Wu, Z., Wang, F., Fan, Z., et al. (2019). Whole-tooth regeneration by allogeneic cell reassociation in pig jawbone. Tissue Eng. Part A **25**: 1202–1212. DOI: 10.1089/ten.tea.2018.0243.
91. Gao, Z.H., Hu, L., Liu, G.L., et al. (2016). Bio-root and implant-based restoration as a tooth replacement alternative. J. Dent. Res. **95**: 642–649. DOI: 10.1177/0022034516639260.
92. Sonoyama, W., Liu, Y., Fang, D., et al. (2006). Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS ONE **1**: e79. DOI: 10.1371/journal.pone.0000079.
93. Wei, F., Song, T., Ding, G., et al. (2013). Functional tooth restoration by allogeneic mesenchymal stem cell-based bio-root regeneration in swine. Stem Cells Dev. **22**: 1752–1762. DOI: 10.1089/scd.2012.0688.
94. He, L., Zhou, J., Chen, M., et al. (2019). Parenchymal and stromal tissue regeneration of tooth organ by pivotal signals reinstated in decellularized matrix. Nat. Mater. **18**: 627–637. DOI: 10.1038/s41563-019-0368-6.
95. Yang, X., Ma, Y., Guo, W., et al. (2019). Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. Theranostics **9**: 2694–2711. DOI: 10.7150/thno.31801.

96. Sui, B.D., Zheng, C.X., Zhao, W.M., et al. (2023). Mesenchymal condensation in tooth development and regeneration: A focus on translational aspects of organogenesis. Physiol. Rev. **103**: 1899–1964. DOI: 10.1152/physrev.00019.2022.
97. Yen, T.H., and Wright, N.A. (2006). The gastrointestinal tract stem cell niche. Stem Cell Rev. **2**: 203–212. DOI: 10.1007/s12015-006-0048-1.
98. Tullie, L., Jones, B.C., De Coppi, P., et al. (2022). Building gut from scratch - progress and update of intestinal tissue engineering. Nat. Rev. Gastroenterol. Hepatol. **19**: 417–431. DOI: 10.1038/s41575-022-00586-x.
99. Raghavan, S., Gilmont, R.R., Miyasaka, E.A., et al. (2011). Successful implantation of bioengineered, intrinsically innervated, human internal anal sphincter. Gastroenterology **141**: 310–319. DOI: 10.1053/j.gastro.2011.03.056.
100. Speer, A.L., Ren, X., McNeill, E.P., et al. (2021). Bioengineering of the digestive tract: Approaching the clinic. Cytotherapy **23**: 381–389. DOI: 10.1016/j.jcyt.2021.02.006.
101. Orlando, G., Domínguez-Bendala, J., Shupe, T., et al. (2013). Cell and organ bioengineering technology as applied to gastrointestinal diseases. Gut **62**: 774–786. DOI: 10.1136/gutjnl-2011-301111.
102. Pentinmikko, N., Iqbal, S., Mana, M., et al. (2019). Notum produced by Paneth cells attenuates regeneration of aged intestinal epithelium. Nature **571**: 398–402. DOI: 10.1038/s41586-019-1383-0.
103. Fan, Y., Huo, X., Guo, B., et al. (2022). Cullin 4b-RING ubiquitin ligase targets IRGM1 to regulate Wnt signaling and intestinal homeostasis. Cell Death Differ. **29**: 1673–1688. DOI: 10.1038/s41418-022-00954-9.
104. Richmond, C.A., Rickner, H., Shah, M.S., et al. (2018). JAK/STAT-1 signaling is required for reserve intestinal stem cell activation during intestinal regeneration following acute inflammation. Stem Cell Rep. **10**: 17–26. DOI: 10.1016/j.stemcr.2017.11.015.
105. Zheng, Y., Song, Y., Han, Q., et al. (2018). Intestinal epithelial cell-specific IGF1 promotes the expansion of intestinal stem cells during epithelial regeneration and functions on the intestinal immune homeostasis. Am. J. Physiol. Endocrinol. Metab. **315**: E638–E649. DOI: 10.1152/ajpendo.00022.2018.
106. Campana, L., Esser, H., Huch, M., et al. (2021). Liver regeneration and inflammation: From fundamental science to clinical applications. Nat. Rev. Mol. Cell Biol. **22**: 608–624. DOI: 10.1038/s41580-021-00373-7.
107. Zhang, L., Ma, X.J., Fei, Y.Y., et al. (2022). Stem cell therapy in liver regeneration: Focus on mesenchymal stem cells and induced pluripotent stem cells. Pharmacol. Ther. **232**: 108004. DOI: 10.1016/j.pharmthera.2021.108004.
108. Stevens, K.R., Scull, M.A., Ramanan, V., et al. (2017). In situ expansion of engineered human liver tissue in a mouse model of chronic liver disease. Sci. Transl. Med. **9**: eaah5505. DOI: 10.1126/scitranslmed.aah5505.
109. Asadi, M., Khalili, M., Lotfi, H., et al. (2021). Liver bioengineering: Recent trends/advances in decellularization and cell sheet technologies towards translation into the clinic. Life Sci. **276**: 119373. DOI: 10.1016/j.lfs.2021.119373.
110. Alwahsh, S.M., Rashidi, H., and Hay, D.C. (2018). Liver cell therapy: is this the end of the beginning. Cell. Mol. Life Sci. **75**: 1307–1324. DOI: 10.1007/s00018-017-2713-8.
111. Tatsumi, K., and Okano, T. (2017). Hepatocyte transplantation: Cell sheet technology for liver cell transplantation. Curr. Trans. Rep. **4**: 184–192. DOI: 10.1007/s40472-017-0156-7.
112. Fujii, M., Yamanouchi, K., Sakai, Y., et al. (2018). In vivo construction of liver tissue by implantation of a hepatic non-parenchymal/adipose-derived stem cell sheet. J. Tissue Eng. Regen. Med. **12**: e287–e295. DOI: 10.1002/term.2424.
113. Tang, X.Y., Wu, S., Wang, D., et al. (2022). Human organoids in basic research and clinical applications. Signal Transduct. Target. Ther. **7**: 168. DOI: 10.1038/s41392-022-01024-9.
114. Miao, Y., Ha, A., de Lau, W., et al. (2020). Next-generation surrogate Wnts support organoid growth and deconvolute frizzled pleiotropy in vivo. Cell Stem Cell **27**: 840–851.e846. DOI: 10.1016/j.stem.2020.07.020.
115. Nguyen, D.T., Althage, M., Magnone, M.C., et al. (2018). Translational strategy: Humanized mini-organs. Drug Discov. Today **23**: 1812–1817. DOI: 10.1016/j.drudis.2018.05.039.
116. Mullard, A. (2023). Mini-organs attract big pharma. Nat. Rev. Drug Discov. **22**: 175–176. DOI: 10.1038/d41573-023-00030-y.
117. Corrò, C., Novellasdemunt, L., and Li, V.S.W. (2020). A brief history of organoids. Am. J. Physiol. Cell Physiol. **319**: C151–c165. DOI: 10.1152/ajpcell.00120.2020.
118. Madl, C.M., and Heilshorn, S.C. (2018). Engineering hydrogel microenvironments to recapitulate the stem cell niche. Annu. Rev. Biomed. Eng. **20**: 21–47. DOI: 10.1146/annurev-bioeng-062117-120954.
119. Yan, K.S., Janda, C.Y., Chang, J., et al. (2017). Non-equivalence of Wnt and R-spondin ligands during Lgr5(+) intestinal stem-cell self-renewal. Nature **545**: 238–242. DOI: 10.1038/nature22313.
120. Puschhof, J., Pleguezuelos-Manzano, C., Martinez-Silgado, A., et al. (2021). Intestinal organoid cocultures with microbes. Nat. Protoc. **16**: 4633–4649. DOI: 10.1038/s41596-021-00589-z.
121. Han, X., Mslati, M.A., Davies, E., et al. (2021). Creating a more perfect union: modeling intestinal bacteria-epithelial interactions using organoids. Cell. Mol. Gastroenterol. Hepatol. **12**: 769–782. DOI: 10.1016/j.jcmgh.2021.04.010.
122. Tallapragada, N.P., Cambra, H.M., Wald, T., et al. (2021). Inflation-collapse dynamics drive patterning and morphogenesis in intestinal organoids. Cell Stem Cell **28**: 1516–1532.e1514. DOI: 10.1016/j.stem.2021.04.002.
123. Meng, F., Shen, C., Yang, L., et al. (2022). Mechanical stretching boosts expansion and regeneration of intestinal organoids through fueling stem cell self-renewal. Cell Regen. **11**: 39. DOI: 10.1186/s13619-022-00137-4.
124. Zhang, W., Huang, G., and Xu, F. (2020). Engineering biomaterials and approaches for mechanical stretching of cells in three dimensions. Front. Bioeng. Biotechnol. **8**: 589590. DOI: 10.3389/fbioe.2020.589590.
125. He, L., Si, G., Huang, J., et al. (2018). Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel. Nature **555**: 103–106. DOI: 10.1038/nature25744.
126. Argentiere, S., Siciliano, P.A., and Blasi, L. (2021). How microgels can improve the impact of organ-on-chip and microfluidic devices for 3d culture: Compartmentalization, single cell encapsulation and control on cell fate. Polymers **13**: 3216. DOI: 10.3390/polym13193216.
127. Rivera, K.R., Pozdin, V.A., Young, A.T., et al. (2019). Integrated phosphorescence-based photonic biosensor (iPOB) for monitoring oxygen levels in 3D cell culture systems. Biosens. Bioelectron. **123**: 131–140. DOI: 10.1016/j.bios.2018.07.035.
128. Park, S.E., Georgescu, A., and Huh, D. (2019). Organoids-on-a-chip. Science **364**: 960–965. DOI: 10.1126/science.aaw7894.
129. Zhang, S., Wan, Z., and Kamm, R.D. (2021). Vascularized organoids on a chip: Strategies for engineering organoids with functional vasculature. Lab Chip **21**: 473–488. DOI: 10.1039/D0LC01186J.
130. Shirure, V.S., Hughes, C.C.W., and George, S.C. (2021). Engineering vascularized organoid-on-a-chip models. Annu. Rev. Biomed. Eng. **23**: 141–167. DOI: 10.1146/annurev-bioeng-090120-094330.
131. Not list. (2022). Organoids and organs on a chip. Nat. Biotechnol. **40**: 472. DOI: 10.1038/s41587-022-01287-1.
132. Baptista, L.S., Porrini, C., Kronemberger, G.S., et al. (2022). 3D organ-on-a-chip: The convergence of microphysiological systems and organoids. Front. Cell Dev. Biol. **10**: 1043117. DOI: 10.3389/fcell.2022.1043117.
133. Wang, Y., Gao, Y., Pan, Y., et al. (2023). Emerging trends in organ-on-a-chip systems for drug screening. Acta Pharm. Sin. B **13**: 2483–2509. DOI: 10.1016/j.apsb.2023.02.006.
134. Skardal, A., Aleman, J., Forsythe, S., et al. (2020). Drug compound screening in single and integrated multi-organoid body-on-a-chip systems. Biofabrication **12**: 025017. DOI: 10.1088/1758-5090/ab6d36.
135. Mittal, R., Woo, F.W., Castro, C.S., et al. (2019). Organ-on-chip models: Implications in drug discovery and clinical applications. J. Cell. Physiol. **234**: 8352–8380. DOI: 10.1002/jcp.27729.
136. Rajan, S.A.P., Aleman, J., Wan, M., et al. (2020). Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform. Acta Biomater. **106**: 124–135. DOI: 10.1016/j.actbio.2020.02.015.
137. Ouchi, R., and Koike, H. (2023). Modeling human liver organ development and diseases with pluripotent stem cell-derived organoids. Front. Cell Dev. Biol. **11**: 1133534. DOI: 10.3389/fcell.2023.1133534.
138. Maldonado, M., Luu, R.J., Ico, G., et al. (2017). Lineage- and developmental stage-specific mechanomodulation of induced pluripotent stem cell differentiation. Stem Cell. Res. Ther. **8**: 216. DOI: 10.1186/s13287-017-0667-2.
139. Mashinchian, O., De Franceschi, F., Nassiri, S., et al. (2022). An engineered multicellular stem cell niche for the 3D derivation of human myogenic progenitors from iPSCs. EMBO J. **41**: e110655. DOI: 10.15252/embj.2022110655.

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AUTHOR CONTRIBUTIONS

Huan Liu and Lei Hu contributed equally to writing the manuscript. Dake Zhang, Xiaogang Wang and Songlin Wang supervised, organized and revised the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.

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