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Review

Aging in 3D: Organoid Systems as Models to Uncover Cellular Senescence and Therapeutic Targets Across Diseases

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Abstract

Aging is a complex biological process characterized by progressive loss of cellular homeostasis, impaired regenerative capacity, and accumulation of senescent cells that collectively predispose tissues to disease. Traditional two-dimensional culture systems and animal models have provided valuable insights but fail to fully recapitulate the spatial organization, cellular heterogeneity, and microenvironmental cues of aging human tissues. Organoid technology—three-dimensional self-organizing structures derived from adult stem cells or pluripotent stem cells has emerged as a transformative platform to model aging in vitro. These mini-tissues retain the architecture, signaling dynamics, and lineage hierarchy of native organs, making them powerful systems to interrogate age-associated cellular phenotypes, DNA damage responses, and senescence programs. This review discusses how organoid models are advancing our understanding of aging biology across multiple organ systems, from the intestines and liver to the brain and lung. We highlighted key molecular pathways driving cellular senescence within organoids—including p16INK4a/p21CIP1 signaling, SASP activation, mitochondrial dysfunction, and epigenetic drift—and how these can be targeted to restore tissue homeostasis. We further discussed how organoids derived from aged tissues, induced pluripotent stem cells, and engineered oncogene systems reveal new therapeutic opportunities to modulate senescence in age-related disorders, cancer, and regenerative medicine. Finally, we discussed emerging integrative tools such as organoid co-cultures, single-cell omics, and senolytics drug screening that are expanding the potential of organoids as translational platforms for anti-aging and disease intervention.

Keywords: organoids; cellular senescence; aging; regenerative medicine; disease modeling; senolytics; molecular targets; 3D culture

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1. Introduction

Aging is a universal biological process characterized by the progressive decline of tissue integrity, stem cell function, and regenerative capacity across organ systems [1,2]. As organisms age, cells accumulate DNA damage, undergo metabolic and epigenetic alterations, and experience chronic stress that together contribute to impaired homeostasis and heightened risk for disease [3,4]. A central hallmark linking aging to disease susceptibility is cellular senescence, a stable cell-cycle arrest program accompanied by widespread chromatin remodeling, mitochondrial dysfunction, and secretion of pro-inflammatory factors collectively known as the senescence-associated secretory phenotype (SASP) [5,6].

Although senescence initially acts as a tumor-suppressive mechanism, its chronic accumulation during aging contributes to tissue dysfunction, inflammation, fibrosis, and cancer initiation [7,8].

Traditional in vitro models such as two-dimensional (2D) monolayer cultures have provided foundational insights into senescence biology but fail to capture the three-dimensional (3D) architecture, multi-lineage complexity, and native signaling gradients of aging tissues [9]. Animal models, while physiologically relevant, are limited by species-specific differences and difficulty in parsing human-specific aging phenomena [10]. These limitations have driven the emergence of organoid technology as a transformative platform to model human aging and senescence in vitro. Organoids are self-organizing, stem cell-derived 3D structures that recapitulate key features of native tissues, including epithelial polarity, cellular heterogeneity, spatial organization, and lineage hierarchy [11]. Organoids derived from adult stem cells, pluripotent stem cells, or patient tissues have enabled researchers to study aging signatures such as stem cell exhaustion, impaired niche signaling, metabolic decline, altered stress responses, and senescence accumulation in controlled experimental settings [12]. Importantly, organoids retain donor-specific epigenetic and transcriptional landscapes, enabling direct comparison of young and aged tissues, modeling organismal aging, and identification of cell-intrinsic drivers of senescence [13]. Furthermore, organoids can be genetically engineered—using tools such as CRISPR/Cas9 to induce senescence through DNA damage, oncogene activation, telomere dysfunction, or mitochondrial stress, allowing mechanistic interrogation of senescence pathways including p53/p21, p16INK4a/Rb, and SASP regulation [14] (Table 1).

Table 1. Key cellular senescence pathways and markers studied using organoid models, their functional outcomes, therapeutic implications, and relevant references.

Pathway/Marker	Organoid Model	Functional Outcome	Therapeutic Implication	References
p16INK4a/Rb	Intestinal, hepatic	Cell cycle arrest, SASP induction	Senolytic targeting, tissue rejuvenation	[15–17]
p53/p21CIP1	Intestinal, cerebral	DNA damage response, apoptosis regulation	Chemotherapy response prediction, OIS modeling	[18]
mTOR signaling	Hepatic, pancreatic	Metabolic regulation, stem cell function	Rapamycin/mTOR modulators, stem cell rejuvenation	[19]
SASP factors (IL-6, IL-8, MMPs)	Multiple tissues	Paracrine tissue remodeling, inflammation	Senomorphic interventions	[20]
DNA damage/γ-H2AX	Intestinal, cerebral	Genomic instability, aging phenotype	Senescence biomarkers, therapeutic monitoring	[21]

Because senescence plays roles in diverse pathologies—spanning cancer, neurodegeneration, metabolic disorders, pulmonary fibrosis, and chronic inflammation—organoid systems have become powerful tools for targeted discovery and drug screening aimed at modulating aging and senescence [4,21]. Recent advances integrating organoids with single-cell omics, spatial transcriptomics, immune co-cultures, and organoid-on-chip platforms further enhance their ability to model the complex microenvironmental cues that govern aging in vivo [22–24]. By leveraging organoids as physiologically relevant, experimentally tractable models, researchers are gaining novel insights into human aging biology and accelerating the development of interventions aimed at improving health span and combating age-associated diseases.

In this review, we discuss how organoid systems provide unprecedented opportunities to dissect the mechanisms of aging and cellular senescence across diverse tissues, highlighting key molecular and cellular pathways uncovered through organoid-based studies. These platforms enable direct interrogation of epithelial-intrinsic aging programs, stem cell dysfunction, and microenvironmental regulation within physiologically relevant 3D contexts. We further examine how integration of organoid models with emerging technologies, including single-cell and spatial omics, functional genomics, and organoid-on-chip systems, has advanced our understanding of senescence heterogeneity and dynamics. Finally, we discuss emerging therapeutic strategies, including senolytic and senomorphic interventions, that are enabled and refined through organoid-based platforms, underscoring their potential to accelerate the translation of aging biology into targeted and personalized therapies.

2. Overview of Organoid Systems

Organoid technology has revolutionized in vitro modeling by enabling the generation of self-organizing, 3D structures that recapitulate key features of native tissues, including multicellular composition, spatial architecture, and functional signaling networks [25]. Organoids are typically derived from two major sources: adult stem cells (ASCs) residing in tissue-specific niches, and pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) (Table 2). Each system offers unique advantages for modeling development, homeostasis, disease mechanisms, and aging (Figure 1).

Table 2. Organoid platforms for modeling aging-associated mechanisms and cellular senescence across tissues. This table summarizes major organoid systems, their stem cell sources, and the specific aging-related mechanisms they enable investigators to interrogate. Rather than broadly listing disease applications, the table highlights key hallmarks of aging—including stem cell exhaustion, genomic instability, mitochondrial dysfunction, altered niche signaling, and inflammaging—and outlines mechanistic questions that can be addressed using each organoid platform. These models facilitate age-stratified analysis and provide controlled systems to dissect epithelial-intrinsic aging processes, microenvironmental remodeling, and age-modified responses to oncogenic or metabolic stress.

Organoid Type	Stem Cell Source	Aging-Associated Mechanisms Modeled	Key Aging Questions Addressed	Representative Applications	References
Intestinal	Adult stem cells	Stem cell exhaustion; Wnt signaling decline; increased TGF-β signaling; SASP accumulation; genomic instability	How does aging alter niche responsiveness and regenerative capacity? How does epithelial age modify oncogene-induced senescence (OIS)?	Colorectal tumor initiation; inflammaging; regenerative decline	[26]
Hepatic	iPSC/Adult stem cells	DNA damage accumulation; metabolic inflexibility; mitochondrial dysfunction; impaired detoxification	Does mitochondrial decline drive age-associated metabolic disease? How does genomic instability influence fibrosis and cancer risk?	Liver fibrosis; metabolic syndrome; hepatocellular carcinoma	[27]
Cerebral	iPSC	Proteostasis decline; mitochondrial dysfunction; oxidative stress; neuroinflammatory signaling	How does aging alter neuronal resilience and protein aggregation? How does inflammaging contribute to neurodegeneration?	Alzheimer's disease; Parkinson's disease	[28]
Pancreatic	iPSC/Adult stem cells	β-cell senescence; oxidative stress; impaired insulin secretion; ER stress	How does aging reduce endocrine regenerative capacity? Can niche modulation reverse β-cell dysfunction?	Type 2 diabetes; pancreatic tumorigenesis	[29]

Table 2. *Cont.*

Organoid Type	Stem Cell Source	Aging-Associated Mechanisms Modeled	Key Aging Questions Addressed	Representative Applications	References
Renal	Adult stem cells/iPSC	Tubular senescence; oxidative stress; reduced repair capacity; fibrotic signaling	How does epithelial aging impair nephron regeneration? What is the role of senescence in fibrosis progression?	Chronic kidney disease; polycystic kidney disease	[30]
Multi-lineage/Co-culture systems	ASC/iPSC + immune/stromal cells	Inflammaging; immune–epithelial crosstalk; niche deterioration; SASP-mediated remodeling	How do age-associated immune changes influence epithelial stem cell fitness and tumor initiation?	Early tumorigenesis; fibrosis; chronic inflammatory disease	[31–33]

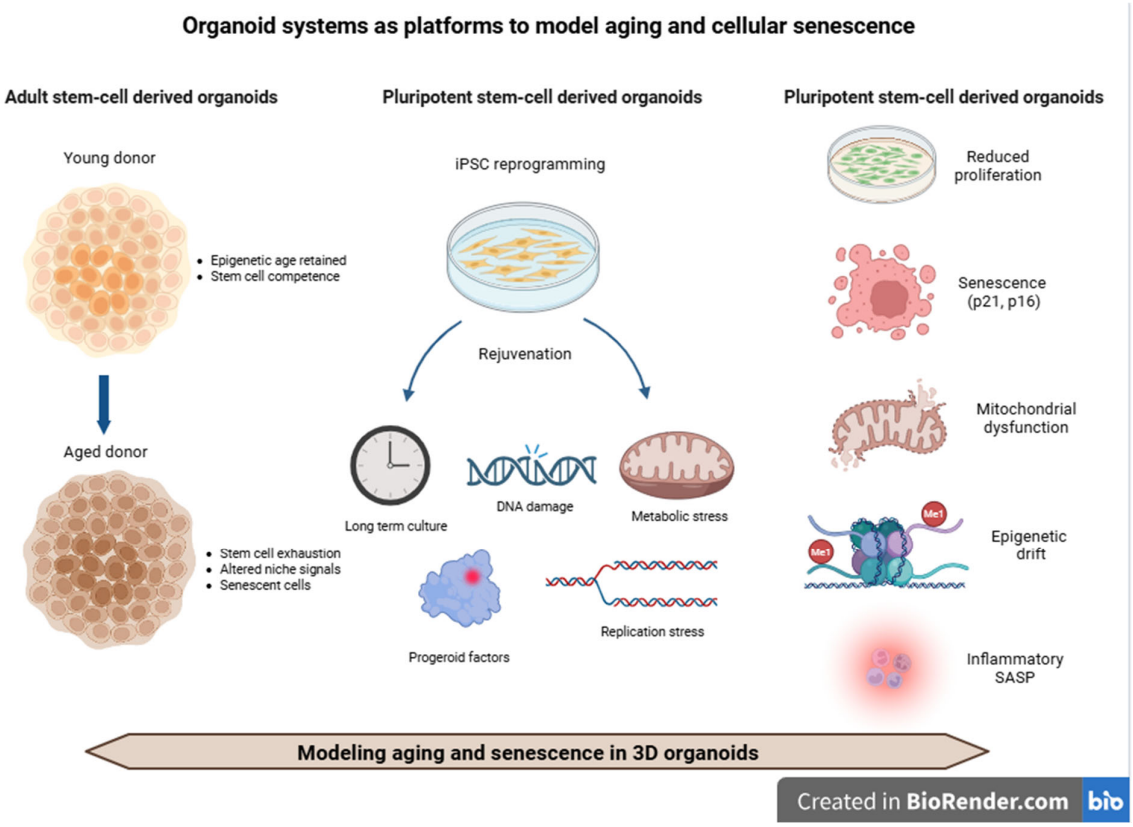


Figure 1. Organoid systems as platforms to model aging and cellular senescence. Organoids derived from adult stem cells (ASCs) and pluripotent stem cells (PSCs) provide complementary approaches to study aging-related processes. ASC-derived organoids retain donor-specific genetic, epigenetic, and aging-associated phenotypes, enabling direct interrogation of stem cell exhaustion, senescence accumulation, and altered niche signaling in aged tissues. In contrast, PSC-derived organoids recapitulate developmental programs and typically resemble fetal tissues but can be engineered to exhibit aging-like features through prolonged culture, DNA damage, metabolic stress, or progeroid manipulation. Together, these systems enable controlled modeling of key hallmarks of aging, including reduced regenerative capacity, mitochondrial dysfunction, genomic instability, epigenetic drift, and senescence-associated secretory phenotypes (SASP), within physiologically relevant 3D tissue contexts. https://app.biorender.com/illustrations/canvas-beta/69753460cde6a4242668b183 (accessed on 17 March 2026).

2.1. Adult Stem Cell-Derived Organoids

ASC-derived organoids are established from tissue-resident stem or progenitor cells that retain intrinsic instructive cues for lineage specification and tissue regeneration. These organoids closely mimic the physiology of the original tissue, including epithelial po-

larity, adult transcriptional identity, and organ-specific functional properties [34]. The reliance on defined niche signals such as Wnt, R-spondin, EGF, BMP inhibition, and Notch modulation enables the ex vivo reconstruction of stem cell behavior and allows precise manipulation of signaling pathways relevant to aging and disease. Importantly, ASC-derived organoids maintain donor-specific epigenetic signatures and aging phenotypes, making them particularly valuable for studying tissue-specific aging, stem cell exhaustion, and senescence accumulation.

2.2. Pluripotent Stem Cell-Derived Organoids

PSC-derived organoids recapitulate stages of embryonic development and are widely used to model organogenesis, developmental defects, and early-onset diseases [34]. These systems rely on stepwise differentiation protocols that mimic embryonic signaling pathways such as Wnt, FGF, TGF-β, and Hedgehog, leading to the generation of highly organized structures such as brain, retinal, intestinal, hepatic, and kidney organoids. Although PSC-derived organoids offer unparalleled developmental modeling capabilities, they often resemble fetal rather than adult tissues and lack aging-associated epigenetic marks, which can limit their application in aging research [35]. Recent strategies, including prolonged culture, DNA damage induction, and metabolic manipulation, have been used to induce aging-like features in PSC-derived organoids, expanding their utility for studying senescence [36].

2.3. Modeling Multicellular Complexity and the Tissue Niche

A critical advantage of organoid systems is their ability to capture aspects of the tissue niche and multicellular interactions. Many organoids retain intrinsic paracrine circuits such as Wnt-producing Paneth cells in intestinal organoids or hepatocyte–cholangiocyte interactions in liver organoids that are essential for maintaining tissue homeostasis and are known to deteriorate with age [37,38]. Advances in co-culture systems have enabled the inclusion of immune cells, fibroblasts, endothelial cells, and microbiota, providing a more physiologically relevant microenvironment to study aging-associated inflammation, stromal remodeling, and immune–epithelial crosstalk [38].

2.4. Comparison with Traditional 2D and Animal Models

Organoid systems overcome several inherent limitations of conventional 2D cell culture and animal models, making them powerful platforms for studying aging, senescence, and complex human diseases. Traditional 2D cultures lack the spatial organization, mechanical cues, and cellular heterogeneity necessary to faithfully model tissue architecture and age-associated functional decline. In contrast, organoids preserve 3D structure, stem cell hierarchies, and lineage relationships that more closely resemble native tissues, enabling investigation of niche-dependent regulation of stem cell behavior and tissue homeostasis [39,40]. Compared with animal models, organoids better capture human-specific aging signatures, including species-specific transcriptional programs, senescence-associated gene expression, and human-relevant drug responses. Their tractability, scalability, and compatibility with genome editing and high-throughput screening make organoids an indispensable platform for aging and senescence research.

Another important advantage of organoid systems is their compatibility with advanced experimental approaches. Organoids can be readily integrated with genome-editing technologies, high-throughput drug screening platforms, and functional genomics tools, enabling systematic interrogation of pathways regulating senescence, stem cell exhaustion, and tissue regeneration [41]. In addition, organoids are highly amenable to multi-omics and imaging-based analyses, including single-cell transcriptomics, epigenomics, spatial

profiling, and live-cell imaging, allowing detailed mechanistic dissection of aging processes at cellular and subcellular resolution [41,42].

Organoids also offer practical and ethical advantages compared with animal models. Their scalability and experimental tractability enable rapid hypothesis testing, precise genetic manipulation, and repeated sampling over time without the confounding effects of whole-organism variability or interspecies differences. While animal models remain essential for studying systemic physiology and organism-level aging, organoids provide a complementary and often more human-relevant system for dissecting cellular and molecular mechanisms underlying age-associated diseases.

Together, these features position organoid systems as an important bridge between simplified in vitro models and in vivo studies, enabling more accurate modeling of human tissue biology and improving the translational relevance of experimental findings.

2.5. Challenges and Limitations

Despite their transformative potential, organoid systems face several conceptual and technical challenges that are particularly relevant for modeling aging and cellular senescence. A major limitation lies in standardization and reproducibility, as organoid derivation efficiency, growth dynamics, and cellular composition can vary substantially depending on donor source, tissue type, passage number, and culture conditions [43].

Long-term culture, which is often required to model chronic aging processes, introduces additional concerns. Extended passaging can lead to culture-induced stress, clonal selection, epigenetic drift, and accumulation of somatic mutations, potentially mimicking or obscuring bona fide aging-associated phenotypes [44–46]. Distinguishing true chronological aging from in vitro–induced senescence, therefore, remains a major conceptual and technical challenge. To address this challenge, aging-associated stem cell exhaustion in organoid systems is defined using comparative, molecular, and functional criteria rather than reliance on single phenotypic markers [47]. In practice, importantly, these transcriptional and epigenetic features align with signatures observed in aged tissues in vivo and are retained across passages, supporting their intrinsic origin. In contrast, diffusion-limited hypoxia or nutrient stress in static cultures typically produces spatially restricted necrosis, acute metabolic stress responses, and reversible growth impairment. Assessment of hypoxia-responsive pathways, combined with the use of perfusion-based microfluidic systems, further aids in distinguishing culture-induced artifacts from stable, age-associated stem cell dysfunction. Moreover, cellular diversity and niche complexity are still incompletely captured in most organoid systems. Although recent co-culture approaches have enabled partial incorporation of immune cells, fibroblasts, and endothelial cells, maintaining stable, long-term multicellular interactions that resemble aged tissue environments remain technically demanding [48–50]. Faithfully modeling age-associated inflammatory niches and SASP requires careful control to avoid non-physiological activation states.

Although animal models remain the preferred systems for studying systemic immune aging, endocrine crosstalk, and pharmacokinetics, the organoid field has made substantial advances in addressing these complex biological processes. Organoid platforms provide a physiologically relevant and dynamically controlled microenvironment that more closely recapitulates human tissue architecture and function compared to traditional in vivo models. Recent developments integrating organoids with immune components, hormonal signaling cues, and microfluidics-based systems have expanded their utility in modeling systemic interactions [51]. Organoids have been successfully applied to study diseases such as cancer and inflammatory bowel disease, while enabling precise experimental manipulation and high-resolution functional readouts. Importantly, their patient-derived nature allows

for individualized disease modeling and therapeutic testing, thereby offering significant potential for personalized medicine approaches.

Emerging technologies provide promising avenues to overcome these limitations. Synthetic and tunable biomaterials allow precise control of matrix stiffness and biochemical signaling, both of which change with age and influence senescence programs. Microfluidic organoid-on-chip platforms enable dynamic perfusion, controlled gradients, and inter-organ communication, improving physiological relevance and long-term stability [52]. Together, these advances are expected to enhance the fidelity and translational relevance of organoid-based aging models. Microfluidic organoid-on-chip platforms enable dynamic perfusion, mechanical cues, and controlled nutrient gradients that improve physiological relevance compared to static cultures [52,53]. However, challenges remain, including device complexity, limited scalability for high-throughput screening, variability in fabrication, and incomplete integration of systemic immune and endocrine components. Addressing these bottlenecks will be essential for translating fluidic organoid systems into robust platforms for aging biology and pharmacokinetic modeling.

2.6. Organoid Systems as Models of Age-Associated Stem Cell Decline, Niche Deterioration, and Senescence Accumulation

A central hallmark of tissue aging is the progressive decline in adult stem cell function, often termed stem cell exhaustion, characterized by reduced self-renewal capacity, altered lineage commitment, and diminished regenerative potential [54,55]. Organoid systems derived from adult stem cells provide a unique platform to model this decline in a controlled ex vivo setting, as they retain donor-specific transcriptional and epigenetic signatures reflective of chronological aging [56]. Comparative studies using organoids derived from young and aged donors have demonstrated persistent reductions in clonogenic efficiency, impaired responsiveness to Wnt and Notch signaling, and increased expression of cell-cycle inhibitors such as CDKN2A/p16INK4a in aged stem cell populations. These intrinsic defects are maintained across passages under identical culture conditions, supporting the interpretation that they reflect bona fide age-associated stem cell dysfunction rather than transient culture stress.

Beyond cell-intrinsic decline, aging is accompanied by deterioration of the stem cell niche, including altered paracrine signaling, extracellular matrix remodeling, and chronic low-grade inflammation ("inflammaging") [57]. Organoid platforms allow experimental manipulation of niche components—such as Wnt ligands, R-spondin, BMP inhibitors, and stromal-derived factors—to causally test how aged microenvironmental cues contribute to stem cell exhaustion [57]. For example, restoration of Wnt signaling in aged intestinal organoids partially rescues proliferative defects, demonstrating that niche deterioration actively drives age-associated functional decline rather than representing an irreversible intrinsic program. Synthetic and tunable extracellular matrices further enable modeling of age-related changes in matrix stiffness and mechanotransduction, both of which influence YAP/TAZ activity, metabolic fitness, and senescence induction.

Organoid systems also permit direct interrogation of senescence accumulation within epithelial stem and progenitor compartments. Aging-associated senescent cells are characterized by stable cell-cycle arrest, activation of p16INK4a/p21 pathways, persistent DNA damage signaling, mitochondrial dysfunction, and acquisition of a SASP. In aged tissue-derived organoids, increased expression of SASP cytokines, accumulation of γH2AX foci, and reduced regenerative capacity have been observed even in the absence of systemic immune or endocrine inputs. This isolation of epithelial-intrinsic aging programs allows discrimination between organism-level influences and cell-autonomous senescence mechanisms.

Importantly, organoids enable distinction between chronological aging signatures and culture-induced stress artifacts. Diffusion-limited hypoxia and nutrient gradients in static 3D cultures can induce stress responses that superficially resemble aging phenotypes [58]. However, comparison of young and aged donor-derived organoids under identical environmental conditions, combined with transcriptomic alignment to in vivo aged tissues, allows identification of conserved aging-specific gene expression programs [59]. Integration of microfluidic perfusion systems further reduces diffusion-related confounders and strengthens modeling fidelity. Together, these approaches position organoids as mechanistically powerful systems to dissect the interplay between intrinsic stem cell decline, niche deterioration, and senescence accumulation that underlie tissue aging.

3. Modeling Cellular Aging in Organoids

Aging is accompanied by progressive and interconnected changes in cellular physiology, stem cell competence, and the integrity of tissue-regenerating niches, ultimately impairing organ function and increasing susceptibility to chronic diseases, degenerative disorders, and cancer. Across multiple tissues, including the intestine, liver, pancreas, and brain, adult stem cells exhibit age-associated declines in proliferative capacity, altered lineage commitment, impaired regenerative responses, and heightened sensitivity to metabolic, inflammatory, and oncogenic stress [60]. Historically, mechanistic insights into tissue aging have largely relied on bulk tissue profiling or in vivo animal models, which, while invaluable, obscure epithelial-intrinsic aging programs due to confounding influences from systemic factors such as immune aging, circulating cytokines, endocrine signaling, and changes in the stromal compartment [61]. These approaches also limit the ability to disentangle cause-and-effect relationships between intrinsic stem cell aging and extrinsic niche alterations. Organoid systems overcome many of these constraints by providing a controlled, genetically tractable, and physiologically relevant ex vivo platform to interrogate how aging reshapes the intrinsic properties of adult stem cells and their immediate microenvironment.

ASC-derived organoids, in particular, have emerged as powerful tools for modeling cellular aging because they retain donor-specific genetic, epigenetic, and transcriptional signatures, including age-associated chromatin states and transcriptional programs [62]. Intestinal organoids derived from aged donors exhibit reduced clonogenicity, delayed growth kinetics, altered differentiation trajectories, and impaired responsiveness to niche-derived signals such as Wnt and Notch, faithfully recapitulating in vivo aging phenotypes [63,64]. Similarly, hepatic and pancreatic organoids have revealed age-related declines in regenerative capacity and increased stress responses that are otherwise masked in whole-organ analyses [65].

Organoid systems further enable high-resolution dissection of hallmarks of cellular aging, including genomic instability, mitochondrial dysfunction, altered nutrient sensing, epigenetic drift, and activation of senescence-associated transcriptional programs. Importantly, age-associated cellular senescence can be studied in a cell-type–specific manner within organoids, allowing direct assessment of senescence markers, cell-cycle arrest, and SASP without confounding contributions from systemic inflammation [66].

In addition to modeling intrinsic aging, organoids offer unique opportunities to investigate how aging alters stem cell–niche interactions. Changes in paracrine signaling, extracellular matrix composition, and mechanical properties of the niche are increasingly recognized as drivers of stem cell dysfunction during aging. By precisely manipulating niche factors and matrix properties ex vivo, organoids enable causal testing of how aged microenvironments contribute to stem cell exhaustion, lineage bias, and senescence accumulation. Collectively, organoid-based aging models provide an unprecedented platform to

dissect epithelial-intrinsic aging mechanisms, separate them from systemic influences, and establish direct links between cellular aging, senescence, and disease susceptibility. These systems, therefore, serve as a critical foundation for identifying age-specific vulnerabilities and therapeutic targets across diverse age-associated diseases.

A major strength of organoid systems in aging research is their ability to preserve cell-autonomous hallmarks of aging. Organoids derived from aged tissues display increased DNA damage accumulation, reduced clonogenic potential, impaired Wnt responsiveness, elevated reactive oxygen species, and molecular signatures of senescence, including upregulated Cdkn2a/p16INK4a and SASP-associated cytokines [67,68]. These changes persist across passages, indicating that stem cell–intrinsic aging programs are retained ex vivo and can be dissected in isolation from immune or endocrine cues. Conversely, organoids derived from young donors provide a rejuvenated baseline against which age-dependent phenotypes can be directly compared, enabling systematic evaluation of pathways such as Wnt, Notch, EGFR, BMP, and YAP that are altered during aging. Organoid-based aging models also allow the controlled introduction of genetic, epigenetic, environmental, and pharmacological perturbations to identify pathways that drive epithelial decline. Studies using intestinal and hepatic organoids have shown that age-related loss of niche-derived Wnt and R-spondin signaling restricts stem cell renewal, while aged stem cells exhibit amplified TGF-β signaling and chromatin remodeling that limit regenerative fitness [69,70]. Additionally, long-term culture systems using induced iPSC-derived organoids enable modeling of premature or accelerated aging syndromes by manipulating telomere maintenance, lamin processing, mitochondrial function, or DNA repair mechanisms [71,72].

Importantly, organoid platforms also facilitate the study of interactions between aging and disease susceptibility, including infection, inflammation, metabolic dysfunction, and cancer initiation. For example, aged intestinal organoids exhibit increased sensitivity to inflammatory cytokines and heightened transcriptional activation of NF-κB and JAK–STAT signaling upon stimulation, which may contribute to age-associated inflammatory bowel disease risk [73]. Similarly, aged colon or pancreatic organoids show differential responses to oncogenic mutations (e.g., *BRAFV600E*, *KRASG12D*), reflecting age-dependent differences in MAPK activation, senescence induction, and differentiation trajectories [74,75]. These discoveries underscore how organoid models can reveal context-specific vulnerabilities and therapeutic targets that arise with epithelial aging. Together, these advances position organoids as a transformative platform for aging research, enabling precise mechanistic dissection of epithelial decline, niche dysregulation, senescence programs, and age-related disease initiation. By integrating organoid-based assays with single-cell transcriptomics, CRISPR editing, and high-Throughput compound screening, researchers can now identify reshapes tissue homeostasis and identify strategies to restore regenerative capacity or prevent age-associated disorders.

4. Senescence Pathways Revealed Through Organoid Models

Cellular senescence is an irreversible growth-arrest program triggered by diverse stressors including DNA damage, telomere attrition, oxidative stress, chromatin disruption, and oncogene activation. While senescence acts as an essential tumor-suppressive barrier, its long-term persistence contributes to chronic inflammation, impaired tissue regeneration, and age-related pathologies through the secretion of pro-inflammatory cytokines, chemokines, and matrix-remodeling enzymes collectively termed the SASP. Despite its importance, studying senescence in vivo has historically been challenging due to tissue heterogeneity, systemic influences, and limited access to early-stage senescent cells. Organoid systems now provide a unique opportunity to interrogate the cell-intrinsic and microenvironment-modulated mechanisms of senescence with unprecedented resolution.

Importantly, the term "senescence marker" refers not to a single definitive molecule but to a constellation of phenotypic and molecular features that collectively define a senescent state. Canonical hallmarks include stable cell-cycle arrest, activation of the p53/p21CIP1 and/or p16INK4a/Rb pathways, persistent DNA damage signaling (e.g., γH2AX foci), senescence-associated β-galactosidase (SA-β-gal) activity, chromatin remodeling, and acquisition of a SASP [76,77]. However, no single marker is sufficient to establish senescence, as individual features such as p16INK4a expression or transient DNA damage responses may also occur in non-senescent stress contexts [78]. In organoid systems, rigorous identification of senescence therefore relies on combinatorial and functional criteria—including durable growth arrest and sustained secretory remodeling—rather than isolated marker expression. Moreover, the relative prominence of specific senescence features may vary across tissue types and organoid platforms, necessitating context-dependent interpretation rather than universal extrapolation.

Organoids faithfully recapitulate epithelial lineage hierarchies and retain the capacity to undergo senescence in response to intrinsic aging, environmental insults, or targeted genetic perturbations. Aged intestinal and hepatic organoids exhibit canonical senescence features including elevated p16INK4a and p21CIP1 expression, increased DNA damage markers (γH2AX), mitochondrial dysfunction, and diminished regenerative capacity [79]. Importantly, these phenotypes persist ex vivo, demonstrating that stem cell-intrinsic aging programs are maintained independent of systemic inflammatory or hormonal inputs. This ability to isolate epithelial-intrinsic drivers makes organoids valuable for dissecting transcriptional, epigenetic, and metabolic mechanisms underlying age-associated senescence (Figure 2).

Organoid models have also been instrumental in uncovering how oncogene-induced senescence (OIS) manifests within epithelial tissues. Organoid-based co-culture systems incorporating fibroblasts, immune cells, or engineered extracellular matrices are illuminating the microenvironmental regulation of senescence. Senescent epithelial organoids secrete SASP factors such as IL-6, IL-8, MMPs, and GDF15, which can remodel the niche, alter stromal behavior, and reinforce senescence in neighboring cells [80,81]. Conversely, niche-derived signals such as Wnt, R-spondin, BMP, and TGF-β profoundly influence the induction, maintenance, or reversal of senescence. For instance, loss of Wnt/R-spondin responsiveness in aged intestinal organoids accelerates senescence, whereas restoring niche cues improves proliferation and reduces stress signaling. These systems highlight how epithelial senescence is a dynamic interplay between intrinsic damage responses and extrinsic niche determinants.

Organoid models also support therapeutic discovery efforts, particularly for senolytic (senescence-clearing) and senomorphic (SASP-modulating) interventions. High-throughput drug screening using organoids derived from aged tissues or OIS-induced systems has identified candidate compounds targeting PI3K/AKT, BCL-2 family proteins, and metabolic vulnerabilities unique to senescent cells [82]. Additionally, CRISPR-based genetic screens in organoids are uncovering regulators of senescence escape, including chromatin modifiers, MAPK modulators, and DNA repair factors, which represent potential targets for preventing early neoplastic transformation [83,84].

Collectively, organoids have emerged as a powerful platform for investigating the full spectrum of senescence biology—from intrinsic aging-related decline and oncogene-induced senescence to microenvironment-driven remodeling and therapeutic targeting. By enabling mechanistic dissection at cellular and tissue levels, organoid models are accelerating the discovery of senescence-associated vulnerabilities relevant to aging, cancer initiation, and regenerative failure.

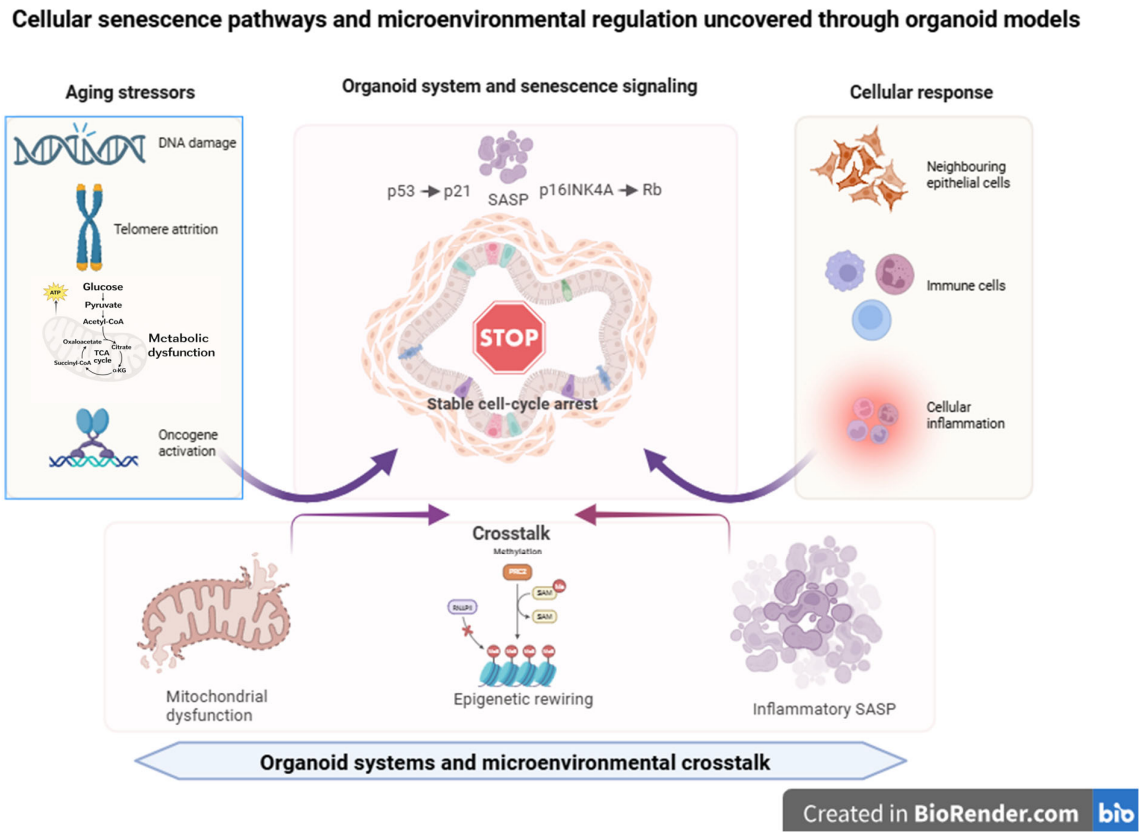


Figure 2. Cellular senescence pathways and microenvironmental regulation uncovered through organoid models. Organoid systems enable detailed dissection of senescence-inducing stimuli, core signaling pathways, and downstream functional consequences within epithelial tissues. Aging-associated stressors, including DNA damage, telomere attrition, metabolic dysfunction, and oncogene activation, converge on canonical p53/p21 and p16INK4a/Rb tumor suppressor pathways to induce stable cell-cycle arrest. Senescent cells exhibit mitochondrial dysfunction, chromatin remodeling, and secretion of senescence-associated secretory phenotype (SASP) factors that remodel the local microenvironment. Organoid co-culture systems further reveal dynamic crosstalk between senescent epithelial cells, stromal fibroblasts, and immune components, highlighting context-dependent roles of senescence in tissue dysfunction, inflammation, and early tumorigenesis. https://app.biorender.com/illustrations/canvas-beta/697543791e425e67f693e5c2 (accessed on 17 March 2026).

5. Organoids as Platforms for Target Discovery

Organoid systems have emerged as powerful and versatile platforms for therapeutic target discovery, effectively bridging the gap between reductionist mechanistic studies and clinically relevant translational research. Their defining features—3D architecture, preservation of tissue-specific stem cell hierarchies, and retention of donor-specific genetic and epigenetic information—enable the interrogation of disease-relevant pathways within a physiologically meaningful context that is difficult to achieve in conventional 2D cultures. These attributes are particularly advantageous for studying aging-related processes, where subtle, cell-intrinsic alterations interact with niche-dependent signals to shape disease susceptibility and therapeutic response. A key strength of organoid-based systems lies in their ability to capture inter-individual heterogeneity, which is especially pronounced in aging tissues. Organoids derived from young versus aged donors, or from patients with age-associated diseases, retain differences in transcriptional programs, epigenetic landscapes, and stress responses, allowing systematic identification of age-specific vulnerabilities and candidate targets [85]. This donor-specific fidelity makes organoids well suited for

uncovering pathways that drive stem cell exhaustion, senescence accumulation, and altered differentiation trajectories during aging.

Organoids also provide a tractable platform to study OIS and early tumorigenic events, processes that are increasingly recognized as critical windows for therapeutic intervention. By introducing defined genetic alterations using genome-editing approaches, such as CRISPR/Cas9, organoids enable direct comparison of oncogenic signaling outcomes in young versus aged epithelial contexts. These studies have revealed that aging can fundamentally alter cellular responses to oncogenic insults, shifting the balance between growth arrest, senescence, and sustained proliferation, thereby uncovering age-dependent therapeutic targets and resistance mechanisms. Importantly, organoids are highly compatible with functional genomics and high-throughput-screening approaches. Pooled or arrayed genetic perturbation screens can be combined with phenotypic readouts such as growth dynamics, lineage composition, senescence markers, and secretory profiles to identify regulators of aging-associated decline and disease progression [86]. Similarly, drug screening in organoid cultures has enabled the discovery of compounds that selectively target senescent cells, modulate senescence-associated secretory phenotypes, or restore aged stem cell function, thereby accelerating the translation of aging biology into therapeutic strategies [87].

Beyond epithelial-intrinsic targets, emerging organoid co-culture systems incorporating immune cells, fibroblasts, and endothelial components allow interrogation of microenvironment- and niche-dependent vulnerabilities. These platforms are particularly relevant for identifying targets linked to inflammaging, stromal remodeling, and immune evasion—hallmarks of age-associated diseases that are poorly modeled in traditional systems. By enabling controlled manipulation of both epithelial and niche compartments, organoids facilitate the discovery of combinatorial and context-dependent therapeutic targets. Collectively, organoid-based platforms provide a scalable and human-relevant framework for identifying therapeutic targets across aging-associated diseases. Their ability to integrate genetic perturbation, functional screening, and patient-specific biology positions organoids as a cornerstone technology for precision target discovery and drug development in the context of aging and cellular senescence.

5.1. Genetic and Molecular Target Identification

Organoids allow precise manipulation of genes, signaling pathways, and microenvironmental factors to identify targets that regulate aging, senescence, and disease progression. CRISPR/Cas9 and RNA interference (RNAi) screens in organoids have been applied to systematically interrogate the roles of tumor suppressors, oncogenes, DNA repair genes, and chromatin regulators in organ-specific contexts. For example, genome-wide CRISPR screens in intestinal organoids have identified modulators of Wnt, Notch, and MAPK signaling that govern stem cell renewal and resistance to senescence, revealing potential targets for rejuvenation strategies and early cancer prevention. Moreover, organoid models derived from aged tissues or engineered to harbor oncogenic mutations (e.g., *BRAFV600E*, *KRASG12D*) enable direct identification of context-dependent vulnerabilities. Studies in aged colon organoids demonstrated that Wnt pathway augmentation can restore proliferative capacity in senescent stem cells, whereas MAPK inhibition selectively suppresses the growth of oncogene-primed cells. These insights are invaluable for designing targeted therapies that selectively modulate senescent or pre-malignant cells while sparing healthy tissue.

5.2. High-Throughput Drug Screening and Senotherapeutics

Organoid cultures are highly compatible with high-throughput drug screening, allowing systematic evaluation of small molecules, biologics, and senolytic or senomorphic compounds in physiologically relevant human tissue models [88]. For instance, screening libraries of BCL-2 inhibitors, PI3K/AKT modulators, or metabolic regulators in aged or senescence-induced organoids have identified candidate compounds that selectively eliminate senescent cells or suppress SASP-associated inflammatory signaling [89]. Importantly, these findings are directly translating to preclinical or clinical studies, as organoids preserve human-specific responses that may not be captured in murine models. In addition to senolytic compounds, organoids have been used to discover regenerative targets that rejuvenate aged tissue stem cells. Manipulating niche factors (e.g., Wnt agonists, BMP inhibitors) in aged intestinal organoids restores stem cell proliferation and mitigates senescence phenotypes, demonstrating the utility of organoids in identifying actionable pathways for tissue rejuvenation.

5.3. Multi-Omics Integration for Target Discovery

The combination of organoid technology with multi-omics profiling—including single-cell transcriptomics, spatial transcriptomics, proteomics, and epigenomics—enables high-resolution mapping of senescence and aging signatures [90]. Such analyses can reveal gene regulatory networks, post-translational modifications, and metabolic dependencies that are unique to senescent or oncogene-primed cells. For example, single-cell RNA sequencing of aged intestinal organoids has uncovered subpopulations of stem cells with elevated inflammatory signaling, chromatin remodeling factors, and SASP-associated genes, which can serve as candidate therapeutic targets [91]. Spatial transcriptomics further allows the identification of cell–cell interaction networks and niche-derived signals that modulate senescence or stem cell function, enhancing the precision of target selection.

5.4. Personalized and Precision Medicine Applications

Patient-derived organoids (PDOs) extend the potential of organoid-based target discovery into personalized medicine. By deriving organoids from healthy, aged, or diseased tissues of individual patients, researchers can identify patient-specific vulnerabilities and screen for drugs tailored to the individual's molecular and epigenetic context [92]. For aging-related interventions, PDOs allow evaluation of senolytic or rejuvenating compounds across genetically diverse human backgrounds, thereby improving the translational relevance of preclinical discoveries.

5.5. Aging-Dependent Vulnerabilities, Senolytic Strategies, and Age-Modulated Oncogenic Responses

A major strength of organoid systems in aging research is their ability to reveal aging-dependent cellular vulnerabilities that are not evident in young tissues or conventional culture systems. Aging is accompanied by alterations in nutrient sensing, mitochondrial metabolism, chromatin organization, and stress response pathways, generating distinct molecular dependencies in aged epithelial stem cells. Organoids derived from aged donors retain these transcriptional and epigenetic features, enabling identification of pathways selectively required for survival or regeneration in aged tissues. For example, aged intestinal organoids display impaired Wnt responsiveness and increased inflammatory signaling, highlighting context-specific therapeutic targets [93].

Organoid models allow tissue-specific testing of senolytic compounds targeting anti-apoptotic regulators such as BCL-2 family proteins or PI3K/AKT signaling, while distinguishing effects on senescent versus functional stem cell compartments. In parallel, modulation of niche factors—including Wnt and BMP signaling—has demonstrated partial

restoration of regenerative capacity in aged organoids. Organoids also uniquely enable interrogation of age-modulated oncogenic responses. The same oncogenic mutation can elicit distinct outcomes depending on epithelial age, with aged tissues exhibiting altered thresholds for senescence induction, DNA damage responses, and proliferative escape [94,95]. These findings position aging as an active modifier of oncogenic fitness landscapes and therapeutic vulnerability. By directly comparing young and aged epithelial backgrounds under controlled genetic perturbations, organoids provide a powerful platform to define age-specific targets and optimize intervention strategies.

5.6. Limitations and Future Directions

Despite their growing utility in therapeutic target discovery, organoid-based systems face several conceptual and technical limitations that must be carefully considered when modeling aging, senescence, and disease. One major challenge is variability and reproducibility, as differences in donor material, tissue source, culture medium composition, extracellular matrix properties, and passage number can substantially influence organoid growth behavior, differentiation state, and drug responses [96]. Such variability complicates cross-study comparisons and poses challenges for standardization in large-scale screening and translational pipelines.

A related limitation concerns the dependence on animal-derived matrices, such as Matrigel, which exhibit batch-to-batch variability and contain ill-defined biochemical and mechanical cues. These matrices can obscure subtle aging-associated phenotypes and confound interpretation of therapeutic effects, particularly when studying mechanosensitive pathways or senescence-related changes in extracellular matrix signaling [97]. The development and adoption of synthetic, tunable biomaterials represent an important future direction for improving reproducibility and physiological relevance.

Another fundamental constraint is the lack of systemic and circulatory inputs. Conventional organoid cultures are largely isolated from endocrine signals, immune surveillance, neuronal inputs, and vascular perfusion, all of which profoundly shape aging trajectories and therapeutic responses in vivo [98]. In particular, the absence of functional vasculature limits long-term culture and drug delivery kinetics, while the lack of immune components restricts the study of immunosenescence, inflammaging, and immune-mediated clearance of senescent cells.

Recent technological advances are beginning to address these challenges. Microfluidic organoid-on-chip platforms enable dynamic perfusion, controlled nutrients and oxygen gradients, and real-time monitoring of tissue responses, thereby improving physiological relevance and long-term stability [99]. Similarly, the generation of vascularized organoids through endothelial co-culture or self-assembly approaches has enhanced tissue maturation, metabolic function, and drug response fidelity [100]. These innovations are particularly promising for modeling chronic aging processes and pharmacokinetics in a human-relevant context. In parallel, advanced co-culture and assembled systems incorporating immune cells, fibroblasts, and other stromal components are expanding the scope of organoid-based target discovery. Such systems enable interrogation of aging-associated inflammatory niches, senescence-associated secretory phenotypes, and stromal–epithelial crosstalk that drive disease progression and therapeutic resistance [101]. Integrating these multicellular platforms with single-cell and spatial multi-omics approaches will further enhance mechanistic resolution and target validation.

Looking ahead, the convergence of organoid technology with functional genomics, artificial intelligence–driven analysis, and patient-derived biobanks is expected to transform target discovery in aging and age-related diseases. Large-scale, standardized organoid repositories representing diverse ages and disease states will enable systematic

identification of age-specific vulnerabilities and facilitate precision therapeutic development [102,103]. In summary, while organoid-based platforms for target discovery face important limitations, ongoing advances in bioengineering, co-culture strategies, and systems integration are rapidly expanding their capabilities. By enabling genetic perturbation, high-throughput drug screening, multi-omics profiling, and patient-specific modeling within a physiologically relevant framework, organoids are poised to play a central role in identifying interventions aimed at restoring tissue homeostasis, mitigating cellular senescence, and preventing age-associated disease and early tumorigenesis.

6. Applications for Disease Modeling

Organoid technology has transformed our ability to model human disease by recapitulating tissue architecture, cellular heterogeneity, and functional responses in vitro. Unlike traditional 2D cultures or animal models, organoids retain patient-specific genetic, epigenetic, and phenotypic characteristics, providing unprecedented opportunities to study aging, cancer, metabolic disorders, infectious diseases, and neurodegeneration [104]. This section highlights key applications of organoids in modeling human disease.

6.1. Modeling Age-Associated Diseases

Aging is a major risk factor for multiple pathologies, including epithelial decline, fibrosis, and cancer. Organoids derived from aged tissues preserve stem cell-intrinsic aging features such as reduced proliferative potential, altered lineage allocation, DNA damage accumulation, and SASP expression. Intestinal organoids from aged donors, for example, have revealed diminished Wnt responsiveness and increased TGF-β signaling, highlighting potential targets for rejuvenation or regenerative therapies [105]. Similarly, hepatic and pancreatic organoids capture age-related declines in metabolic function, stress responses, and regenerative capacity, providing models to study organ-specific aging mechanisms (Table 3).

Table 3. Organoid-based models for investigating aging-associated diseases table summarizes key organoid platforms used to study age-related diseases and highlights the aging-associated mechanisms that can be investigated using these systems.

Disease Area	Organoid Model	Aging-Associated Mechanism Studied	Key Insights from Organoid Studies
Colorectal cancer	Intestinal organoids	Stem cell exhaustion, altered Wnt signaling, oncogene-induced senescence	Reveals age-dependent susceptibility to tumor initiation and altered responses to oncogenic mutations
Liver diseases	Hepatic organoids	Mitochondrial dysfunction, metabolic decline, genomic instability	Enables study of metabolic aging and fibrosis progression
Neurodegenerative diseases	Cerebral organoids	Proteostasis decline, oxidative stress, neuroinflammation	Model age-related protein aggregation and neuronal vulnerability
Diabetes and metabolic disorders	Pancreatic organoids	β-cell senescence, oxidative stress, metabolic dysregulation	Allows investigation of endocrine aging and impaired insulin secretion
Chronic kidney disease	Renal organoids	Tubular senescence, inflammatory signaling	Provides insight into impaired regenerative capacity in aging kidneys
Infectious diseases	Intestinal/airway organoids	Age-related immune decline and epithelial barrier dysfunction	Enables study of increased infection susceptibility in aged tissues

6.2. Cancer Modeling

Patient-derived and genetically engineered organoids are widely used to study tumor initiation, progression, and therapy resistance. In colorectal, pancreatic, gastric, and liver cancers, organoids accurately recapitulate histological features, driver mutations, and signaling pathway dependencies. Importantly, organoids derived from aged or oncogene-primed tissues demonstrate age-dependent differences in responses to mutations, revealing how aging reshapes tumor suppressive mechanisms, senescence induction, and microenvironmental interactions. These models enable dissection of OIS, identification of escape pathways, and preclinical testing of targeted therapies. Importantly, aging alters not only mutation frequency but also the cellular response to oncogenic activation. In aged epithelial organoids, oncogene induction (e.g., *BRAFV600E* or *KRASG12D*) produces distinct senescence thresholds, stress signaling dynamics, and proliferative outcomes compared with young counterparts, reflecting age-dependent remodeling of tumor suppressive barriers. These findings indicate that aging reshapes oncogenic fitness landscapes, influencing whether cells undergo durable senescence, apoptosis, or malignant progression. Organoid systems uniquely enable age-stratified genetic perturbation experiments within controlled human epithelial contexts, allowing direct interrogation of how chronological aging modifies tumor initiation trajectories.

6.3. Infectious Disease Modeling

Organoids have become powerful tools for studying host–pathogen interactions. Human intestinal, airway, hepatic, and brain organoids have been used to model viral, bacterial, and parasitic infections, capturing tissue-specific infection patterns, immune responses, and pathogen-induced cellular remodeling [106]. For example, intestinal organoids have been used to study enteric viruses like rotavirus and SARS-CoV-2, providing insights into epithelial tropism, replication dynamics, and antiviral responses while preserving donor-specific variability [107]. Aging organoids further allow examination of age-dependent susceptibility to infection, reflecting increased vulnerability observed in older populations.

6.4. Metabolic and Genetic Disorders

Organoids derived from patients with monogenic or metabolic diseases faithfully recapitulate disease-specific phenotypes and facilitate functional assays for drug screening. Hepatic organoids from patients with alpha-1 antitrypsin deficiency, familial hypercholesterolemia, or Wilson's disease exhibit defective protein processing, lipid accumulation, or copper metabolism, providing platforms to test gene-corrective therapies or pharmacological interventions. Similarly, pancreatic and kidney organoids have been used to model cystic fibrosis, diabetes, and polycystic kidney disease, allowing evaluation of therapeutic responses in a patient-specific context.

6.5. Neurodegenerative Diseases

Cerebral organoids derived from human iPSCs allow modeling of neurodevelopmental and neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and microcephaly. These organoids capture critical features such as neuronal subtype specification, cortical layering, synapse formation, and disease-relevant protein aggregation. Integration with aging or stress-inducing protocols enables modeling of late-onset neurodegenerative processes, which are difficult to replicate in conventional cell cultures or animal models.

6.6. Immune and Microbiome Interactions

Co-culture of organoids with immune cells, fibroblasts, or microbiota components enables the study of tissue–immune interactions and host–microbe dynamics. Immune-competent intestinal and lung organoids have been used to examine inflammatory responses, immune-mediated tissue damage, and the impact of aging on immune surveillance [108,109]. Similarly, microbial colonization of intestinal organoids allows investigation of microbiome-driven metabolic and inflammatory alterations, revealing mechanisms by which aging and dysbiosis contribute to disease susceptibility.

6.7. Mechanistic Modeling of Age-Associated Disease Pathways

Beyond modeling individual diseases, organoid systems provide a platform to dissect shared mechanisms that drive age-associated pathologies across tissues. Aging is characterized by genomic instability, mitochondrial dysfunction, altered nutrient sensing, epigenetic drift, and chronic low-grade inflammation, which collectively increase disease susceptibility. Organoids derived from aged tissues retain many of these intrinsic hallmarks, enabling mechanistic analysis of how accumulated cellular damage translates into organ-specific dysfunction. For example, aged intestinal and hepatic organoids exhibit increased DNA damage signaling and reduced repair capacity, reflecting age-related genomic instability. Similarly, pancreatic and liver organoids demonstrate mitochondrial and metabolic alterations that correlate with diminished regenerative potential and stress resilience. Because organoids isolate epithelial-intrinsic aging from systemic influences, they allow direct testing of whether such metabolic and genomic defects are primary drivers of pathology. Aging is accompanied by progressive accumulation of somatic mutations, telomere attrition, and impaired DNA repair capacity, leading to persistent activation of DNA damage response pathways and increased genomic instability [110]. In aged organoid systems, elevated γH2AX foci, altered ATM/ATR signaling, and reduced repair efficiency have been observed under identical culture conditions, indicating intrinsic age-associated defects rather than acute experimental damage [111]. These comparative young-versus-aged organoid studies uniquely allow separation of chronic genomic decline from transient stress-induced DNA damage, thereby positioning organoids as reductionist platforms to interrogate how accumulated genomic instability drives stem cell exhaustion and tumor initiation.

Mitochondrial dysfunction is another central hallmark of aging, characterized by impaired oxidative phosphorylation, increased reactive oxygen species (ROS) production, and altered metabolic flexibility. Aged pancreatic, hepatic, and intestinal organoids exhibit metabolic rewiring and reduced regenerative fitness consistent with in vivo aging phenotypes. Because organoids isolate epithelial compartments from systemic inflammatory and endocrine confounders, they allow direct testing of whether mitochondrial dysfunction acts as a primary driver of stem cell decline or as a secondary consequence of organismal aging. Such mechanistic dissection is difficult to achieve in whole-animal models where systemic variables obscure cell-intrinsic effects.

Organoid systems also model inflammaging, a persistent inflammatory state that contributes to fibrosis, tumorigenesis, and impaired regeneration. Cytokine exposure or immune co-culture approaches reveal how sustained NF-κB and JAK–STAT activation promotes stem cell dysfunction and senescence accumulation [112]. Importantly, comparative studies of young versus aged organoids show that identical genetic or environmental insults produce divergent outcomes depending on epithelial age, including altered senescence thresholds and proliferative responses. These findings underscore that aging actively reshapes disease trajectories rather than simply increasing disease incidence. By integrating intrinsic stem cell decline, niche deterioration, and inflammatory remodeling, organoid systems position aging as a mechanistic driver of pathology rather than a passive background

factor. An additional systemic mechanism increasingly recognized as a hallmark of aging is clonal hematopoiesis of indeterminate potential (CHIP), characterized by age-associated expansion of hematopoietic stem cell clones harboring mutations in genes such as DNMT3A, TET2, or ASXL1 [113–115]. These mutant clones contribute to chronic low-grade inflammation, altered immune surveillance, and increased risk of cardiovascular disease, metabolic dysfunction, and malignancy. Although organoid systems do not recapitulate hematopoietic clonal expansion directly, they provide a controlled platform to model the downstream consequences of CHIP-associated inflammatory environments [116]. Co-culture approaches incorporating mutant macrophages or exposure to cytokine profiles characteristic of CHIP (e.g., elevated IL-1β, IL-6) enable interrogation of how systemic inflammatory signaling influences epithelial stem cell fitness, senescence accumulation, and tumor initiation. In this context, organoids can serve as reductionist systems to dissect how hematopoietic aging indirectly accelerates dysfunction across non-hematopoietic organs [117].

6.8. Advantages of Organoid Systems for Modeling Aging and Disease

Organoid systems offer several distinct advantages over conventional 2D cell culture and animal models, positioning them as uniquely powerful platforms for studying aging, senescence, and complex human diseases. A primary advantage is the retention of human-specific tissue characteristics, including species-specific transcriptional programs, signaling dependencies, and epigenetic landscapes that are often poorly conserved in animal models. This human fidelity is particularly important for aging research, as many aging-associated pathways, immune responses, and drug sensitivities differ substantially between humans and commonly used model organisms. Unlike traditional 2D cultures, which impose artificial polarity and uniform exposure to nutrients and growth factors, organoids preserve 3D architecture, stem cell hierarchies, and cellular heterogeneity that more closely resemble native tissues. This structural and cellular complexity allows organoids to model lineage dynamics, cell–cell interactions, and niche-dependent regulation of stem cell behavior—features that are essential for understanding age-associated declines in tissue regeneration and the emergence of disease states. Advances in genome editing and scalable culture formats have made it possible to perform targeted and genome-wide perturbation studies directly in human organoids, uncovering functional dependencies that are often missed in 2D systems. Similarly, drug screening in organoids provides more predictive assessments of therapeutic efficacy and toxicity by capturing tissue-specific responses and inter-individual variability.

Another key advantage is the compatibility of organoids with multi-omics and advanced imaging-based approaches, enabling deep mechanistic dissection of complex phenotypes [118]. Single-cell transcriptomics, epigenomics, proteomics, and spatial profiling can be integrated with live-cell imaging and lineage tracing to resolve how aging and disease processes unfold at cellular and subcellular resolution within intact tissue-like structures. While animal models remain indispensable for studying whole-organism physiology, organoids provide a complementary and often superior platform for dissecting human-specific mechanisms underlying multifactorial diseases, including cancer, degenerative disorders, and age-associated pathologies. Together, these advantages make organoids particularly well suited for modeling complex diseases in which human-specific physiology, cellular interactions, and dynamic stem cell behavior play central roles. By bridging the gap between simplified in vitro systems and in vivo models, organoids enable more accurate mechanistic insights and improve the translational relevance of experimental findings.

7. Emerging Technologies Specifically Enabling Mechanistic Dissection of Aging and Senescence in Organoids

Emerging technologies have transformed organoid systems from static tissue mimetics into dynamic platforms for dissecting the mechanisms of aging and cellular senescence. Rather than serving solely as structural replicas of tissues, modern organoid models now enable longitudinal, mechanistic, and age-stratified interrogation of stem cell exhaustion, senescence heterogeneity, inflammaging, and age-modified oncogenic responses. The integration of high-resolution multi-omics, genome engineering, bioengineering approaches, and computational modeling has shifted organoid-based aging research from descriptive phenotyping toward causal and predictive analysis of age-associated decline [119,120].

One major technological advance is the application of single-cell and spatial multi-omics to organoid systems. Single-cell transcriptomics, epigenomics, and chromatin accessibility profiling allow precise mapping of age-associated cellular states, lineage trajectories, and senescence programs within heterogeneous organoid populations [121]. Spatially resolved transcriptomic and proteomic approaches further preserve architectural context, enabling the study of how aging alters cell–cell interactions, niche signaling, and spatial organization within tissues [122]. Together, these approaches dissect how aging manifests at the level of individual cells and tissue microdomains. Advances in live imaging and lineage tracing have also transformed the study of aging in organoids. Long-term time-lapse imaging, combined with fluorescent reporters and inducible genetic labeling, allows direct observation of stem cell dynamics, clonal competition, and senescence onset over extended culture periods [123]. These tools are particularly valuable for distinguishing gradual, age-related functional decline from acute stress responses or culture-induced artifacts.

The incorporation of microfluidic and organoid-on-chip technologies represents another critical innovation. These platforms enable controlled perfusion, mechanical stimulation, and real-time monitoring of tissue responses, addressing key limitations of static organoid cultures such as hypoxia, nutrient gradients, and waste accumulation. For aging research, organoid-on-chip systems facilitate modeling of chronic stress, long-term drug exposure, and inter-organ communication, offering a more faithful approximation of in vivo aging environments. Emerging bioengineering approaches, including synthetic and tunable extracellular matrices, allow precise control of mechanical stiffness, ligand presentation, and matrix remodeling—parameters that are increasingly recognized as key regulators of cellular aging and senescence. By decoupling biochemical and biophysical cues, these systems enable causal testing of how age-associated changes in the tissue microenvironment drive stem cell dysfunction and disease susceptibility.

In parallel, genome engineering and functional genomics tools have been adapted for use in organoids to systematically interrogate aging-associated pathways. CRISPR-based perturbation strategies, including pooled loss-of-function, gain-of-function, and epigenome-editing screens, allow high-throughput identification of regulators of senescence, stress resistance, and regenerative decline in a tissue-specific context [124]. Integration of these approaches with phenotypic and multi-omic readouts is accelerating the discovery of actionable targets in aging biology. Finally, advances in computational modeling and artificial intelligence are beginning to play an important role in organoid-based aging research. Machine learning approaches applied to high-content imaging, single-cell datasets, and drug screening outputs enable pattern recognition, trajectory inference, and predictive modeling of aging phenotypes and therapeutic responses [125]. These computational frameworks are poised to enhance data integration and translational decision-making in complex, multidimensional organoid datasets.

Together, these emerging technologies are transforming organoids into sophisticated platforms for studying aging as a dynamic, multifactorial process (Table 4). By enabling

precise manipulation, longitudinal analysis, and systems-level integration, they substantially enhance the power of organoid models to uncover mechanisms of aging, identify therapeutic targets, and predict intervention outcomes.

Table 4. Emerging technologies advancing aging research in organoid systems. This table summarizes key experimental and computational technologies that enable mechanistic investigation of aging and senescence in organoid models.

Technology	Application in Organoid Systems	Aging-Related Questions Addressed
Single-cell RNA sequencing	Identifies transcriptional heterogeneity in organoids	What cell populations drive aging and senescence?
Spatial transcriptomics	Maps spatial cell–cell interactions within organoids	How does aging alter tissue architecture and niche signaling?
CRISPR functional genomics	Genome-wide perturbation screening	Which genes regulate senescence, DNA repair, and stem cell decline?
Organoid-on-chip/microfluidics	Perfusion, nutrient gradients, inter-organ communication	How do chronic stress and vascular decline influence aging tissues?
Advanced live-cell imaging	Long-term monitoring of stem cell dynamics	How does senescence develop over time in aging tissues?
Metabolomics and mitochondrial assays	Measurement of metabolic activity	How does metabolic decline drive aging phenotypes?
Artificial intelligence and machine learning	Analysis of multi-omics and imaging data	Can aging trajectories and drug responses be predicted?

7.1. Single-Cell and Spatial Omics

In the context of aging research, single-cell and spatial omics technologies are particularly powerful for resolving senescence heterogeneity, age-dependent lineage bias, and stem cell exhaustion trajectories within organoid systems [125]. Single-cell RNA sequencing (scRNA-seq), single-cell ATAC-seq, and spatial transcriptomics have revolutionized our understanding of cellular heterogeneity in organoids. These technologies allow dissection of age-associated transcriptional and epigenetic changes at the resolution of individual cells, uncovering subpopulations of stem cells, progenitors, and differentiated cells with distinct aging signatures [126]. For example, scRNA-seq in aged intestinal organoids has identified stem cell subsets with elevated inflammatory signaling and diminished Wnt responsiveness, pinpointing potential targets for rejuvenation [127]. Spatial transcriptomics further enables mapping of microenvironmental interactions and niche-derived signals that influence senescence and tissue decline, providing a more holistic understanding of tissue aging.

7.2. CRISPR-Based Functional Genomics

For aging biology, CRISPR-based perturbation strategies enable direct testing of genes that regulate senescence thresholds, DNA repair decline, mitochondrial fitness, and niche responsiveness in aged versus young organoids [128]. CRISPR/Cas9 technologies facilitate targeted genetic manipulation within organoids, enabling high-throughput functional screens to identify regulators of aging, senescence, and stress responses. Pooled or arrayed CRISPR screens allow interrogation of thousands of genes, revealing pathways that control stem cell proliferation, senescence induction, and DNA repair. For instance, CRISPR-mediated knockout of Wnt modulators or DNA damage response genes in aged intestinal organoids can accelerate or reverse senescence, providing mechanistic insight and highlighting potential therapeutic targets [129].

7.3. Microfluidics and Organ-on-Chip Platforms

Because aging is profoundly influenced by chronic stress exposure, altered perfusion, and systemic inflammation, microfluidic organoid-on-chip platforms are uniquely positioned to model long-term aging-associated microenvironmental remodeling. Integration of organoids with microfluidic devices—so-called "organ-on-chip" systems—enhances physiological relevance by introducing dynamic fluid flow, nutrient gradients, mechanical forces, and vascular-like perfusion [130]. These platforms enable modeling of age-associated changes in tissue perfusion, shear stress responses, and nutrient metabolism, which are critical for studying aging in vivo-like contexts. For example, microfluidic intestinal organoids allow investigation of age-dependent epithelial barrier function, stem cell dynamics, and responses to inflammatory or metabolic stressors under controlled flow conditions.

Beyond single-tissue modeling, recent advances in organoid-on-chip engineering have enabled incorporation of endothelial layers, perfusable microvascular networks, and immune components, thereby improving tissue maturation and functional stability. Such systems facilitate more physiologically relevant drug exposure profiles and allow partial modeling of pharmacokinetic and pharmacodynamic parameters that are otherwise absent in static organoid cultures [131]. Multi-organ chip platforms further extend this concept by linking different organoid types (e.g., liver–gut, liver–brain, or immune–epithelium systems) to simulate aspects of inter-organ communication, systemic inflammation, and metabolic crosstalk that contribute to organismal aging. These developments are particularly significant for aging research, where chronic low-grade inflammation, altered nutrient sensing, and vascular decline collectively shape tissue vulnerability and therapeutic response.

Despite these advances, several bottlenecks currently limit the widespread adoption of fluidic organoid platforms. Device fabrication and operation remain technically complex, often requiring specialized engineering expertise and limiting accessibility across laboratories. Variability in microfluidic chip design and materials introduces challenges in reproducibility and standardization, complicating cross-study comparisons. Additionally, scalability and throughput remain lower than conventional organoid cultures, restricting large-scale screening applications [132]. While incorporation of vascular and immune components has improved physiological relevance, fully recapitulating systemic endocrine regulation, circulating immune aging, and whole-body pharmacokinetics remain challenging. Long-term stability of multi-cellular co-culture systems under continuous flow conditions also requires further optimization. Addressing these technical and translational challenges through standardized platforms, modular chip design, and integration with high-throughput workflows will be critical for realizing the full potential of fluidic organoid systems in aging and therapeutic modeling.

7.4. Imaging and Live-Cell Monitoring

Live-cell imaging approaches are especially valuable for aging research, as they enable longitudinal visualization of gradual functional decline, clonal competition, and progressive senescence accumulation in individual organoids. Advanced imaging techniques, including confocal microscopy, light-sheet microscopy, and high-content live-cell imaging, permit real-time monitoring of aging processes in organoids. These approaches enable visualization of senescence markers, DNA damage foci, mitochondrial dynamics, autophagy, and proliferation in three dimensions over time [133]. Time-lapse imaging of aged organoids has revealed dynamic heterogeneity in senescence induction, niche remodeling, and stem cell replacement, highlighting mechanisms of tissue resilience and decline [134].

7.5. Metabolic and Functional Assays

Given that mitochondrial dysfunction and metabolic inflexibility are central hallmarks of aging, integration of organoids with metabolic profiling platforms provides direct functional assessment of age-associated bioenergetic decline [135]. Aging is tightly linked to alterations in metabolism and mitochondrial function. Organoids are increasingly used with metabolomics, mitochondrial flux analysis, and bioenergetic profiling to quantify age-associated changes in energy homeostasis and reactive oxygen species generation. These assays allow correlation of functional decline with molecular changes, facilitating the identification of metabolic vulnerabilities and targets for intervention.

7.6. Artificial Intelligence and Computational Modeling

In aging-focused organoid studies, AI-driven integration of multi-dimensional datasets enables reconstruction of senescence trajectories, prediction of regenerative potential, and identification of age-specific therapeutic vulnerabilities [136]. Integration of organoid data with machine learning and computational modeling enables predictive analysis of aging trajectories, drug responses, and senescence induction. High-dimensional datasets from single-cell omics, imaging, and functional assays can be analyzed to identify biomarkers of aging, predict regenerative potential, and optimize therapeutic interventions. Computational models also allow simulation of organoid behavior under different perturbations, improving experimental design and accelerating discovery. Recent advances in AI and integrative multi-omics are rapidly transforming organoid-based aging research from descriptive profiling toward predictive and mechanistic modeling [137]. Machine-learning frameworks applied to scRNA-seq, single-cell ATAC-seq, spatial transcriptomics, and high-content imaging datasets now enable identification of senescence-associated cellular states, inference of lineage trajectories, and reconstruction of gene regulatory networks at unprecedented resolution [138]. In organoid systems, AI-driven image analysis has been used to quantify morphological aging phenotypes, classify drug responses, and predict functional decline based on multidimensional phenotypic signatures. Integration of transcriptomic, epigenomic, proteomic, and metabolomic layers further allows mapping of age-dependent regulatory circuits and identification of context-specific therapeutic vulnerabilities. Importantly, computational modeling approaches, including deep learning and graph-based network inference, are being leveraged to predict senescence induction, stem cell exhaustion trajectories, and responsiveness to senolytic or senomorphic compounds in PDOs. These integrative strategies position organoids not only as experimental platforms but also as data-rich systems for systems-level modeling of human aging and therapeutic response.

7.7. Multi-Organ and Co-Culture Systems

Multi-organ and co-culture systems are particularly relevant for aging research, as they allow modeling of inflammaging, immune–epithelial crosstalk, and inter-organ metabolic communication that shape organismal aging. Emerging co-culture and multi-organoid platforms facilitate the study of inter-organ interactions and systemic aging effects. For example, co-culturing intestinal organoids with immune cells or fibroblasts enables modeling of age-associated inflammation and niche deterioration, while coupling liver and pancreatic organoids in microfluidic systems allows investigation of metabolic crosstalk during aging [139]. These approaches offer a more integrative perspective on organism-level aging processes. Collectively, these technologies reposition organoids as mechanistic systems for studying aging as a dynamic, multi-layered process rather than a static phenotype. By enabling age-stratified perturbation, longitudinal monitoring, and systems-level integration,

emerging tools enhance the ability of organoid models to uncover causal drivers of stem cell exhaustion, senescence accumulation, and age-modified disease trajectories.

8. Therapeutic and Translational Potential of Organoid Models

Organoid systems extend beyond their utility as experimental models of aging and disease biology to serve as powerful platforms for therapeutic development and translational research. By faithfully recapitulating key aspects of human tissue architecture, cellular heterogeneity, and patient-specific genetic and epigenetic contexts, organoids enable more predictive assessment of drug efficacy, toxicity, and resistance mechanisms than traditional preclinical models [140]. These attributes are particularly valuable for aging-associated diseases, where subtle cell-intrinsic defects and context-dependent signaling critically shape therapeutic outcomes. One of the most impactful translational applications of organoid technology is in preclinical drug discovery and validation. PDOs have been shown to reliably mirror clinical drug responses across multiple disease settings, including colorectal, pancreatic, gastric, and liver cancers, as well as non-malignant disorders. In the context of aging, organoids enable screening for compounds that restore aged stem cell function, modulate senescence-associated pathways, or selectively eliminate senescent cells, thereby facilitating the identification of candidate senotherapeutics with improved human relevance [141]. Organoid models also provide a robust framework for precision medicine, allowing therapeutic strategies to be tailored to individual patients based on organoid-derived functional responses. Biobanked organoids generated from diverse patient populations preserve inter-individual variability and can be used to predict treatment sensitivity, optimize drug combinations, and stratify patients for clinical trials. This approach is particularly promising for age-associated diseases, where heterogeneity in biological aging trajectories often leads to variable treatment responses.

Beyond pharmacological testing, organoids are increasingly being explored in regenerative and cell-based therapies. Advances in long-term expansion, genetic stability, and differentiation control have enabled the generation of transplantable organoid-derived tissues for epithelial repair and regeneration. For aging tissues, organoid-based regenerative strategies offer the potential to replenish dysfunctional stem cell pools, restore tissue integrity, and counteract degenerative decline, although significant challenges related to engraftment, integration, and safety remain. Organoids further serve as valuable platforms for evaluating therapeutic toxicity and off-target effects in a tissue-specific manner. Compared with animal models, human organoids provide more accurate assessments of drug-induced injury, particularly for tissues such as liver, intestine, and kidney that are highly susceptible to age-related functional decline and adverse drug reactions [142]. This capability is especially relevant in elderly populations, where altered pharmacokinetics and increased vulnerability necessitate careful evaluation of therapeutic safety.

Looking forward, the integration of organoid models with genome editing, multi-omics profiling, and computational modeling is expected to further enhance their translational impact. Combining organoid-based functional data with patient clinical information and machine learning approaches may enable more accurate prediction of therapeutic responses and identification of biomarkers linked to aging and senescence [143]. As organoid technologies continue to mature, they are poised to play an increasingly central role in bridging basic aging research and clinical intervention. In summary, organoid systems represent a transformative platform for therapeutic discovery and translational research. By enabling human-relevant drug testing, personalized treatment strategies, and regenerative approaches within a controlled yet physiologically meaningful framework, organoids hold substantial promise for improving outcomes in aging-related diseases and advancing precision medicine.

8.1. Drug Discovery and High-Throughput Screening

Organoids are increasingly employed in high-throughput drug screening to identify small molecules, biologics, and senolytic compounds that selectively target aged, diseased, or oncogene-primed cells. Patient-derived and genetically engineered organoids enable the evaluation of therapeutic efficacy, toxicity, and resistance mechanisms in a human-relevant system [140]. For example, aged intestinal and hepatic organoids have been used to screen senolytic compounds such as BCL-2 inhibitors, which selectively eliminate senescent cells and attenuate SASP-mediated inflammation, highlighting strategies to ameliorate age-related tissue dysfunction [144].

Furthermore, organoids derived from cancer patients allow personalized drug screening, identifying therapeutics that target tumor-specific vulnerabilities while sparing normal tissue. This approach has been particularly successful in colorectal, pancreatic, and liver cancers, where patient-derived organoids faithfully predict responses to chemotherapy, targeted therapy, and immunotherapy. Integrating organoids with automated platforms, robotics, and multi-well assays enhances throughput and reproducibility, accelerating drug discovery pipelines.

8.2. Senolytic and Senomorphic Therapeutics

Senescence contributes to aging-related tissue dysfunction, chronic inflammation, and cancer progression. Organoid models provide a controlled platform for testing senolytic (cell-eliminating) and senomorphic (SASP-modulating) therapies. Genetic or pharmacologic induction of senescence in organoids allows the identification of compounds that specifically target senescent cells without affecting proliferating stem cells [145]. Additionally, organoids allow mechanistic evaluation of how therapeutic interventions modulate secretory phenotypes and tissue microenvironmental interactions, providing insights into potential systemic effects.

8.3. Gene and Cell-Based Therapies

Organoids are ideal platforms for evaluating gene editing and regenerative strategies. CRISPR/Cas9-mediated correction of disease-causing mutations in patient-derived organoids has been successfully demonstrated in cystic fibrosis, metabolic liver disorders, and monogenic intestinal diseases [146,147]. Edited organoids can then be expanded and transplanted into preclinical models to restore tissue function, offering a roadmap for future cell-based regenerative therapies. Additionally, co-culture with immune cells or vascular networks allows testing of gene- and cell-based therapies under physiologically relevant conditions.

8.4. Modeling Therapy Response and Resistance

Organoids provide a predictive platform to study drug resistance mechanisms in aged and diseased tissues. Aging and senescence can alter cellular metabolism, stress responses, and signaling pathways, affecting therapeutic responses. Organoid models allow systematic evaluation of these factors, enabling identification of combinatorial therapies to overcome resistance [148,149]. This is particularly relevant in oncology, where organoids derived from aged or genetically complex tumors can predict patient-specific responses to chemotherapeutics, targeted inhibitors, and immune-modulating agents.

8.5. Integration with Personalized and Precision Medicine

PDOs are at the forefront of personalized medicine, enabling tailoring of therapeutic interventions based on individual patient biology. By capturing donor-specific genetics, epigenetics, and aging phenotypes, PDOs allow evaluation of therapy efficacy, toxicity,

and optimal dosing in vitro before clinical administration. This approach is particularly valuable in elderly patients or those with comorbidities, where standard therapies may be less effective or more toxic.

8.6. Future Directions and Clinical Translation

Despite their promise, several challenges remain for translating organoid-based discoveries into clinical practice, including long-term culture stability, scalability, reproducibility, and integration with immune and vascular systems [150]. Furthermore, combining organoid models with multi-omics, imaging, and computational modeling will accelerate identification of actionable targets and predictive biomarkers for aging-related diseases, cancer, and regenerative interventions (Figure 3).

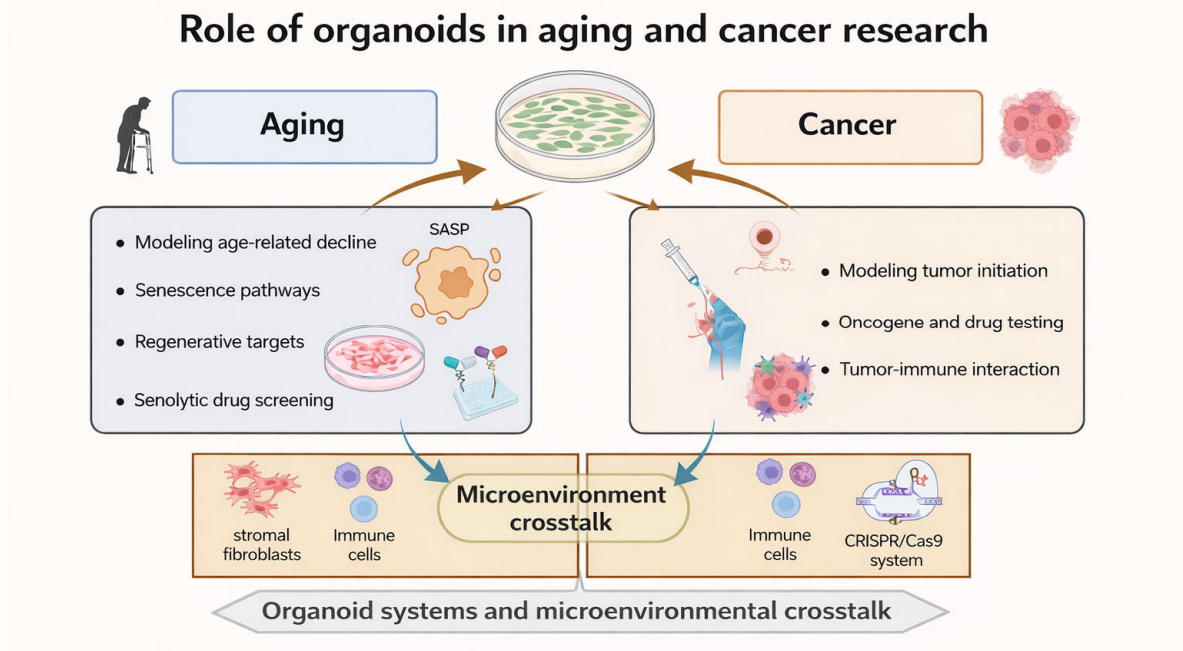


Figure 3. Applications of 3D organoid systems in aging and cancer research. 3D organoid models enable physiologically relevant investigation of aging-associated tissue decline and cancer biology. In aging research, organoids derived from young and aged tissues are used to study stem cell exhaustion, cellular senescence, and regenerative capacity, and to screen senolytic and senomorphic compounds. In cancer research, patient-derived and genetically engineered organoids facilitate modeling of tumor initiation and progression, functional interrogation of oncogenic drivers, and assessment of drug responses and resistance. Across both contexts, integration of stromal and immune components allows analysis of microenvironmental crosstalk that influences tissue homeostasis and disease evolution, supporting translational target discovery and precision medicine applications. https://app.biorender.com/illustrations/canvas-beta/69759fff023658e17ca5bb58 (accessed on 17 March 2026).

In summary, organoid technology offers an unparalleled platform for therapeutic discovery, testing, and translational research. By integrating genetic manipulation, high-throughput screening, personalized modeling, and regenerative approaches, organoids provide a versatile system to bridge basic biological insights with clinical applications. Their use promises to improve treatment strategies for age-associated pathologies, cancer, metabolic disorders, and other human diseases, accelerating the move toward precision medicine.

9. Challenges, Limitations, and Future Perspectives

While organoid technology has revolutionized the study of aging, senescence, and disease biology, several challenges and limitations remain that need to be addressed to fully realize their translational potential. At the same time, ongoing technological innovations provide promising avenues to overcome these barriers and expand the scope of organoid research.

9.1. Technical and Biological Limitations

1. Limited vascularization and immune components: Traditional organoids lack functional vasculature and immune cell populations, which are critical for modeling systemic aging, inflammatory responses, and tissue regeneration [151]. The absence of vascular perfusion limits nutrient and oxygen delivery, impacting long-term culture stability and cellular metabolism.
2. Incomplete tissue maturation: Organoids often resemble fetal or immature tissue rather than fully adult tissue, which can limit their ability to accurately model late-onset diseases or age-associated phenotypes [152]. Strategies to accelerate maturation, such as growth factor modulation, mechanical cues, or co-culture with stromal cells, are under investigation.
3. Donor and culture variability: Inter-donor heterogeneity and differences in culture conditions (e.g., matrix composition, growth factors, oxygen levels) can lead to variable organoid formation efficiency and phenotype reproducibility [153]. Standardized protocols are essential to reduce experimental variability, especially for high-throughput drug screening and translational applications.
4. Limited lifespan and senescence artifacts: Prolonged culture can introduce artificial senescence or genetic drift, complicating interpretation of age-related phenotypes [154]. Careful optimization of passage number and culture duration is crucial for maintaining physiologically relevant aging features.
5. The widespread reliance on animal-derived extracellular matrices such as Matrigel introduces batch-to-batch variability and poorly defined biochemical cues, complicating cross-study comparisons and large-scale translational applications [155,156]. Another key challenge is the incomplete maturation and architectural complexity of many organoid models. While ASC-derived organoids retain aspects of tissue identity, they frequently lack higher-order structures such as vasculature, innervation, and lymphatic components, all of which play critical roles in aging, metabolic regulation, and immune surveillance [157]. The absence of perfusable vascular networks can result in diffusion-limited hypoxia, nutrient gradients, and waste accumulation in long-term cultures, potentially confounding interpretations of senescence and stress-associated phenotypes.
6. A further limitation is the insufficient representation of systemic aging cues. Endocrine signals, circulating metabolites, immune aging (immunosenescence), and neurohumoral regulation profoundly influence tissue aging in vivo but are largely absent in conventional organoid cultures. Consequently, organoids primarily capture cell-intrinsic and local niche–dependent aging mechanisms, while organismal drivers of aging and inter-organ communication remain underrepresented [158].

9.2. Modeling Systemic and Multi-Organ Aging

Aging is a multifactorial process involving interactions between organs, tissues, and systemic factors such as hormones, the immune system, and the microbiome. Current organoid models are primarily tissue-specific and often lack integration with systemic components. Emerging multi-organoid and organ-on-chip platforms, which link organoids

from different tissues under controlled flow conditions, offer a potential solution for studying inter-organ communication and systemic aging effects [159].

9.3. Integration with Microenvironmental and Niche Factors

Organoids typically lack complete representation of extracellular matrix (ECM) complexity, stromal interactions, and mechanical forces present in vivo. These niche signals are critical for stem cell maintenance, senescence regulation, and tissue homeostasis [160]. Advances in synthetic hydrogels, ECM engineering, and co-culture systems are enabling more faithful recapitulation of the tissue microenvironment, which is essential for modeling aging and disease.

9.4. Translational and Clinical Challenges

Despite their predictive potential, clinical translation of organoid-based discoveries faces several hurdles:

- Scalability: Producing organoids at clinical scale remains challenging, particularly for regenerative therapies or personalized medicine applications.
- Regulatory compliance: Safety, reproducibility, and standardization must meet regulatory standards for therapeutic use.
- Cost and accessibility: Organoid culture is resource-intensive, limiting broad adoption in some research or clinical settings.

9.5. Future Directions

1. Vascularized and immune-competent organoids: Incorporation of vasculature, immune cells, and other niche components will improve physiological relevance and allow modeling of inflammation, immune surveillance, and systemic aging.
2. Advanced multi-omics integration: Combining single-cell transcriptomics, proteomics, epigenomics, and metabolomics will enable comprehensive mapping of aging networks and identification of robust therapeutic targets.
3. Personalized aging models: Patient-derived organoids from different age groups and genetic backgrounds will facilitate individualized studies of aging, senescence, and drug response, advancing precision medicine approaches.
4. Automation and high-throughput platforms: Robotics, microfluidics, and organ-on-chip integration will allow large-scale screening of drugs, senolytic compounds, and regenerative therapies in physiologically relevant contexts.
5. Computational modeling and AI integration: Machine learning algorithms can analyze complex organoid datasets to predict aging trajectories, therapy responses, and optimal intervention strategies, accelerating translational research [161,162]. AI-driven frameworks—including deep learning, trajectory inference algorithms, and graph-based network modeling—enable integration of transcriptomic, epigenomic, proteomic, and metabolic layers to reconstruct aging trajectories and identify regulatory circuits that drive stem cell exhaustion or senescence induction. In organoid-based model systems, machine learning–driven image analysis has increasingly been applied to extract quantitative information from complex morphological features. These computational approaches enable the classification of aging-associated phenotypes, such as changes in organoid size, shape, structural organization, and cellular heterogeneity. By analyzing large image datasets, machine learning models can also quantify drug-induced phenotypic responses and identify subtle morphological changes that may not be easily detected through manual observation. Importantly, these image-derived phenotypic signatures can be integrated with predictive algo-

rithms to forecast therapeutic sensitivity, thereby providing a powerful platform for high-throughput drug screening and precision medicine applications.

10. Conclusions

Organoid technology offers unprecedented opportunities to study aging, senescence, and disease in human-relevant systems. It has emerged as a transformative paradigm in biomedical research, offering unprecedented opportunities to model human aging, cellular senescence, and disease progression within physiologically relevant 3D systems. By recapitulating native tissue architecture, cellular heterogeneity, and stem cell hierarchies, organoids bridge the long-standing gap between simplified in vitro cultures and complex in vivo models, enabling more precise dissection of epithelial-intrinsic aging mechanisms across multiple organ systems [163]. This human-relevant fidelity is particularly critical for aging research, where species-specific differences and systemic confounders often limit the translational value of traditional models. Organoids derived from aged tissues, as well as genetically or environmentally engineered to model senescence, have yielded important insights into age-associated alterations in signaling pathways, stem cell competence, and microenvironmental regulation of tissue homeostasis. These systems have clarified how aging reshapes proliferative capacity, lineage commitment, and stress responses, while revealing context-dependent roles for OIS and SASP in tissue decline and disease initiation [5,85]. Importantly, organoid-based models allow aging-related phenotypes to be studied in isolation from systemic influences, thereby enabling causal mechanistic interrogation that is difficult to achieve in vivo.

The integration of organoid platforms with emerging technologies—including single-cell and spatial multi-omics, CRISPR-based functional genomics, organoid-on-chip systems, advanced live imaging, and computational modeling—has further expanded their analytical power [164]. These approaches enable longitudinal, high-resolution, and systems-level analysis of aging processes, transforming organoids from descriptive models into predictive platforms for hypothesis testing and therapeutic discovery [165]. As a result, organoids now provide a versatile framework to uncover novel regulators of senescence, identify age-specific vulnerabilities, and interrogate dynamic stem cell–niche interactions. Looking ahead, organoid systems hold exceptional promises for the development and translation of senescence-modulating therapies, including senolytic and senomorphic agents, regenerative strategies, and patient-tailored interventions. Their compatibility with high-throughput drug screening, functional genomics, and patient-derived biobanks positions organoids as key drivers of precision medicine approaches for age-associated diseases. Furthermore, organoid-based modeling of therapy resistance, toxicity, and tissue-specific responses is poised to improve clinical decision-making, particularly in elderly populations where treatment efficacy and safety remain major challenges.

Despite ongoing limitations related to standardization, maturation, and systemic integration, rapid advances in bioengineering, multicellular co-culture, and microphysiological systems are steadily enhancing the physiological relevance and translational utility of organoid models. Continued efforts to harmonize protocols, develop synthetic matrices, and incorporate immune and vascular components will be essential to fully realize the potential of organoids as comprehensive aging models [166,167]. In summary, organoid systems represent a paradigm shift in aging and disease research. They provide human-relevant, experimentally tractable platforms capable of elucidating fundamental mechanisms of cellular aging, uncovering novel therapeutic targets, and guiding the development of interventions aimed at mitigating senescence and preserving tissue resilience across the lifespan. As these technologies continue to evolve, organoids are poised to play a

central role in translating insights from aging biology into effective, personalized therapies that address the growing burden of age-related disease.

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Abbreviations

The following abbreviations are used in this manuscript:

2D	Two-dimensional
3D	Three-dimensional
SASP	Senescence-associated secretory phenotype
OIS	Oncogene-induced senescence
ASCs	Adult stem cells
PSCs	Pluripotent stem cells (such as ESCs)
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
RNAi	RNA interference
PDOs	Patient-derived organoids
scRNA-seq	Single-cell RNA sequencing
ECM	Extracellular matrix

References

1. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. *Cell* **2013**, *153*, 1194–1217. [CrossRef]
2. Partridge, L. The New Biology of Ageing. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 147–154. [CrossRef] [PubMed]
3. Goodell, M.A.; Rando, T.A. Stem Cells and Healthy Aging. *Science* **2015**, *350*, 1199–1204. [CrossRef]
4. Kirkland, J.L.; Tchkonia, T. Cellular Senescence: A Translational Perspective. *eBioMedicine* **2017**, *21*, 21–28. [CrossRef]
5. Campisi, J. Aging, Cellular Senescence, and Cancer. *Annu. Rev. Physiol.* **2013**, *75*, 685–705. [CrossRef]
6. Di Micco, R.; Krizhanovsky, V.; Baker, D.; d'Adda di Fagagna, F. Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 75–95. [CrossRef] [PubMed]
7. Campisi, J.; d'Adda di Fagagna, F. Cellular Senescence: When Bad Things Happen to Good Cells. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 729–740. [CrossRef]
8. Baker, D.J.; Childs, B.G.; Durik, M.; Wijers, M.E.; Sieben, C.J.; Zhong, J.; Saltness, R.A.; Jeganathan, K.B.; Verzosa, G.C.; Pezeshki, A. Naturally Occurring p16Ink4a-Positive Cells Shorten Healthy Lifespan. *Nature* **2016**, *530*, 184–189. [CrossRef]
9. Edmondson, R.; Broglie, J.J.; Adcock, A.F.; Yang, L. Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors. *Assay Drug Dev. Technol.* **2014**, *12*, 207–218. [CrossRef]
10. Dutta, S.; Sengupta, P. Men and Mice: Relating Their Ages. *Life Sci.* **2016**, *152*, 244–248. [CrossRef] [PubMed]
11. Sato, T.; Vries, R.G.; Snippert, H.J.; Van De Wetering, M.; Barker, N.; Stange, D.E.; Van Es, J.H.; Abo, A.; Kujala, P.; Peters, P.J. Single Lgr5 Stem Cells Build Crypt-Villus Structures In Vitro without a Mesenchymal Niche. *Nature* **2009**, *459*, 262–265. [CrossRef]
12. Lancaster, M.A.; Knoblich, J.A. Organogenesis in a Dish: Modeling Development and Disease Using Organoid Technologies. *Science* **2014**, *345*, 1247125. [CrossRef] [PubMed]
13. Pentinmikko, N.; Iqbal, S.; Mana, M.; Andersson, S.; Cognetta, A.B., III; Suciu, R.M.; Roper, J.; Luopajärvi, K.; Markelin, E.; Gopalakrishnan, S. Notum Produced by Paneth Cells Attenuates Regeneration of Aged Intestinal Epithelium. *Nature* **2019**, *571*, 398–402. [CrossRef]

14. Jothi, D.; Kulka, L.A.M. Strategies for Modeling Aging and Age-Related Diseases. *npj Aging* **2024**, *10*, 32. [CrossRef]
15. Chao, C.-C.; Shen, P.-W.; Tzeng, T.-Y.; Kung, H.-J.; Tsai, T.-F.; Wong, Y.-H. Human iPSC-Derived Neurons as a Platform for Deciphering the Mechanisms behind Brain Aging. *Biomedicines* **2021**, *9*, 1635. [CrossRef]
16. Richter, K.M.; Wrage, M.; Krekeler, C.; De Oliveira, T.; Conradi, L.-C.; Menck, K.; Bleckmann, A. Model Systems to Study Tumor-Microbiome Interactions in Early-Onset Colorectal Cancer. *EMBO Mol. Med.* **2025**, *17*, 395–413. [CrossRef] [PubMed]
17. Fujii, M.; Shimokawa, M.; Date, S.; Takano, A.; Matano, M.; Nanki, K.; Ohta, Y.; Toshimitsu, K.; Nakazato, Y.; Kawasaki, K. A Colorectal Tumor Organoid Library Demonstrates Progressive Loss of Niche Factor Requirements during Tumorigenesis. *Cell Stem Cell* **2016**, *18*, 827–838. [CrossRef]
18. Lu, X.; Yang, J.; Xiang, Y. Modeling Human Neurodevelopmental Diseases with Brain Organoids. *Cell Regen.* **2022**, *11*, 1. [CrossRef] [PubMed]
19. Ogrodnik, M.; Miwa, S.; Tchkonia, T.; Tiniakos, D.; Wilson, C.L.; Lahat, A.; Day, C.P.; Burt, A.; Palmer, A.; Anstee, Q.M. Cellular Senescence Drives Age-Dependent Hepatic Steatosis. *Nat. Commun.* **2017**, *8*, 15691. [CrossRef]
20. Beumer, J.; Artegiani, B.; Post, Y.; Reimann, F.; Gribble, F.; Nguyen, T.N.; Zeng, H.; Van den Born, M.; Van Es, J.H.; Clevers, H. Enteroendocrine Cells Switch Hormone Expression along the Crypt-to-Villus BMP Signalling Gradient. *Nat. Cell Biol.* **2018**, *20*, 909–916. [CrossRef]
21. Ritschka, B.; Storer, M.; Mas, A.; Heinzmann, F.; Ortells, M.C.; Morton, J.P.; Sansom, O.J.; Zender, L.; Keyes, W.M. The Senescence-Associated Secretory Phenotype Induces Cellular Plasticity and Tissue Regeneration. *Genes Dev.* **2017**, *31*, 172–183. [CrossRef]
22. González-Gualda, E.; Baker, A.G.; Fruk, L.; Muñoz-Espín, D. A Guide to Assessing Cellular Senescence In Vitro and In Vivo. *FEBS J.* **2021**, *288*, 56–80. [CrossRef]
23. Suhito, I.R.; Sunil, C.; Tay, A. Engineering Human Immune Organoids for Translational Immunology. *Bioact. Mater.* **2025**, *44*, 164–183. [CrossRef]
24. Zhou, L.; Huang, J.; Li, C.; Gu, Q.; Li, G.; Li, Z.A.; Xu, J.; Zhou, J.; Tuan, R.S. Organoids and Organs-on-Chips: Recent Advances, Applications in Drug Development, and Regulatory Challenges. *Med* **2025**, *6*, 100667. [CrossRef]
25. Clevers, H. Modeling Development and Disease with Organoids. *Cell* **2016**, *165*, 1586–1597. [CrossRef]
26. Sato, T.; Stange, D.E.; Ferrante, M.; Vries, R.G.; Van Es, J.H.; Van Den Brink, S.; Van Houdt, W.J.; Pronk, A.; Van Gorp, J.; Siersema, P.D. Long-Term Expansion of Epithelial Organoids from Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium. *Gastroenterology* **2011**, *141*, 1762–1772. [CrossRef] [PubMed]
27. Lancaster, M.A.; Renner, M.; Martin, C.-A.; Wenzel, D.; Bicknell, L.S.; Hurles, M.E.; Homfray, T.; Penninger, J.M.; Jackson, A.P.; Knoblich, J.A. Cerebral Organoids Model Human Brain Development and Microcephaly. *Nature* **2013**, *501*, 373–379. [CrossRef]
28. Takebe, T.; Sekine, K.; Enomura, M.; Koike, H.; Kimura, M.; Ogaeri, T.; Zhang, R.-R.; Ueno, Y.; Zheng, Y.-W.; Koike, N. Vascularized and Functional Human Liver from an iPSC-Derived Organ Bud Transplant. *Nature* **2013**, *499*, 481–484. [CrossRef] [PubMed]
29. Barker, N.; Huch, M.; Kujala, P.; van de Wetering, M.; Snippert, H.J.; van Es, J.H.; Sato, T.; Stange, D.E.; Begthel, H.; van den Born, M. Lgr5+ ve Stem Cells Drive Self-Renewal in the Stomach and Build Long-Lived Gastric Units In Vitro. *Cell Stem Cell* **2010**, *6*, 25–36. [CrossRef] [PubMed]
30. Takasato, M.; Er, P.X.; Chiu, H.S.; Maier, B.; Baillie, G.J.; Ferguson, C.; Parton, R.G.; Wolvetang, E.J.; Roost, M.S.; Chuva de Sousa Lopes, S.M. Kidney Organoids from Human iPS Cells Contain Multiple Lineages and Model Human Nephrogenesis. *Nature* **2015**, *526*, 564–568. [CrossRef]
31. Sato, T.; Clevers, H. Primary Mouse Small Intestinal Epithelial Cell Cultures. In *Epithelial Cell Culture Protocols*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 319–328.
32. Yin, X.; Farin, H.F.; Van Es, J.H.; Clevers, H.; Langer, R.; Karp, J.M. Niche-Independent High-Purity Cultures of Lgr5+ Intestinal Stem Cells and Their Progeny. *Nat. Methods* **2014**, *11*, 106–112. [CrossRef] [PubMed]
33. Eiraku, M.; Takata, N.; Ishibashi, H.; Kawada, M.; Sakakura, E.; Okuda, S.; Sekiguchi, K.; Adachi, T.; Sasai, Y. Self-Organizing Optic-Cup Morphogenesis in Three-Dimensional Culture. *Nature* **2011**, *472*, 51–56. [CrossRef] [PubMed]
34. McCracken, K.W.; Catá, E.M.; Crawford, C.M.; Sinagoga, K.L.; Schumacher, M.; Rockich, B.E.; Tsai, Y.-H.; Mayhew, C.N.; Spence, J.R.; Zavros, Y. Modelling Human Development and Disease in Pluripotent Stem-Cell-Derived Gastric Organoids. *Nature* **2014**, *516*, 400–404. [CrossRef] [PubMed]
35. Khorraminejad-Shirazi, M.; Dorvash, M.; Estedlal, A.; Hoveidaei, A.H.; Mazloomrezaei, M.; Mosaddeghi, P. Aging: A Cell Source Limiting Factor in Tissue Engineering. *World J. Stem Cells* **2019**, *11*, 787. [CrossRef]
36. Aspal, M.; Pushlar, N.; Melameka, M.; McVicar, R.N.; Smith, E.; Ogunyamoju, T.; Kumar, M.; Marth, J.D.; Chun, J.; Snyder, E.Y. Modeling the Aging Human Lung: Generation of a Senescent Human Lung Organoid Culture System. *bioRxiv* **2025**. [CrossRef]
37. Broutier, L.; Mastrogiovanni, G.; Verstegen, M.M.; Francies, H.E.; Gavarró, L.M.; Bradshaw, C.R.; Allen, G.E.; Arnes-Benito, R.; Sidorova, O.; Gaspersz, M.P. Human Primary Liver Cancer–Derived Organoid Cultures for Disease Modeling and Drug Screening. *Nat. Med.* **2017**, *23*, 1424–1435. [CrossRef]

38. Nozaki, K.; Mochizuki, W.; Matsumoto, Y.; Matsumoto, T.; Fukuda, M.; Mizutani, T.; Watanabe, M.; Nakamura, T. Co-Culture with Intestinal Epithelial Organoids Allows Efficient Expansion and Motility Analysis of Intraepithelial Lymphocytes. *J. Gastroenterol.* **2016**, *51*, 206–213. [CrossRef]
39. Dave, R.; Pandey, K.; Patel, R.; Gour, N.; Bhatia, D. Leveraging 3D Cell Culture and AI Technologies for Next-Generation Drug Discovery. *Cell Biomater.* **2025**, *1*, 100050. [CrossRef]
40. Poornima, K.; Francis, A.P.; Hoda, M.; Eladl, M.A.; Subramanian, S.; Veeraraghavan, V.P.; El-Sherbiny, M.; Asseri, S.M.; Hussamuldin, A.B.A.; Surapaneni, K.M. Implications of Three-Dimensional Cell Culture in Cancer Therapeutic Research. *Front. Oncol.* **2022**, *12*, 891673. [CrossRef]
41. Liu, X.; Zhou, Z.; Zhang, Y.; Zhong, H.; Cai, X.; Guan, R. Recent Progress on the Organoids: Techniques, Advantages and Applications. *Biomed. Pharmacother.* **2025**, *185*, 117942. [CrossRef]
42. Yang, S.; Hu, H.; Kung, H.; Zou, R.; Dai, Y.; Hu, Y.; Wang, T.; Lv, T.; Yu, J.; Li, F. Organoids: The Current Status and Biomedical Applications. *MedComm* **2023**, *4*, e274. [CrossRef] [PubMed]
43. Wang, Q.; Yuan, F.; Zuo, X.; Li, M. Breakthroughs and challenges of organoid models for assessing cancer immunotherapy: A cutting-edge tool for advancing personalised treatments. *Cell Death Discov.* **2025**, *11*, 222. [CrossRef]
44. Kretzschmar, K.; Clevers, H. Organoids: Modeling Development and the Stem Cell Niche in a Dish. *Dev. Cell* **2016**, *38*, 590–600. [CrossRef] [PubMed]
45. Bhaduri, A.; Andrews, M.G.; Mancia Leon, W.; Jung, D.; Shin, D.; Allen, D.; Jung, D.; Schmunk, G.; Haeussler, M.; Salma, J. Cell Stress in Cortical Organoids Impairs Molecular Subtype Specification. *Nature* **2020**, *578*, 142–148. [CrossRef]
46. Kapetanos, K.; Asimakopoulos, D.; Christodoulou, N.; Vogt, A.; Khan, W. Chronological Age Affects MSC Senescence in Vitro—A Systematic Review. *Int. J. Mol. Sci.* **2021**, *22*, 7945. [CrossRef]
47. Sun, X.; Sun, F.; Zhang, Y.; Qu, J.; Zhang, W.; Liu, G.-H. A Narrative Review of Organoids for Investigating Organ Aging: Opportunities and Challenges. *J. Bio-X Res.* **2023**, *6*, 3–14. [CrossRef]
48. Llorente, C. The Imperative for Innovative Enteric Nervous System–Intestinal Organoid Co-Culture Models: Transforming GI Disease Modeling and Treatment. *Cells* **2024**, *13*, 820. [CrossRef]
49. Neal, J.T.; Li, X.; Zhu, J.; Giangarra, V.; Grzeskowiak, C.L.; Ju, J.; Liu, I.H.; Chiou, S.-H.; Salahudeen, A.A.; Smith, A.R. Organoid Modeling of the Tumor Immune Microenvironment. *Cell* **2018**, *175*, 1972–1988. [CrossRef]
50. Magré, L.; Verstegen, M.M.; Buschow, S.; Van Der Laan, L.J.; Peppelenbosch, M.; Desai, J. Emerging Organoid-Immune Co-Culture Models for Cancer Research: From Oncoimmunology to Personalized Immunotherapies. *J. Immunother. Cancer* **2023**, *11*, e006290. [CrossRef]
51. Kamroo, A.; Kakroudi, M.H.; Sarmadian, A.J.; Firouzabadi, A.; Mousavi, S.; Yazdanpanah, N.; Saleki, K.; Rezaei, N. Immunosenescence and Organoids: Pathophysiology and Therapeutic Opportunities. *Immun. Ageing* **2025**, *22*, 46. [CrossRef]
52. Chauhdari, T.; Zaidi, S.A.; Su, J.; Ding, Y. Organoids Meet Microfluidics: Recent Advancements, Challenges, and Future of Organoids-on-Chip. *Vitr. Models* **2025**, *4*, 71–88. [CrossRef]
53. Gjorevski, N.; Sachs, N.; Manfrin, A.; Giger, S.; Bragina, M.E.; Ordóñez-Morán, P.; Clevers, H.; Lutolf, M.P. Designer Matrices for Intestinal Stem Cell and Organoid Culture. *Nature* **2016**, *539*, 560–564. [CrossRef]
54. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. Hallmarks of Aging: An Expanding Universe. *Cell* **2023**, *186*, 243–278. [CrossRef] [PubMed]
55. Azar, J.; Bahmad, H.F.; Daher, D.; Moubarak, M.M.; Hadadeh, O.; Monzer, A.; Al Bitar, S.; Jamal, M.; Al-Sayegh, M.; Abou-Kheir, W. The Use of Stem Cell-Derived Organoids in Disease Modeling: An Update. *Int. J. Mol. Sci.* **2021**, *22*, 7667. [CrossRef] [PubMed]
56. Zhu, Y.; Lin, N.; Li, J.; Zhang, H.; Zhang, P.; Cheng, X.; Yang, Q.; Liu, L. Organoids in Genetic Disorders: From Disease Modeling to Translational Applications. *Stem Cell Rev. Rep.* **2025**, *21*, 2578–2596. [CrossRef] [PubMed]
57. Fane, M.; Weeraratna, A.T. How the Ageing Microenvironment Influences Tumour Progression. *Nat. Rev. Cancer* **2020**, *20*, 89–106. [CrossRef]
58. Volkmer, E.; Drosse, I.; Otto, S.; Stangelmayer, A.; Stengele, M.; Kallukalam, B.C.; Mutschler, W.; Schieker, M. Hypoxia in Static and Dynamic 3D Culture Systems for Tissue Engineering of Bone. *Tissue Eng. Part A* **2008**, *14*, 1331–1340. [CrossRef]
59. Beerman, I.; Bhattacharya, D.; Zandi, S.; Sigvardsson, M.; Weissman, I.L.; Bryder, D.; Rossi, D.J. Functionally Distinct Hematopoietic Stem Cells Modulate Hematopoietic Lineage Potential during Aging by a Mechanism of Clonal Expansion. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 5465–5470. [CrossRef]
60. Ermolaeva, M.; Neri, F.; Ori, A.; Rudolph, K.L. Cellular and Epigenetic Drivers of Stem Cell Ageing. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 594–610. [CrossRef]
61. Sharpless, N.E.; Sherr, C.J. Forging a Signature of In Vivo Senescence. *Nat. Rev. Cancer* **2015**, *15*, 397–408. [CrossRef]
62. Scesa, G.; Adami, R.; Bottai, D. iPSC Preparation and Epigenetic Memory: Does the Tissue Origin Matter? *Cells* **2021**, *10*, 1470. [CrossRef]
63. Sprangers, J.; Zaalberg, I.C.; Maurice, M.M. Organoid-based modeling of intestinal development, regeneration, and repair. *Cell Death Differ.* **2021**, *28*, 95–107. [CrossRef] [PubMed]

64. Shay, J.E.; Yilmaz, Ö.H. Dietary and Metabolic Effects on Intestinal Stem Cells in Health and Disease. *Nat. Rev. Gastroenterol. Hepatol.* **2025**, *22*, 23–38. [CrossRef]
65. Peng, W.C.; Logan, C.Y.; Fish, M.; Anbarchian, T.; Aguisanda, F.; Álvarez-Varela, A.; Wu, P.; Jin, Y.; Zhu, J.; Li, B. Inflammatory Cytokine TNFα Promotes the Long-Term Expansion of Primary Hepatocytes in 3D Culture. *Cell* **2018**, *175*, 1607–1619. [CrossRef]
66. Kuehnemann, C.; Wiley, C.D. Senescent cells at the crossroads of aging, disease, and tissue homeostasis. *Aging Cell* **2024**, *23*, e13988. [CrossRef] [PubMed]
67. Hu, J.L.; Todhunter, M.E.; LaBarge, M.A.; Gartner, Z.J. Opportunities for Organoids as New Models of Aging. *J. Cell Biol.* **2018**, *217*, 39–50. [CrossRef]
68. Yao, Q.; Cheng, S.; Pan, Q.; Yu, J.; Cao, G.; Li, L.; Cao, H. Organoids: Development and applications in disease models, drug discovery, precision medicine, and regenerative medicine. *MedComm* **2024**, *5*, e735. [CrossRef]
69. Merenda, A.; Fenderico, N.; Maurice, M.M. Wnt signaling in 3D: Recent advances in the applications of intestinal organoids. *Trends Cell Biol.* **2020**, *30*, 60–73. [CrossRef]
70. Igarashi, R.; Oda, M.; Okada, R.; Yano, T.; Takahashi, S.; Pastuhov, S.; Matano, M.; Masuda, N.; Togasaki, K.; Ohta, Y.; et al. Generation of human adult hepatocyte organoids with metabolic functions. *Nature* **2025**, *641*, 1248–1257. [CrossRef]
71. Yu, P.; Liu, B.; Dong, C.; Chang, Y. Induced Pluripotent Stem Cells-Based Regenerative Therapies in Treating Human Aging-Related Functional Decline and Diseases. *Cells* **2025**, *14*, 619. [CrossRef] [PubMed]
72. Miller, J.D.; Ganat, Y.M.; Kishinevsky, S.; Bowman, R.L.; Liu, B.; Tu, E.Y.; Mandal, P.K.; Vera, E.; Shim, J.; Kriks, S. Human iPSC-Based Modeling of Late-Onset Disease via Progerin-Induced Aging. *Cell Stem Cell* **2013**, *13*, 691–705. [CrossRef]
73. Capeling, M.M.; Chen, B.; Aliar, K.; Penna, E.; Lopez, V.I.; Foo, C.; Rost, S.; Holokai, L.; Tong, X.; Phillips, D.; et al. Dictionary of human intestinal organoid responses to secreted niche factors at single cell resolution. *Nat. Commun.* **2026**, *17*, 1527. [CrossRef] [PubMed]
74. Krauß, D.; Moreno-Viedma, V.; Adachi-Fernandez, E.; de Sá Fernandes, C.; Genger, J.W.; Fari, O.; Blauensteiner, B.; Kirchhofer, D.; Bradaric, N.; Gushchina, V.; et al. EGFR controls transcriptional and metabolic rewiring in KRASG12D colorectal cancer. *EMBO Mol. Med.* **2025**, *17*, 1355. [CrossRef] [PubMed]
75. Chatsirisupachai, K.; Lagger, C.; de Magalhães, J.P. Age-associated differences in the cancer molecular landscape. *Trends Cancer* **2022**, *8*, 962–971. [CrossRef] [PubMed]
76. Kumari, R.; Jat, P. Mechanisms of cellular senescence: Cell cycle arrest and senescence associated secretory phenotype. *Front. Cell Dev. Biol.* **2021**, *9*, 645593. [CrossRef]
77. Yang, H.; Liu, D.; Qiu, L.; Wang, R.; Zhang, C.; Yu, D.; Zhong, Q.; Yuki, N.; Song, Z.; Zhu, T. Reprogramming Cellular Senescence and Aging Clocks for Advanced Cancer Immunotherapy. *Mol. Cancer* **2025**, *24*, 237. [CrossRef]
78. Safwan-Zaiter, H.; Wagner, N.; Wagner, K.-D. P16INK4A—More than a Senescence Marker. *Life* **2022**, *12*, 1332. [CrossRef]
79. Sorrentino, V.; Romani, M.; Mouchiroud, L.; Beck, J.S.; Zhang, H.; D'Amico, D.; Moullan, N.; Potenza, F.; Schmid, A.W.; Rietsch, S. Enhancing Mitochondrial Proteostasis Reduces Amyloid-β Proteotoxicity. *Nature* **2017**, *552*, 187–193. [CrossRef]
80. Wang, B.; Han, J.; Elisseeff, J.H.; Demaria, M. The senescence-associated secretory phenotype and its physiological and pathological implications. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 958–978. [CrossRef]
81. Takasugi, M.; Yoshida, Y.; Ohtani, N. Cellular senescence and the tumour microenvironment. *Mol. Oncol.* **2022**, *16*, 3333–3351. [CrossRef]
82. Zuo, J.; Fang, Y.; Wang, R.; Liang, S. High-throughput solutions in tumor organoids: From culture to drug screening. *Stem Cells* **2025**, *43*, sxae070. [CrossRef]
83. Mukhare, R.; Gandhi, K.A.; Kadam, A.; Raja, A.; Singh, A.; Madhav, M.; Chaubal, R.; Pandey, S.; Gupta, S. Integration of organoids with CRISPR screens: A narrative review. *Biol. Cell* **2025**, *117*, e70006. [CrossRef]
84. Geurts, M.H.; Clevers, H. CRISPR engineering in organoids for gene repair and disease modelling. *Nat. Rev. Bioeng.* **2023**, *1*, 32–45. [CrossRef]
85. Torrens-Mas, M.; Perelló-Reus, C.; Navas-Enamorado, C.; Ibargüen-González, L.; Sanchez-Polo, A.; Segura-Sampedro, J.J.; Masmiquel, L.; Barcelo, C.; Gonzalez-Freire, M. Organoids: An emerging tool to study aging signature across human tissues. modeling aging with patient-derived organoids. *Int. J. Mol. Sci.* **2021**, *22*, 10547. [CrossRef] [PubMed]
86. Li, S.; Agudelo Garcia, P.A.; Aliferis, C.; Becich, M.J.; Calyeca, J.; Cosgrove, B.D.; Elisseeff, J.; Farzad, N.; Fertig, E.J.; Glass, C. Advancing Biological Understanding of Cellular Senescence with Computational Multiomics. *Nat. Genet.* **2025**, *57*, 2381–2394. [CrossRef]
87. Xue, C.; Sun, F.; Zhu, H.; Shi, J.; Wang, J.; Sun, Q.; Tong, M.; Gu, X.; Han, Q.; Shi, W. Advancements, strategies, and challenges in organoid-based drug evaluation for tissue engineering and regenerative medicine. *Engineering*, 2025, *in press*. [CrossRef]
88. Su, Y.; Chen, Y.; Wei, G.; Shi, Y. Organoid-based novel technology for antitumor drug screening. *View* **2025**, *6*, 20250107. [CrossRef]
89. Glaviano, A.; Foo, A.S.; Lam, H.Y.; Yap, K.C.; Jacot, W.; Jones, R.H.; Eng, H.; Nair, M.G.; Makvandi, P.; Geoerger, B.; et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol. Cancer* **2023**, *22*, 138. [CrossRef]

90. Wu, Y.; Zhang, F.; Du, F.; Huang, J.; Wei, S. Combination of tumor organoids with advanced technologies: A powerful platform for tumor evolution and treatment response. *Mol. Med. Rep.* **2025**, *31*, 140. [CrossRef]
91. Li, T.; Yin, J.; Hao, Y.; Gao, W.; Li, Q.; Feng, Q.; Tao, B.; Hao, M.; Liu, Y.; Lin, C.; et al. Single-cell sequencing and organoids: Applications in organ development and disease. *Mol. Biomed.* **2025**, *6*, 134. [CrossRef]
92. Sun, J.; Huang, S.; Zhang, Q.; Lei, L.; Nie, H. Development of organoids for drug research and tissue regeneration. *Precis. Med. Eng.* **2025**, *2*, 100045. [CrossRef]
93. Cui, H.; Tang, D.; Garside, G.B.; Zeng, T.; Wang, Y.; Tao, Z.; Zhang, L.; Tao, S.I. Wnt signaling mediates the aging-induced differentiation impairment of intestinal stem cells. *Stem Cell Rev. Rep.* **2019**, *15*, 448–455. [CrossRef] [PubMed]
94. Trastus, L.A.; d'Adda di Fagagna, F. The complex interplay between aging and cancer. *Nat. Aging* **2025**, *5*, 350–365. [CrossRef] [PubMed]
95. Xie, Z.; Zhan, Q. The complex interplay between aging and cancer: Unraveling the clues. *Med. Rev.* **2024**, *4*, 2–4. [CrossRef]
96. Silverman, L.I.; Flanagan, F.; Rodriguez-Granrose, D.; Simpson, K.; Saxon, L.H.; Foley, K.T. Identifying and managing sources of variability in cell therapy manufacturing and clinical trials. *Regen. Eng. Transl. Med.* **2019**, *5*, 354–361. [CrossRef]
97. Wang, M.; Yu, H.; Zhang, T.; Cao, L.; Du, Y.; Xie, Y.; Ji, J.; Wu, J. In-Depth Comparison of Matrigel Dissolving Methods on Proteomic Profiling of Organoids. *Mol. Cell. Proteom.* **2022**, *21*, 100181. [CrossRef] [PubMed]
98. Minciullo, P.L.; Catalano, A.; Mandraffino, G.; Casciaro, M.; Crucitti, A.; Maltese, G.; Morabito, N.; Lasco, A.; Gangemi, S.; Basile, G. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. *Arch. Immunol. Ther. Exp.* **2016**, *64*, 111–126. [CrossRef]
99. Liu, K.; Chen, X.; Fan, Z.; Ren, F.; Liu, J.; Hu, B. From Organoids to Organoids-on-a-Chip: Current Applications and Challenges in Biomedical Research. *Chin. Med. J.* **2025**, *138*, 792–807. [CrossRef]
100. Zhang, S.; Wan, Z.; Kamm, R.D. Vascularized Organoids on a Chip: Strategies for Engineering Organoids with Functional Vasculature. *Lab A Chip* **2021**, *21*, 473–488. [CrossRef]
101. Cuenca, M.V.; Bulut, M.; Mummery, C.L.; Orlova, V.V. Vascularization of Organoid Microenvironments: Perfusable Networks for Organoid Growth and Maturation. *Curr. Opin. Biomed. Eng.* **2025**, *34*, 100586. [CrossRef]
102. LeSavage, B.L.; Suhar, R.A.; Broguiere, N.; Lutolf, M.P.; Heilshorn, S.C. Next-Generation Cancer Organoids. *Nat. Mater.* **2022**, *21*, 143–159. [CrossRef]
103. Zhou, Z.; Cong, L.; Cong, X. Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. *Front. Oncol.* **2021**, *11*, 762184. [CrossRef] [PubMed]
104. Schutgens, F.; Clevers, H. Human Organoids: Tools for Understanding Biology and Treating Diseases. *Annu. Rev. Pathol. Mech. Dis.* **2020**, *15*, 211–234. [CrossRef] [PubMed]
105. Tian, C.-M.; Yang, M.-F.; Xu, H.-M.; Zhu, M.-Z.; Yue, N.-N.; Zhang, Y.; Shi, R.-Y.; Yao, J.; Wang, L.-S.; Liang, Y.-J.; et al. Stem cell-derived intestinal organoids: A novel modality for IBD. *Cell Death Discov.* **2023**, *9*, 255. [CrossRef]
106. Meirelles, L.A.; Persat, A. Decoding host-microbe interactions with engineered human organoids. *EMBO J.* **2025**, *44*, 1569. [CrossRef] [PubMed]
107. Gebert, J.T.; Scribano, F.; Engevik, K.A.; Perry, J.L.; Hyser, J.M. Gastrointestinal organoids in the study of viral infections. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2023**, *324*, G51–G59. [CrossRef]
108. Wang, J.; Tao, X.; Zhu, J.; Dai, Z.; Du, Y.; Xie, Y.; Chu, X.; Fu, G.; Lei, Z. Tumor organoid-immune co-culture models: Exploring a new perspective of tumor immunity. *Cell Death Discov.* **2025**, *11*, 195. [CrossRef]
109. Harter, M.F.; Recaldin, T.; Gjorevski, N. Organoids as Models of Immune-Organ Interaction. *Cell Rep.* **2025**, *44*, 116214. [CrossRef]
110. Vijg, J. From DNA damage to mutations: All roads lead to aging. *Ageing Res. Rev.* **2021**, *68*, 101316. [CrossRef]
111. Anglada, T.; Repullés, J.; Espinal, A.; LaBarge, M.A.; Stampfer, M.R.; Genescà, A.; Martín, M. Delayed γH2AX foci disappearance in mammary epithelial cells from aged women reveals an age-associated DNA repair defect. *Aging* **2019**, *11*, 1510. [CrossRef]
112. Sarapultsev, A.; Gusev, E.; Komelkova, M.; Utepova, I.; Luo, S.; Hu, D. JAK-STAT Signaling in Inflammation and Stress-Related Diseases: Implications for Therapeutic Interventions. *Mol. Biomed.* **2023**, *4*, 40. [CrossRef]
113. Evans, M.A.; Walsh, K. Clonal Hematopoiesis, Somatic Mosaicism, and Age-Associated Disease. *Physiol. Rev.* **2023**, *103*, 649–716. [CrossRef] [PubMed]
114. Mejia-Ramirez, E.; Florian, M.C. Understanding Intrinsic Hematopoietic Stem Cell Aging. *Haematologica* **2019**, *105*, 22. [CrossRef]
115. Fuster, J.J.; MacLauchlan, S.; Zuriaga, M.A.; Polackal, M.N.; Ostriker, A.C.; Chakraborty, R.; Wu, C.-L.; Sano, S.; Muralidharan, S.; Rius, C. Clonal Hematopoiesis Associated with TET2 Deficiency Accelerates Atherosclerosis Development in Mice. *Science* **2017**, *355*, 842–847. [CrossRef] [PubMed]
116. Avagyan, S.; Zon, L.I. Clonal Hematopoiesis and Inflammation–the Perpetual Cycle. *Trends Cell Biol.* **2023**, *33*, 695–707. [CrossRef]
117. Cobo, I.; Tanaka, T.; Glass, C.K.; Yeang, C. Clonal Hematopoiesis Driven by DNMT3A and TET2 Mutations: Role in Monocyte and Macrophage Biology and Atherosclerotic Cardiovascular Disease. *Curr. Opin. Hematol.* **2022**, *29*, 1–7. [CrossRef]
118. Artegiani, B.; Hendriks, D. Organoids from Pluripotent Stem Cells and Human Tissues: When Two Cultures Meet Each Other. *Dev. Cell* **2025**, *60*, 493–511. [CrossRef]

119. Sibilio, P.; De Smaele, E.; Paci, P.; Conte, F. Integrating multi-omics data: Methods and applications in human complex diseases. *Biotechnol. Rep.* **2025**, *48*, e00938. [CrossRef]
120. Baião, A.R.; Cai, Z.; Poulos, R.C.; Robinson, P.J.; Reddel, R.R.; Zhong, Q.; Vinga, S.; Gonçalves, E. A technical review of multi-omics data integration methods: From classical statistical to deep generative approaches. *Brief. Bioinform.* **2025**, *26*, bbaf355. [CrossRef]
121. Kester, L.; Van Oudenaarden, A. Single-Cell Transcriptomics Meets Lineage Tracing. *Cell Stem Cell* **2018**, *23*, 166–179. [CrossRef]
122. Kim, D.; Youn, J.; Kim, J.; Lee, J.; Yoon, J.; Kim, D.S. From Organoid Culture to Manufacturing: Technologies for Reproducible and Scalable Organoid Production. *npj Biomed. Innov.* **2026**, *3*, 12. [CrossRef]
123. Stuart, T.; Satija, R. Integrative Single-Cell Analysis. *Nat. Rev. Genet.* **2019**, *20*, 257–272. [CrossRef] [PubMed]
124. Larsson, L.; Frisén, J.; Lundeberg, J. Spatially Resolved Transcriptomics Adds a New Dimension to Genomics. *Nat. Methods* **2021**, *18*, 15–18. [CrossRef]
125. Longo, S.K.; Guo, M.G.; Ji, A.L.; Khavari, P.A. Integrating Single-Cell and Spatial Transcriptomics to Elucidate Intercellular Tissue Dynamics. *Nat. Rev. Genet.* **2021**, *22*, 627–644. [CrossRef]
126. Ritsma, L.; Ellenbroek, S.I.; Zomer, A.; Snippert, H.J.; De Sauvage, F.J.; Simons, B.D.; Clevers, H.; Van Rheenen, J. Intestinal Crypt Homeostasis Revealed at Single-Stem-Cell Level by In Vivo Live Imaging. *Nature* **2014**, *507*, 362–365. [CrossRef]
127. Fan, R. Single-Cell and Spatial Omics for Mapping Cellular Senescence in Health, Aging And Disease. *Innov. Aging* **2023**, *7*, 473. [CrossRef]
128. Azani, A.; Sharafi, M.; Doachi, R.; Akbarzadeh, S.; Lorestani, P.; Haji Kamanaj Olia, A.; Zahed, Z.; Gharedaghi, H.; Ghasrsaz, H.; Foroozand, H.; et al. Applications of CRISPR-Cas9 in Mitigating Cellular Senescence and Age-Related Disease Progression. *Clin. Exp. Med.* **2025**, *25*, 237. [CrossRef]
129. Lamprecht, J.; Sanad, S.A.; Kusumbe, A.P.; Habib, S.J. The Wnt–NAD+ Axis in Cancer, Aging, and Tissue Regeneration. *Trends Cell Biol.* **2025**, *36*, 154–174. [CrossRef]
130. Surina, S.; Chmielewska, A.; Pratscher, B.; Freund, P.; Rodríguez-Rojas, A.; Burgener, I.A. Organ-on-a-Chip: A Roadmap for Translational Research in Human and Veterinary Medicine. *Int. J. Mol. Sci.* **2025**, *26*, 10753. [CrossRef]
131. Cai, H.; Wang, D.; Zhao, Y.; Yang, C. Recent Advances in Microfluidic Chip Technology for Laboratory Medicine: Innovations and Artificial Intelligence Integration. *Biosensors* **2026**, *16*, 104. [CrossRef] [PubMed]
132. Fan, X.; Hou, K.; Liu, G.; Shi, R.; Wang, W.; Liang, G. Strategies to overcome the limitations of current organoid technology-engineered organoids. *J. Tissue Eng.* **2025**, *16*, 20417314251319475. [CrossRef] [PubMed]
133. Shroff, H.; Testa, I.; Jug, F.; Manley, S. Live-cell imaging powered by computation. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 443–463. [CrossRef]
134. Rabinowitz, Z.M.; Cui, L. Detecting Cellular Senescence In Vivo: Imagining Imaging Better. *Aging Cancer* **2023**, *4*, 97–110. [CrossRef]
135. Zhang, X.; Gao, Y.; Zhang, S.; Wang, Y.; Pei, X.; Chen, Y.; Zhang, J.; Zhang, Y.; Du, Y.; Hao, S. Mitochondrial Dysfunction in the Regulation of Aging and Aging-Related Diseases. *Cell Commun. Signal.* **2025**, *23*, 290. [CrossRef]
136. Bai, L.; Wu, Y.; Li, G.; Zhang, W.; Zhang, H.; Su, J. AI-Enabled Organoids: Construction, Analysis, and Application. *Bioact. Mater.* **2024**, *31*, 525–548. [CrossRef] [PubMed]
137. Wen, J. Refining the generation, interpretation and application of multi-organ, multi-omics biological aging clocks. *Nat. Aging* **2025**, *5*, 1897–1913. [CrossRef]
138. Zhu, S.; Hua, H.; Chen, S. Rigorous integration of single-cell ATAC-seq data using regularized barycentric mapping. *Nat. Mach. Intell.* **2025**, *7*, 1461–1477. [CrossRef]
139. Bogoslowski, A.; An, M.; Penninger, J.M. Incorporating immune cells into organoid models: Essential for studying human disease. *Organoids* **2023**, *2*, 140–155. [CrossRef]
140. Abbasian, M.H.; Sobhani, N.; Sisakht, M.M.; D'Angelo, A.; Sirico, M.; Roudi, R. Patient-derived organoids: A game-changer in personalized cancer medicine. *Stem Cell Rev. Rep.* **2025**, *21*, 211–225. [CrossRef]
141. Zhu, Z.; Cheng, Y.; Liu, X.; Ding, W.; Liu, J.; Ling, Z.; Wu, L. Advances in the development and application of human organoids: Techniques, applications, and future perspectives. *Cell Transplant.* **2025**, *34*, 09636897241303271. [CrossRef] [PubMed]
142. Wang, D.; Villenave, R.; Stokar-Regenscheit, N.; Clevers, H. Human organoids as 3D in vitro platforms for drug discovery: Opportunities and challenges. *Nat. Rev. Drug Discov.* **2026**, *25*, 204–226. [CrossRef] [PubMed]
143. Balkhair, O.; Albalushi, H. Artificial Intelligence in Organoid-Based Disease Modeling: A New Frontier in Precision Medicine. *Biomimetics* **2025**, *10*, 845. [CrossRef]
144. Kolesnichenko, M. Rejuvenating the aging gut by targeting senescence: Organ rejuvenation. *Nat. Aging* **2026**, *6*, 32–33. [CrossRef] [PubMed]
145. Zhu, Y.; Tchkonia, T.; Pirtskhalava, T.; Gower, A.C.; Ding, H.; Giorgadze, N.; Palmer, A.K.; Ikeno, Y.; Hubbard, G.B.; Lenburg, M. The Achilles' Heel of Senescent Cells: From Transcriptome to Senolytic Drugs. *Aging Cell* **2015**, *14*, 644–658. [CrossRef]
146. Takebe, T.; Wells, J.M. Organoids by Design. *Science* **2019**, *364*, 956–959. [CrossRef]

147. Bulcaen, M.; Kortleven, P.; Liu, R.B.; Maule, G.; Dreano, E.; Kelly, M.; Ensinck, M.M.; Thierie, S.; Smits, M.; Ciciani, M.; et al. Prime editing functionally corrects cystic fibrosis-causing CFTR mutations in human organoids and airway epithelial cells. *Cell Rep. Med.* **2024**, *5*, 101544. [CrossRef]
148. Kumar, A.; Thirumurugan, K. Understanding cellular senescence: Pathways involved, therapeutics and longevity aiding. *Cell Cycle* **2023**, *22*, 2324–2345. [CrossRef]
149. Hautefort, I.; Poletti, M.; Papp, D.; Korcsmaros, T. Everything you always wanted to know about organoid-based models (and never dared to ask). *Cell. Mol. Gastroenterol. Hepatol.* **2022**, *14*, 311–331. [CrossRef]
150. Takebe, T.; Wells, J.M.; Helmrath, M.A.; Zorn, A.M. Organoid center strategies for accelerating clinical translation. *Cell Stem Cell* **2018**, *22*, 806–809. [CrossRef] [PubMed]
151. Papp, D.; Korcsmaros, T.; Hautefort, I. Revolutionizing immune research with organoid-based co-culture and chip systems. *Clin. Exp. Immunol.* **2024**, *218*, 40–54. [CrossRef] [PubMed]
152. Tenreiro, M.F.; Muotri, A.R. Reconstructing human corticogenesis: Insights from cerebral organoids into neurodevelopment and disease modeling. *Dev. Cell*, 2026; *in press*. [CrossRef]
153. Mohammadi, S.; Morell-Perez, C.; Wright, C.W.; Wyche, T.P.; White, C.H.; Sana, T.R.; Lieberman, L.A. Assessing donor-to-donor variability in human intestinal organoid cultures. *Stem Cell Rep.* **2021**, *16*, 2364–2378. [CrossRef]
154. Yamauchi, S.; Takahashi, A. Cellular Senescence: Mechanisms and Relevance to Cancer and Aging. *J. Biochem.* **2025**, *177*, 163–169. [CrossRef] [PubMed]
155. Wolff, L.; Hendrix, S. Rethinking Matrigel: The Complex Journey to Matrix Alternatives in Organoid Culture. *Adv. Sci.* **2025**, *12*, e08734. [CrossRef]
156. Aisenbrey, E.A.; Murphy, W.L. Synthetic alternatives to Matrigel. *Nat. Rev. Mater.* **2020**, *5*, 539–551. [CrossRef]
157. Heinzelmann, E.; Piraino, F.; Costa, M.; Roch, A.; Norkin, M.; Garnier, V.; Homicsko, K.; Brandenberg, N. iPSC-derived and Patient-Derived Organoids: Applications and challenges in scalability and reproducibility as pre-clinical models. *Curr. Res. Toxicol.* **2024**, *7*, 100197. [CrossRef]
158. Murrow, L.M.; Weber, R.J.; Gartner, Z.J. Dissecting the stem cell niche with organoid models: An engineering-based approach. *Development* **2017**, *144*, 998–1007. [CrossRef]
159. Zhao, Y.; Landau, S.; Okhovatian, S.; Liu, C.; Lu, R.X.Z.; Lai, B.F.L.; Wu, Q.; Kieda, J.; Cheung, K.; Rajasekar, S.; et al. Integrating organoids and organ-on-a-chip devices. *Nat. Rev. Bioeng.* **2024**, *2*, 588–608. [CrossRef]
160. Hofer, M.; Lutolf, M.P. Engineering Organoids. *Nat. Rev. Mater.* **2021**, *6*, 402–420. [CrossRef]
161. Ramesan, S.P.; Poonacha, J.B.; Pathirana, D.; Merkt, S.; Maass, C.; Hasenauer, J.; Reckzeh, E.S. Next-generation discovery: Empowering organoid research with machine learning, artificial intelligence, and mathematical modeling. *Trends Biotechnol.* **2026**. [CrossRef]
162. Yang, J.; Fischer, N.G.; Ye, Z. Revolutionising oral organoids with artificial intelligence. *Biomater. Transl.* **2024**, *5*, 372.
163. Suarez-Martinez, E.; Suazo-Sanchez, I.; Celis-Romero, M.; Carnero, A. 3D and organoid culture in research: Physiology, hereditary genetic diseases and cancer. *Cell Biosci.* **2022**, *12*, 39. [CrossRef] [PubMed]
164. Quintard, C.; Tubbs, E.; Jonsson, G.; Jiao, J.; Wang, J.; Werschler, N.; Laporte, C.; Pitaval, A.; Bah, T.-S.; Pomeranz, G. A Microfluidic Platform Integrating Functional Vascularized Organoids-on-Chip. *Nat. Commun.* **2024**, *15*, 1452. [CrossRef] [PubMed]
165. Ong, H.T.; Karatas, E.; Poquillon, T.; Grenci, G.; Furlan, A.; Dilasser, F.; Raffi, S.B.M.; Blanc, D.; Drimaracci, E.; Mikec, D.; et al. Digitalized organoids: Integrated pipeline for high-speed 3D analysis of organoid structures using multilevel segmentation and cellular topology. *Nat. Methods* **2025**, *22*, 1343–1354. [CrossRef] [PubMed]
166. Liu, H.; Zhang, X.; Liu, J.; Qin, J. Vascularization of engineered organoids. *BMEMat* **2023**, *1*, e12031. [CrossRef]
167. Shirure, V.S.; Hughes, C.C.; George, S.C. Engineering vascularized organoid-on-a-chip models. *Annu. Rev. Biomed. Eng.* **2021**, *23*, 141–167. [CrossRef] [PubMed]

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