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REVIEW ARTICLE

Bioengineered materials-driven construction of musculoskeletal organoids in aging research: Strategies, applications, and future perspectives

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Abstract

Age-related deterioration of the musculoskeletal (MSK) system drives loss of mobility, lower quality of life, and escalates healthcare costs in older adults. Organoids are three-dimensional, self-organizing constructs that recapitulate key aspects of tissue architecture and function, providing a promising platform to model human MSK aging with higher physiological relevance than conventional two-dimensional cultures and many animal models. Current reliance on decellularized extracellular matrices as scaffolds constrains reproducibility and limits the ability to tune biochemical and biophysical cues. In contrast, engineered matrices allow for precise control over composition, mechanics, and degradability, thereby supporting organoid formation, maturation, and the induction of aging-related phenotypes. This review specifically focuses on MSK organoids and presents a conceptual synthesis linking biomaterial parameters to core MSK aging hallmarks and functional validation assays. We synthesize current strategies for constructing aging MSK organoids and delineate biomaterials design principles to recapitulate aged MSK microenvironments. We examine the key structural, mechanical, and biochemical material properties that influence organoid formation and the establishment of an aging-related microenvironment. Finally, we discuss smart material platforms and strategies for multi-tissue integration, assessing their potential to facilitate the

exploration of mechanistic insights and therapeutic testing, as well as to enhance the accuracy and translational relevance of *in vitro* aging models.

Keywords: Aging model; Musculoskeletal organoid; Aging niches; *In vitro* senescence; Biomaterials

1. Introduction

Aging-related changes in the musculoskeletal (MSK) system are key contributors to decreased function, lower quality of life, and increased societal costs in older adults. Conditions such as osteoporosis, sarcopenia, and osteoarthritis (OA) are widespread and often occur together, creating a complex health challenge in elderly care.[1] While traditional models have greatly contributed to our understanding of MSK aging, they have significant drawbacks. Two-dimensional (2D) cell cultures do not mimic the three-dimensional (3D) environment, mechanical forces, or cell–cell and cell–matrix interactions found in native tissues, making it difficult to accurately reflect aging processes. Animal models, while useful, often differ from humans in genetics, anatomy, tissue microstructure, immune systems, and aging patterns, which hinders the translation of research into clinical practice. Therefore, new model systems that better emulate human MSK aging are needed to strengthen mechanistic understanding and accelerate therapeutic development.

As an emerging *in vitro* 3D model, organoids recapitulate key aspects of tissue architecture, cellular diversity, and functions by enabling stem or tissue-specific adult cells to self-organize and develop *in vitro*. Organoids provide a valuable platform for MSK research, as they can mimic essential features of tissues such as bone, cartilage, and muscle, encompassing extracellular matrix (ECM) formation, response to mechanical stress, and tissue-specific functions.[2] Specifically, aging MSK organoids are 3D tissue models designed to replicate the complex biological changes that occur in bone, cartilage/joint, muscle, tendon, ligament, and intervertebral disc tissues with age. This review also expands the scope to include craniofacial structures, such as the periodontal ligament, alveolar bone, and temporomandibular joint. These models allow researchers to study diseases such as osteoporosis and sarcopenia in a setting that closely resembles the natural tissue environment, offering a more physiologically relevant alternative to traditional 2D cell cultures[3]. Using patient-derived cells, native-like biochemical and biophysical cues, and dynamic culture conditions, MSK organoids provide a personalized model to explore how the MSK system ages, thereby advancing the development of new treatments for age-related conditions.

The application of organoids in aging research faces several challenges, primarily in replicating the complex and prolonged aging processes typical of living organisms. Existing models often fall short in mimicking aging-related conditions such as chronic inflammation, matrix stiffening, and stem cell decline.[4,5] Standard organoid culture methods lack critical elements that modulate aging, such as engineered ECM components and controlled oxidative/metabolic stress, highlighting the need for novel techniques to effectively drive and manage aging processes within organoids.[6]

Notably, several recent reviews have focused on aging organoids or MSK organoid models, but this current work differs from previous reviews in key aspects. Previous reviews on aging organoids provide broad overviews of organoid applications across tissues but lack a dedicated focus on MSK-specific aging mechanisms and biomaterial design principles.[2,7-9] In contrast, this review synthesizes biomaterial science, MSK aging biology, and organoid engineering, with a specific focus on how engineered matrix parameters (biochemical, biomechanical, structural) can be tuned to recapitulate aged MSK microenvironments. Rather than introducing a formal framework, we offer an organizing, materials-driven roadmap that maps matrix design variables to MSK aging hallmarks and associated functional validation assays, and we highlight tissue-specific construction strategies for physiologically relevant aging MSK organoid models.

Motivated by the significant gaps in current *in vitro* models of MSK aging, this review explores the emerging intersection of biomaterials, MSK organoids, and tissue assemblies in aging research. We summarize and integrate approaches for engineering aging-relevant MSK organoid systems across tissue types, and distill rational design principles to support mechanistic studies and therapeutic testing, ultimately aiming to improve the accuracy and translational relevance of *in vitro* MSK aging models.

2. Aging mechanisms and senescent organoid construction

2.1. Core aging mechanisms in MSK tissues

Aging of the MSK system involves a range of interconnected molecular and cellular changes that occur across multiple levels.[10] These changes can be broadly grouped into three

categories aligned with established hallmarks of aging, each reflecting the specific biological effects of the changes.

2.1.1. Primary hallmarks

Primary hallmarks are core drivers of aging, directly leading to cellular dysfunction. These include genomic instability, such as accumulated DNA damage, telomere shortening—which reduces the regenerative potential of stem cells—and epigenetic changes, like methylation drift in genes involved in bone, muscle, and cartilage formation.[11,12]

Cellular senescence emerges from multiple, interconnected mechanisms. Although activation of the DNA-damage response via the ataxia-telangiectasia mutated (ATM)/ataxia-telangiectasia and Rad-3 (ATR)–p53–p21 pathway can induce cell cycle arrest, this does not invariably lead to permanent exit from the cell cycle; sustained activation of p53 and p21 together with reinforcing signals is typically required to entrench the senescent state.[13,14] Mitochondrial DNA (mtDNA) mutations elevate reactive oxygen species (ROS) levels, thereby exacerbating DNA damage.[15] Oxidative stress activates the p38 mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways, promoting senescence-associated secretory phenotype (SASP) expression.[16] Abnormal mechanical stress can further induce ROS and senescence through multiple mechanosensing pathways, including the activation of the c-Jun N-terminal kinase pathway through Piezo1/transient receptor potential cation channel subfamily V member 4-mediated calcium influx.[17] In addition, inflammatory cytokines such as interleukin (IL)-1β and IL-6 enhance SASP production through signal transducer and activator of transcription 3-mediated positive feedback loops.[18,19]

2.1.2. Antagonistic hallmarks

Initially, antagonistic hallmarks may act as adaptive responses to tissue damage, but their sustained activation accelerates the aging process. Mitochondrial dysfunction, for instance, leads to ROS accumulation in muscle fibers and bone cells, and disrupted nutrient-sensing pathways (such as imbalances between mechanistic target of rapamycin activation and sirtuin 1 [SIRT1] inhibition) contribute to tissue loss and metabolic issues.[20] Age-related mtDNA damage further increases ROS production, triggering pathways that induce cell cycle arrest and SASP expression. Concurrently, reduced nicotinamide adenine dinucleotide (NAD^+) levels diminish SIRT1 activity, worsening inflammation and protein degradation signals, thereby hastening cellular aging.[21] The aging process is also influenced by gut microbiota dysbiosis, which reduces short-chain fatty acid production, thereby affecting muscle metabolism and inflammation through the gut–muscle axis and contributing to age-related decline via a "microbiota–metabolism–inflammation" mechanism.[22,23]

2.1.3. Integrative hallmarks

As the final outcomes of aging, integrative hallmarks of aging are associated with tissue decline and the emergence of senescent features. This includes the depletion of stem cells, such as increased quiescence of muscle satellite cells;[24] changes in intercellular communication, notably heightened inflammation involving factors like IL-6 and tumor necrosis factor alpha (TNF-α);[25] and remodeling of the ECM, such as collagen crosslinking and glycation in tendons and cartilage.[26,27] Together, these changes compromise tissue integrity and alter its mechanical properties (Figure 1A).

2.2. Engineering strategies of senescent MSK organoids

Creating aging MSK organoids that closely replicate natural physiological conditions requires a systematic approach. This involves selecting appropriate cell sources, developing effective methods to induce aging, and establishing a comprehensive validation system that assesses multiple phenotypic features. Table 1 shows a summary of the associations between the three categories of aging hallmarks and their manifestations in key MSK cell types, signaling pathways, and experimental readouts.

2.2.1. Cell source selection

In terms of cell sources, researchers typically use four main types: Primary MSK lineages, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and embryonic stem cells (ESCs) (Figure 1B). Primary MSK cells, such as myoblasts or osteoblasts from elderly donors, retain many *in vivo* aging characteristics but are limited by their reduced proliferative ability and inter-donor variability.[28,29] Differentiating stem cells into muscle or bone lineages, combined with gene editing tools such as clustered regularly interspaced short palindromic repeats to introduce aging-related mutations, offers a valuable method for creating genetic aging models.[30] In addition, MSCs and iPSC-derived mesenchymal progenitor cells are well-established sources for constructing cartilage organoids, providing versatile options for modeling aging-related cartilage degeneration.[31]

2.2.2. Senescence induction strategies

Four principal strategies are commonly used to induce aging phenotypes in organoids (Figure 1C). The first is genetic manipulation, such as using lentiviral-mediated overexpression of aging-associated regulators to induce cell cycle arrest and cellular senescence in organoids.[32] The second is genotoxic stressors, such as the application of ROS generators, like hydrogen peroxide, to recapitulate the mitochondrial dysfunction and redox imbalance observed in aged tissues.[33] The third is proliferative exhaustion,

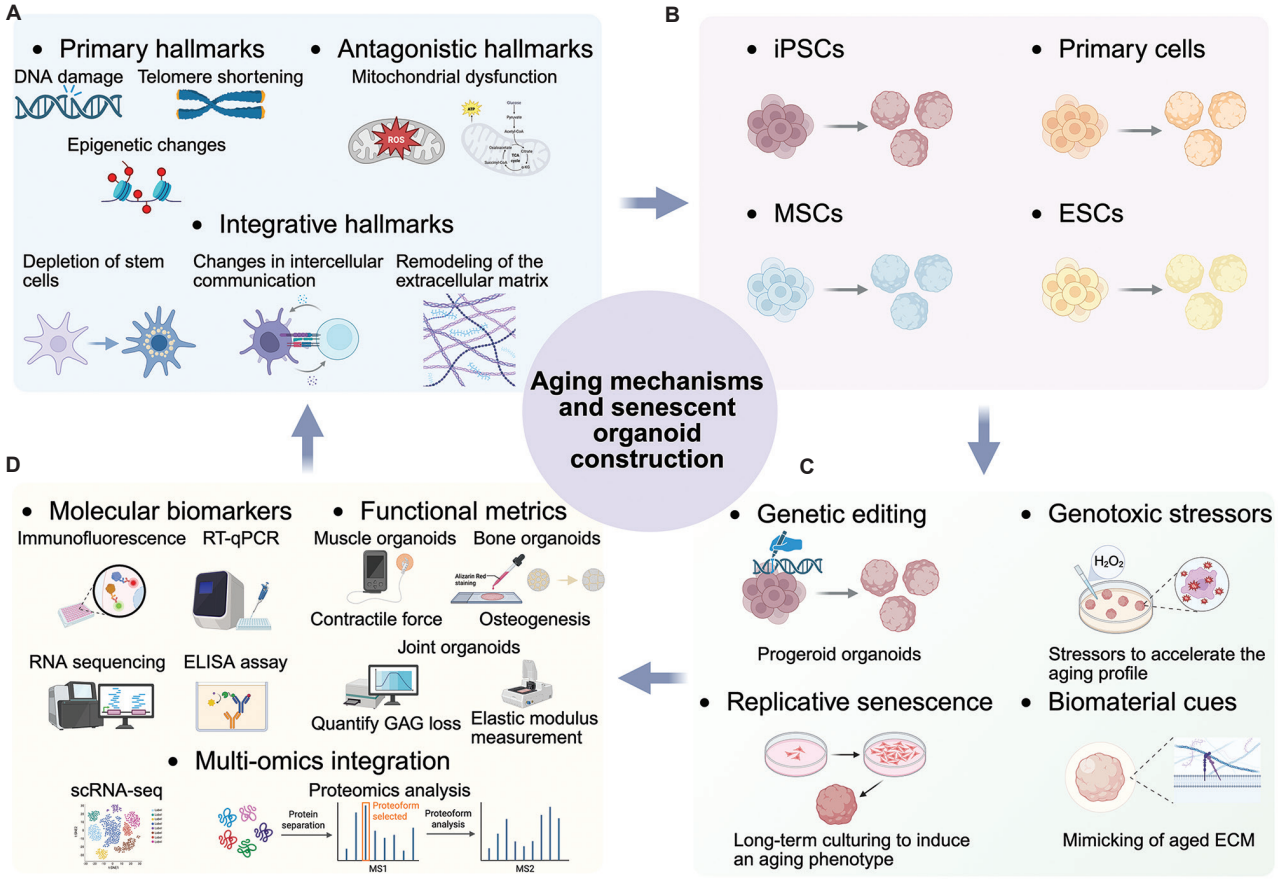

Figure 1. Mechanisms of aging and strategies for constructing senescent organoids. (A) Core aging mechanisms in MSK tissues. (B) Cell sources for aging organoids. (C) Senescence induction strategies. (D) Validation of senescent phenotypes. Created in BioRender. ZENG, Q. (2026) https://BioRender.com/d9x8vc2.

Abbreviations: ECM: Extracellular matrix; ELISA: Enzyme-linked immunosorbent assay; ESCs: Embryonic stem cells; GAG: Glycosaminoglycans; iPSC: Induced pluripotent stem cells; MSCs: Mesenchymal stem cells; MSK: Musculoskeletal; RT-qPCR: Real-time quantitative polymerase chain reaction; scRNA-seq: Single-cell RNA sequencing.

Table 1. Mapping of aging hallmark categories to their associated musculoskeletal cell types, core signaling pathways, and measurable readouts

Hallmark category	Specific MSK cell types	Core signaling pathways	Measurable readouts
Primary hallmarks	Osteoblasts, chondrocytes, muscle satellite cells, tenocytes	ATM/ATR–p53–p21, mtDNA-ROS, epigenetic methylation drift	SA-β-Gal activity, p16/p21 expression, telomere length, DNA damage foci, methylation-based aging clocks
Antagonistic hallmarks	Osteocytes, myocytes, chondrocytes	mTOR/SIRT1, NF-κB, p38 MAPK	ROS levels, NAD^+/NADH ratio, inflammatory cytokine (IL-1β, IL-6) secretion, nutrient-sensing efficiency
Integrative hallmarks	Mesenchymal stem cells, synovial fibroblasts, tenocytes	STAT3, JNK, YAP/TAZ	Stem cell quiescence rate, ECM stiffness, collagen crosslinking degree, tendon/ligament elastic modulus, SASP factor profile

Abbreviations: ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia and Rad3-related; ECM: Extracellular matrix; IL: Interleukin; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; mTOR: Mechanistic target of rapamycin; mtDNA: Mitochondrial DNA; NAD^+: Nicotinamide adenine dinucleotide (oxidized form); NADH: Nicotinamide adenine dinucleotide (reduced form); NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: Reactive oxygen species; SA-β-Gal: Senescence-associated beta-galactosidase; SASP: Senescence-associated secretory phenotype; SIRT1: Sirtuin 1; STAT3: Signal transducer and activator of transcription 3; YAP: Yes-associated protein; TAZ: Transcriptional coactivator with PDZ-binding motif.

such as extended *in vitro* passaging to induce replicative senescence and associated functional decline.[34,35] The fourth approach is biomechanical induction. Culturing organoids in stiff hydrogels or on rigid substrates replicates aged-tissue mechanics and activates integrin–focal adhesion kinase/Src and RhoA–Rho-Rho kinase signaling.[36,37] This raises

cytoskeletal tension, drives the nuclear translocation of Yes-associated protein and transcriptional coactivator with PDZ-binding motif and linker of nucleoskeleton and cytoskeleton-mediated nuclear deformation, impairs DNA repair, increases mitochondrial stress and ROS, and triggers senescence programs in MSK cells.[38,39] These approaches may be applied alone or in combination to model discrete aspects of aging and to facilitate mechanistic studies and therapeutic testing.

Biomechanical induction is preferred for modeling age-related matrix stiffening in cartilage, tendon, or bone tissues, as it recapitulates physiological mechanical cues without disrupting lineage fidelity.[40,41] ROS-based genotoxic stress is suitable for mimicking oxidative damage-driven senescence in muscle or chondrocytes but requires careful dosage control to avoid acute cytotoxicity that precludes stable senescence.[42,43] Proliferative exhaustion is ideal for long-term aging studies but should be limited to 10–15 passages for MSCs or myoblasts to prevent alterations in lineage commitment.[44,45] Genetic manipulation offers precise control over aging-related pathways and is often combined with biomechanical or genotoxic cues to enhance physiological relevance.[46,47] Potential artifacts include non-physiological senescence induced by supraphysiological hydrogen peroxide concentrations, lineage drift from excessive passaging, and artificial stiffening-related cellular responses that do not recapitulate *in vivo* aging dynamics. These concerns can be mitigated through rigorous validation against senescence biomarkers using functional assays specific to the target MSK tissue.[48,49]

2.3. Validation of senescent phenotypes

For phenotypic validation, a comprehensive multi-level assessment system is essential. At the molecular level, key markers such as senescence-associated β-galactosidase (SA-β-Gal) activity, p16 and p21 expression, profiles of SASP factors, and tissue-specific marker expression should be evaluated.[50] Functionally, organoids should be tested for reduced contractility in muscle models, disrupted mineralization in bone models, and loss of glycosaminoglycans (GAG) along with weakened mechanical properties in cartilage models. At the systems level, single-cell RNA sequencing (scRNA-seq) can reveal cellular heterogeneity, while proteomics analysis can identify patterns of ECM degradation, collectively providing a thorough characterization of the senescent state within the organoids[7] (Figure 1D). Genomic and epigenetic analyses provide powerful tools for strengthened validation. Techniques such as DNA methylation profiling (using Illumina EPIC arrays or bisulfite sequencing) and the application of methylation-based aging clocks enable the detection of durable, genome-wide aging signatures. These signatures complement transcriptomic and proteomic readouts, distinguishing true epigenetic aging from transient stress responses.[51,52]

2.3.1. Molecular biomarkers

The established organoid models must be validated at the molecular level to ensure that they accurately replicate *in vivo* aging characteristics. Analysis of SA-β-Gal is used to detect increased lysosomal activity, a hallmark of aging, and is a classic method for identifying senescent cells.[50] This is typically measured through staining and spectral analysis. Second, transcriptional profiling techniques, such as quantitative real-time polymerase chain reaction or RNA-seq, are employed to assess the expression of cell cycle inhibitors like $p16^{INK4A}$ and $p21^{CIP1}$, confirming the activation of critical aging pathways.[19] Moreover, a comprehensive evaluation of the SASP factors (including IL-1β, matrix metalloproteinase [MMP] 3, and transforming growth factor beta [TGF-β]) using multiplex immunoassays like Luminex or enzyme-linked immunosorbent assay (ELISA) is crucial for gauging their expression and secretion levels, which reflect inflammatory and matrix degradation states in the aging microenvironment.[53] Multidimensional, quantitative validation of these molecular markers provides a robust biological foundation for the applications of aging organoid models, ensuring their reliability and relevance in mimicking the aging process.

2.3.2. Functional metrics

Functional validation is a critical aspect of assessing the physiological relevance of aging organoid models, and it requires tissue-specific testing protocols. For muscle organoids, using electrical stimulation devices to induce contraction enables the measurement of maximum contractile force and fatigue tolerance, while microforce sensors can capture force decay curves, providing important information on muscle dysfunction observed in sarcopenia patients.[35] In bone organoids, Alizarin red staining can be used to quantify the area of calcified nodules, and dynamic osteogenesis assays can enable the assessment of possible declines in mineralization rates, which are key indicators of reduced bone formation capacity associated with aging.[54] For cartilage organoids, dimethylmethylene blue can be used to quantify the loss of GAGs. Atomic force microscopy,[55] microcompressometers,[56] and nanoindentation[57] can be employed to measure aging-associated changes in the elastic modulus of the organoids. These pathological changes mimic the degeneration characteristics seen in OA. The above functional tests, when combined with molecular biomarker analyses, form a comprehensive system for validating aging phenotypes. Functional ECM peptide assays can be employed to quantify markers of matrix turnover and damage, such as C-telopeptide (collagen degradation fragments), N-terminal propeptide, and matrix-derived D- and F-fragment peptides.[58,59] These

biochemical readouts provide sensitive, quantitative measures of ECM remodeling that complement histological and biomechanical evaluations. This system spans molecular changes to functional impairments, enabling a thorough understanding of the aging process in organoid models.

2.3.3. Multi-omics integration

System-level phenotypic validation aims to elucidate the intricate molecular networks and cellular dynamics within aging organoid models by integrating multi-omics data, thereby overcoming the limitations of single-marker analysis to provide comprehensive biological insights. ScRNA-seq is a powerful tool that can be used to resolve cellular heterogeneity at the single-cell level, uncovering aging-related transcriptomic features, such as those associated with stem cell depletion and altered fate determination.[60] For example, in aging muscle organoids, scRNA-seq can be used to identify expression patterns of genes related to cell cycle arrest in satellite cells. In aging bone organoids, this technique may reveal osteoblast subpopulations transitioning into senescent precursors.

Moreover, proteomics analysis, especially quantitative proteomics focusing on ECM components, can be employed to systematically identify signatures of aging-related matrix degradation. This includes increased collagen crosslinking, accumulation of elastin degradation products, and elevated MMP activity. This data can not only support transcriptomic findings but also construct comprehensive regulatory networks linking gene expression to protein function.[61,62] In addition, epigenetic aging assays and established longevity biomarkers, such as genome-wide DNA-methylation profiling using Illumina 450K/EPIC arrays or whole-genome bisulfite sequencing, enable the application of validated epigenetic clocks (e.g., Horvath, PhenoAge, GrimAge, and bespoke *in vitro* clocks) to quantify biological age and clock acceleration in organoids.[63,64]

By integrating differentially expressed genes from scRNA-seq with key pathway changes detected through proteomics, we can construct molecular network maps of aging. This approach provides a detailed multidimensional view of MSK aging mechanisms and enables thorough validation of organoid models' aging phenotypes from a systems biology perspective.

3. Material parameter optimization for recapitulating aging microenvironments

Developing organoid models that faithfully replicate aging tissues often requires meticulous control of their matrix material properties. By precisely tuning parameters such as stiffness, porosity, degradation rate, and biochemical composition, researchers can mimic the dynamic tissue changes that occur naturally with age. This careful regulation ensures that the microenvironment within the organoid accurately reflects the evolving characteristics of aging tissues, creating a more realistic and effective platform for studying age-related biological processes.

3.1. Biochemical properties

Accurately modulating the biochemical properties of biomaterials is essential for constructing organoid models that faithfully replicate age-related alterations in tissue ECM and the dynamic distribution of soluble signals within the aging microenvironment (Figure 2A).

3.1.1. ECM composition

Senescent tissues exhibit characteristic changes in their ECM composition, which can actively transmit pro-senescence signals to resident cells.[68] Recreating these biochemical features within *in vitro* materials offers a strategic approach to actively induce organoids into a senescent state, thereby providing a physiologically relevant platform for studying tissue aging and senescence mechanisms. For example, modifying the ratio of collagen I to collagen III can effectively replicate the reduction in fiber size and loss of mechanical integrity observed in tendon aging.[26] Similarly, in cartilage organoids, reducing the content of GAGs or hyaluronic acid can mimic key features of degeneration, such as proteoglycan loss and decreased water retention.[69,70]

Pericellular matrix (PCM), the specialized matrix immediately surrounding individual MSK cells such as chondrocytes and osteocytes, differs compositionally and mechanically from the bulk ECM and critically regulates mechanotransduction, nutrient diffusion, and local growth factor presentation.[71,72] Reproducing PCM features, such as perlecan, hyaluronan, collagen VI content, and local stiffness, in organoid models, therefore, enhances physiological fidelity and modulates cell susceptibility to senescence.[73]

3.1.2. Soluble factor gradients

Throughout the aging process, the spatial distribution and temporal dynamics of signaling molecules, including growth factors, cytokines, and chemokines, undergo substantial changes.[74,75] These signaling changes play a pivotal role in dictating cellular fate decisions, encompassing proliferation, differentiation, and senescence. Precise *in vitro* manipulation of the release kinetics of such factors within biomaterials offers a promising strategy to actively induce or accelerate aging phenotypes in organoid models, thereby enhancing their physiological relevance in the study of tissue aging.

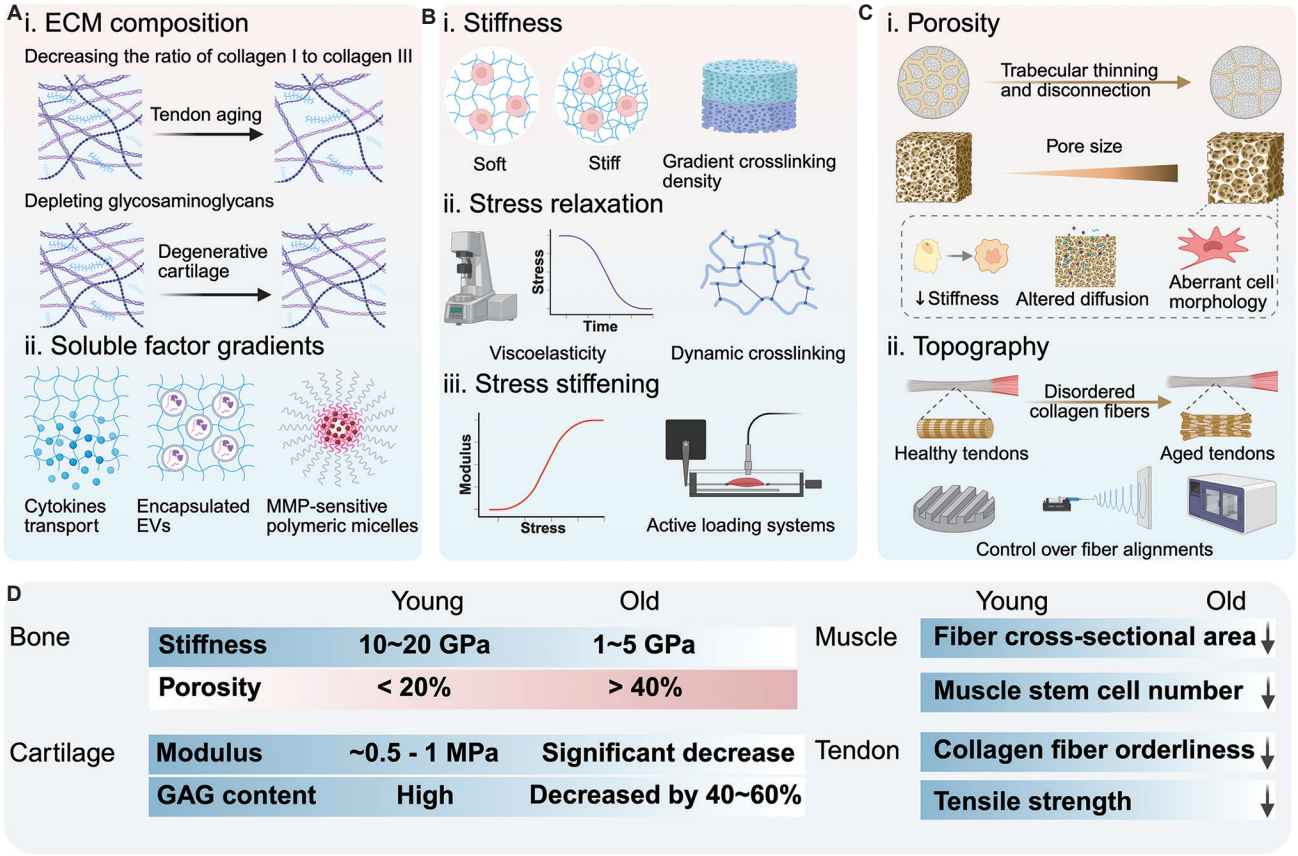

Figure 2. Modulation of material parameters for aging microenvironments. (A) Biochemical properties. (B) Biomechanical properties. (C) Structural properties. (D) Quantitative/semiquantitative comparison of material properties between young and aged tissues. Data adapted from references.[65-67] Created in BioRender. ZENG, Q. (2026) https://BioRender.com/cwou8ar.
Abbreviations: ECM: Extracellular matrix; EVs: Extracellular vesicles; MMP: Matrix metalloproteinases.

Inflammaging is a fundamental hallmark of aging, wherein persistent low-grade inflammatory signals, such as IL-6, TNF-α, and IL-1β, can contribute to the induction of cellular senescence and drive the SASP.[76] In addition, the expression and activity levels of many growth factors, including fibroblast growth factors, hepatocyte growth factor, and vascular endothelial growth factor, are often dysregulated in aging tissues.[77,78] Disrupting their normal delivery patterns, such as changing dosage or timing, can interfere with tissue homeostasis and potentially promote or accelerate the aging process. Furthermore, controlled release of low-dose ROS inducers may cause varying degrees of oxidative damage and senescence.[79] Technologies such as microfluidic gradient generators and micro-patterned release systems are widely employed,[80] enabling controlled modulation and detection of soluble factor concentrations.

Microenvironment-responsive delivery systems (e.g., MMP-sensitive polymeric micelles) hold potential for hydrophobic drug delivery[81,82] and can be engineered to release SASP factors in organoid culture systems, enabling investigations into the bystander effect. In addition, coating materials with extracellular vesicles derived from senescent osteoblasts can be employed to mimic *in vivo* signaling amplification and enable long-range paracrine communication, offering a novel approach to studying the effects of SASP on neighboring cells.[83]

Inhibiting ECM fragmentation is essential when modeling aged microenvironments, as proteolytic matrikines drive inflammation and senescence.[84] Practical strategies to achieve this include the use of protease-resistant or tunable-cleavage biomaterials, as well as the application of decoy receptors or antibodies to neutralize bioactive fragments.[85,86] In addition, incorporating mechanisms that clear these fragments can further enhance the fidelity of aging models and enable the evaluation of interventions targeting fragment-driven inflammation.

Together, these biomaterial-based biochemical modulation strategies create a comprehensive framework that ranges from static modifications of ECM composition to dynamic regulation of signaling pathways, which can be leveraged to develop more realistic and functional senescent microenvironments in organoid models.

3.2. Biomechanical properties

The biomechanical properties of matrix materials directly influence cellular mechano-sensing and fate, as well as overall tissue function (Figure 2B). Therefore, it is critical to precisely control the materials' biomechanical properties when constructing organoid models of the aging microenvironment.

3.2.1. Stiffness

Matrix stiffness plays a crucial role in guiding cellular behavior and tissue function.[87] Aged or osteoarthritic cartilage often shows a 2-–10-fold increase in local PCM stiffness (typically ranging from ~10–100 kPa to several hundred kPa).[88,89] ECM stiffening is a hallmark of cartilage aging, and it is established that matrix mechanics directly influence chondrocyte function through epigenetic regulation.[90,91] By engineering hydrogels with varying stiffness to emulate young and aged cartilage environments, researchers found that culturing aged chondrocytes on softer matrices restored a youthful phenotype, suggesting that the effects of aging on chondrocytes were reversible.[92] Conversely, matrix stiffening promotes the methylation of the α-Klotho gene promoter through mechanotransduction pathways, leading to decreased α-Klotho expression and accelerated chondrocyte senescence.[93] These findings demonstrate that matrix mechanical properties, particularly stiffness, can modulate cellular aging states through epigenetic mechanisms. By fine-tuning the degree of photopolymerization in hydrogels like methacrylated gelatin, researchers can achieve precise, spatial-temporal control of matrix stiffness, allowing the replication of the increased tissue rigidity characteristic of age-related conditions like osteosclerosis.[94] Such hydrogel platforms facilitate detailed studies on how mechanical variations affect cellular aging mechanisms and mechanotransduction pathways, thereby enhancing our understanding of tissue degeneration during the aging process. Ultimately, these engineered systems offer crucial insights into the dynamic interplay between mechanical cues and cellular processes that drive age-associated tissue deterioration.

3.2.2. Stress relaxation

Stress relaxation properties regulate cell behaviors such as migration, proliferation, and differentiation.[95] Hydrogels with faster stress relaxation promote MSC migration, proliferation, and osteogenic differentiation, whereas hydrogels with slower relaxation restrict cellular activities.[96] Since cell proliferation is closely linked to aging, regulating cellular proliferative states by controlling stress relaxation can be leveraged to construct models of aging. In addition, rapidly relaxing matrices facilitate cell spreading and focal adhesion formation, which activates the Yes-associated protein signaling pathway to enhance cell proliferation and survival.[97] This mechanism highlights how mechanical cues influence the balance between cellular "youth," characterized by proliferation, and "aging," marked by cellular stasis. Materials with rapid stress relaxation tend to preserve stem cell pluripotency, whereas slower-relaxing matrices promote senescence.[98-100] Therefore, the latter are useful for studying mechano-memory, namely the persistence of phenotype induced by prior mechanical exposure, and associated cytoskeletal reorganization in aging tissues.[101,102]

Stress-relaxing hydrogels offer notable benefits in organoid culture by allowing tissues to undergo morphogenesis and growth in a more natural environment, as these flexible substrates reduce the physical constraints imposed by rigid materials, thereby enhancing the tissues' developmental potential.[103] Stress relaxation dynamics can be modulated by introducing and tuning dynamic covalent crosslinks, such as reversible borate bonds. Creating matrices with viscoelastic properties that mimic the ECM of aging tissues characterized by slower stress relaxation can prompt cells or organoids to display aging-related features like functional decline and decreased proliferation. However, there are few studies that directly establish a connection between material viscoelasticity and the development of aging organoid models.

3.2.3. Stress stiffening

Many biological soft-tissues, including skin, blood vessels, and the cytoskeleton, display stress stiffening behavior, serving as a crucial mechanical adaptation that helps prevent excessive deformation and tissue damage under physiological loads.[104] During aging, tissue ECM often undergoes compositional and structural changes that generally lead to increased tissue stiffness and a decrease in the tissue's mechanical adaptability.[105] Previous studies suggest that the stress stiffening response changes with age. For instance, aged blood vessels often become more brittle and exhibit diminished stress stiffening behavior, making them more susceptible to damage during blood pressure fluctuations.[106]

3D scaffolds that mimic the mechanical environment of aged tissues can be created by developing hydrogels with specific stress stiffening behaviors through modifications of crosslink density, network architecture, or the incorporation of responsive polymers. Organoid culture on such matrices could facilitate investigations into the timing and level of senescence marker expression, providing insights into the effects of age-related changes in tissue mechanical properties on cellular aging.

In dynamic culture systems, periodic stretching or compression is often applied to hydrogels with stress stiffening properties, causing the local stiffness experienced by organoid cells to fluctuate dynamically during loading.

This unique and potentially non-physiological, time-varying mechanical cue acts as a continuous source of stress, disrupting normal mechanotransduction pathways and potentially promoting or accelerating cellular senescence.

3.3. Structural properties

Accurately replicating the morphological and functional decline of aging tissues in biomimetic organoid models requires precise regulation of the material's structural properties (Figure 2C).

3.3.1. Porosity

The porosity and pore size of 3D scaffolds influence nutrient diffusion, cell migration, cell–cell interactions, and cells' perception of local matrix stiffness.[107] Porosity and pore size can be tuned to mirror aged tissue architecture, thereby influencing scaffold mechanics and cell behavior. Trabecular bone is intrinsically porous (roughly 50–90% depending on anatomical location), with osteoporotic degeneration producing further increases in porosity and trabecular separation.[108] Cortical bone displays low porosity in healthy adults (5–10%), which rises with aging and disease (Figure 2D).[109] Articular cartilage contains a large fraction of interstitial fluid (apparent porosity nd diseaseosteoporotic degeneration producing further increases in porosity and by organoid cel.[110] Tendons show low fibrillar-scale porosity (5–10%) under normal conditions, whereas tendinopathy is associated with increased matrix disruption and apparent porosity.[111,112] By adjusting scaffold pore structures, researchers could generate confined microenvironments with limited nutrient supply to organoids, thereby mimicking the stress conditions faced by cells in tissues with age-related reductions in perfusion (e.g., vascular rarefaction) and potentially promoting aging processes. However, few studies have explored this approach.

Furthermore, increasing the porosity in biomimetic trabecular bone scaffolds beyond 40% significantly decreases the compressive modulus and fatigue resistance,[113] which could be used to model microstructural damage and mechanical deterioration characteristic of osteoporosis. It is essential to recognize that porosity should not be viewed as the sole driver of complex disorders, such as osteoporosis, which are often caused by multiple contributing factors, including hormonal dysregulation, genetic susceptibility, and altered cell signaling. Instead, porosity should be treated as a tunable physical cue that directly affects cellular mechanotransduction, mass transport of nutrients and metabolites, cell–matrix interactions, and downstream cell fate decisions, which could be leveraged to mimic the functional decline observed in aging tissues. Systematic modulation of scaffold porosity allows investigators to dissect the specific mechanistic contributions of this design parameter to MSK aging within a broader, multifactorial context.

In addition, advanced fabrication techniques, such as 3D printing, can be employed to create Haversian canal-like structures with radial pore gradients,[114] effectively simulating the native bone microenvironment responsible for nutrient transport and cell migration.

3.3.2. Topography

Biomaterial topography, including features such as grooves, pillar arrays, and fibrous structures, influences cell behavior, encompassing morphology, polarity, nuclear deformation, and gene expression.[115] During aging, the tissue architecture and organization of tissue ECM are altered due to abnormal collagen crosslinking and fiber alignment within the ECM, compromising normal tissue function.[116]

Designing matrices that replicate the abnormal topologies of aging ECM could disrupt normal cellular signaling, induce stress, and promote aging phenotypes. Such altered topological characteristics may be more conducive to aging phenotypes compared to the structural features of young tissues.[117] Although some research has examined how topology influences stem cell differentiation, its application in constructing aging organoids is still in its early stages.

In young, healthy tendons, collagen fibers are highly ordered and aligned, while in aged tissues, these fibers become less orderly and exhibit increased crosslinking.[118] Techniques such as electrospinning, photolithography, and self-assembly enable precise control over fiber alignment and spatial distribution, allowing for the *in vitro* simulation of the transition from ordered fibers to a less ordered arrangement. Additionally, recent work has shown that force-triggered, rapid microstructure formation on hydrogel surfaces can direct cellular orientation and guide aligned growth.[119]

The above strategies, including the design of pore architectures and fiber topographies, establish an engineering framework that enables the construction of pro-aging microenvironments. This precise regulation of structural parameters provides a robust platform for investigating tissue degeneration and regenerative capacities during aging, thereby advancing our understanding of tissue aging processes and facilitating the development of anti-aging interventions.

4. Tissue-specific strategies for building aging MSK organoids

The current literature employs diverse terminology for 3D MSK culture systems, reflecting the spectrum of structural and functional complexity across these models.

In this review, an "organoid" is defined as a 3D construct derived from stem or progenitor cells that self-organize to recapitulate key structural and functional features of native MSK tissues. "Organoid-like models" refer to scaffold-supported engineered constructs that mimic tissue-specific aging phenotypes, relying on biomaterial scaffolds for structural integrity and microenvironmental regulation. "Microtissues" denote simplified 3D systems tailored to replicate discrete biological functions or pathological changes of aging MSK tissues. This classification aligns with the core characteristics of each model system included herein, facilitating clear discussion of their respective utility in aging research.

Aging of the MSK system presents as a spectrum of interrelated degenerative changes. In addition to common age-related diseases, including osteoporosis, OA, and sarcopenia, older adults also develop shoulder[120] and lumbar disorders, such as rotator cuff pathology[121] and intervertebral disc degeneration.[122] These conditions together contribute to a "mobility impairment syndrome" that undermines function, increases fall and fracture risk, and reduces quality of life.

The pathophysiological core of these conditions comprises ECM degradation and disorganization, establishment of the SASP, which includes both pro- and anti-inflammatory factors, chronic low-grade inflammation, and dysregulated mechanotransduction, culminating in progressive structural and functional decline.[123] To recapitulate this multifactorial pathology *in vitro*, biomaterial-based organoid technologies are emerging as a key solution. In this section, we examine models of osteoporosis, OA, and sarcopenia, emphasizing how biomimetic material design, rigorously defined co-culture architectures, and integrated dynamic mechanical and biochemical cues can recreate tissue-specific aging microenvironments and intercellular signaling to enable mechanistic investigations and therapeutic screening (Table 2).

Major MSK tissues differ fundamentally in ECM composition, structure, and mechanical properties. Bone relies on a highly mineralized matrix for its high rigidity, whereas cartilage forms a compression-resistant, viscoelastic matrix through the presence of proteoglycans and Type II collagen. Skeletal muscle and tendons derive functions from highly oriented muscle fibers or collagen bundles.[138] These differences dictate organoid design strategies: Bone organoids require high-stiffness, mineralizable materials such as hydroxyapatite (HAp)-doped hydrogels; muscle organoids prioritize topologies that guide cell alignment, for example, directionally electrospun fibers; joint-mimicking models must integrate soft–hard heterogeneous regions, which calls for scaffolds such as layered hydrogels.[9] Addressing these distinctions through material and structural design is essential for constructing physiologically relevant, tissue-specific aging organoids.

It is worth mentioning that craniofacial and periodontal models form an important extension of MSK model systems. *In vitro* periodontal ligament, alveolar bone, and temporomandibular joint constructs, established using periodontal ligament fibroblasts, osteoblast–osteoclast co-cultures, and mechanically loaded condylar cartilage, respectively, can recapitulate processes such as periodontitis-associated alveolar bone loss, root resorption, and temporomandibular joint degeneration.[139,140] These models can be employed to capture impaired orthodontic tooth movement driven by periodontal ligament fibroblast senescence and altered receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin signaling, and support quantitative readouts including resorption pits, MMP activity, and micro-computed tomography (μCT) bone loss.[141,142] Such platforms can enable the testing of senolytics, anti-proteolytic agents, and mechanotransduction modulators in clinically relevant craniofacial aging contexts, and the data generated have profound implications for MSK aging research and disease treatment.

4.1. Modeling bone aging using osteoporosis

4.1.1. Pathological hallmarks

Bone aging is characterized by reduced trabecular thickness and connectivity, increased trabecular separation, and an accumulation of microdamage and unrepaired microcracks, leading to higher fracture risks due to increased bone fragility.[143] At the cellular level, aging perturbs bone homeostasis by shifting the balance toward enhanced osteoclastic resorption and diminished osteoblastic formation, thereby promoting progressive structural degeneration.[144] Specifically, bone marrow-derived MSCs decline in number and shift toward adipogenic differentiation, while osteoblasts and osteocytes exhibit increased apoptosis and impaired functions, such as mechano-sensing and intercellular communication. At the same time, osteoclast activity is heightened, and vascularization, along with neurotrophic support, diminishes, contributing to overall skeletal deterioration.[144]

Research shows that DNA damage, telomere shortening, mitochondrial dysfunction, and epigenetic changes in aging bone tissue drive cells into irreversible senescence. These senescent cells secrete proinflammatory factors through the NF-κB pathway, which impairs osteogenesis and promotes osteoclast activity, resulting in bone mass decline.[145] In addition, aging-related mitochondrial dysfunction lowers the NAD^+/Nicotinamide adenine dinucleotide (reduced form) ratio, increases ROS, and worsens cellular damage. At the same time, key signaling pathways, such as the Wnt/

Table 2. Aging musculoskeletal organoids and organoid-like models

Tissue type	Cell source	Scaffold design	Induction strategies	Purpose/results of modeling aging	Category	References
Trabecular bone organoids	Primary female osteoblastic and osteoclastic cells	Human fibrin and micro-trabeculae	Exposure to analog microgravity	To simulate the bone loss process	Organoid-like	124
Trabecular bone organoids	Murine osteogenic cells and marrow cells	Demineralized bone pieces	RANKL, M-CSF, and osteogenic agents	To model localized bone remodeling by enhancing osteoclastic resorption while concurrently impairing osteoblastic bone formation	Organoid-like	125
Cartilaginous organoids	BMSCs	Scaffold-free	IL-1β induced inflammation	To better understand the transcriptomic changes in the organoids induced by the microenvironment	Organoid	126
Cartilage organoids	Human BMSCs	Scaffold-free	IL-1β, IL-6, and TNF-α induced inflammation	The inhibition of C/EBPβ-activating kinases could reverse the degradative processes	Organoid	38
Cartilage models	Chondrocytes	Gelatin–alginate scaffolds	200 μM hydrogen peroxide	3D models more closely mimicked the molecular characteristics of naturally aged human cartilage	Organoid-like	33
Cartilage organoids	Human primary articular chondrocytes	Collagen	Subjected to radiation and/or mechanical loading	To conduct rapid initial screening of anti-senescence compounds in a high-throughput manner	Organoid-like	127
Cartilage models	Human chondrocytes	PEG-based hydrogel	Mechanical compression; dexamethasone, IL-1Ra, rapamycin, celecoxib treatment	Mechanical overload induced OA phenotype in the tissue	Organoid-like	128
Cartilage models	Equine chondrocytes	Fibrin	Exposure to TNF-α and IL-1β and steroid (triamcinolone) treatment	To establish an *in vitro* OA model	Organoid-like	129
Cartilage and synovium models	Human articular chondrocytes and synovial fibroblasts	Fibrin	Synovial fluid from OA patients	To investigate monocyte extravasation to the synovium	Organoid-like	130
Osteochondral tissue models	Human iPSCs	Gelatin	IL-1β stimulation	The presence of bone aggravated cartilage degeneration	Organoid-like	31
Osteochondral tissue models	Human MSCs	Gelatin	IL-1β stimulation	IL-1β treatment of either bone or cartilage induced degenerative responses in the other tissue, suggesting their crosstalk	Organoid-like	131
Cartilage organoid	Synovial mesenchymal stromal cell	Scaffold-free	Hypoxic microenvironment	To model OA and test potential treatments	Organoid	132
Skeletal muscle	C2C12 cell	Gelatin	Cardiotoxin treatment	Cardiotoxin induced structural destruction and reduced passive tension in engineered muscle tissue	Microtissue	133
Skeletal muscle organoid	Human pluripotent stem cells	Scaffold-free	Chronic TNF-α treatment	To mimic a sarcopenia-like phenotype	Organoid	134
3D skeletal muscle model	MPC	Fibrin–Matrigel	Cardiotoxin	To study cell-autonomous changes in skeletal muscle during aging, regeneration, and intervention	Organoid-like	135

(Cont'd...)

Table 2. *(Continued)*

Tissue type	Cell source	Scaffold design	Induction strategies	Purpose/results of modeling aging	Category	References
3D skeletal muscle model	Primary human myoblasts	Collagen–Matrigel	Replicative senescence	To study aging and test drugs	Organoid-like	35
3D skeletal muscle model	Human myoblasts	Fibrin–Matrigel	TNF-α	To assess morphological and functional changes in the aging process of muscular tissue	Organoid-like	136
Skeletal muscle organoid	C2C12	dECM and Collagen	Shear-induced patterning and aging microenvironment	For advancing MSK regenerative therapies following traumatic injuries	Organoid-like	117
3D skeletal muscle model	Fibroblasts, MSC, and hSKMB	Collagen and Matrigel	Hydrogen peroxide	To study inflammatory and oxidative muscle injury induced by tumor-derived signals	Organoid-like	137

Abbreviations: BMSCs: Bone marrow–derived mesenchymal stem cells; C/EBPβ: CCAAT/enhancer-binding protein beta; dECM: Decellularized extracellular matrix; IL-1β: Interleukin-1 beta; IL-1Ra: Interleukin-1 receptor antagonist; IL-6: Interleukin-6; iPSCs: Induced pluripotent stem cells; M-CSF: Macrophage colony-stimulating factor; MPC: Muscle progenitor cells; MSCs: Mesenchymal stem cells; OA: Osteoarthritis; PEG: Polyethylene glycol; RANKL: Receptor activator of nuclear factor kappa-B ligand; TNF-α: Tumor necrosis factor alpha.

β-catenin, Hedgehog, Notch, TGF-β/bone morphogenetic protein, and fibroblast growth factor pathways, become less active with age, further disrupting bone formation and promoting bone resorption.[53]

Bone aging is marked by increased cellular senescence across multiple bone cell types, for example, upregulation of $p16^{INK4a}$, $p21^{CIP1}$, and p53 in osteoblasts, osteocytes, and marrow myeloid cells,[144,145] accompanied by elevated secretion of SASP factors (IL-6, IL-8, MCP-1, TNF-α) and common senescence-associated features such as telomere shortening, increased SA-β-gal activity, and mitochondrial ROS.[53]

To faithfully model human osteoporosis and bone aging *in vitro*, it is crucial to replicate their fundamental pathological features—namely, diminished or gradient mineralization and microstructural deterioration resulting from an imbalance between bone formation and resorption.[146] Creating such disease models involves integrating disease-specific stimuli into physiological systems to mimic cellular and molecular-level pathological changes.

4.1.2. Construction strategies

The core of osteoporosis is imbalanced bone remodeling, which can be modeled by simultaneously activating osteoclasts and inhibiting osteoblast activity through external stimuli, such as estrogen deficiency, RANKL/macrophage colony-stimulating factor overexpression, or simulated microgravity.[147]

Osteoporotic bone tissue shows uneven mineralization. In the trabecular bone organoids established by Iordachescu *et al.*,[124] density differences were used to identify compositional gradients (Figure 3A), highlighting the need for spatially controlled mineralization to mimic localized remodeling imbalances. To achieve this, researchers have proposed the use of gradient-mineralized materials to better replicate the complex microenvironment of osteoporotic bone.[146]

Gradient mineralization materials can be fabricated using chemical techniques that regulate mineral deposition and distribution to create spatial variations in mineral content. For example, controlling the mineralization process with simulated body fluid (SBF) or its variants enables the formation of gradients. Employing 10× concentrated SBF, a gradient from the bottom to the top of fiber mats was achieved by adjusting addition timing and tilt angle (Figure 3B),[148] and a similar gradient along the scaffold's length could be obtained by regulating the addition rate of 10× SBF and scaffold inclination (Figure 3C).[149] In addition, introducing amorphous calcium phosphate nanocluster suspensions into crosslinked poly(ethylene glycol)-dimethacrylate/hyaluronic acid hydrogels via gravity-driven permeation produced a continuous gradient of HAp content within the hydrogel matrix.[156]

Materials with gradient mineralization can also be fabricated using physical methods. Sequential layering of solutions at different concentrations, such as layer-by-layer coating of HAp and poly(lactic-co-glycolic acid) solutions or spin-coating of HAp/polycaprolactone (PCL) suspensions (Figure 3D), can create gradients of mineral content along the scaffold from bottom to top or along its length.[150,157] Gradients can also form via diffusion along polymer chains and solvent permeation. For instance, applying PCL solution onto HAp/PCL films induces HAp density gradients through swelling (Figure 3E).[151] Nanoparticle sedimentation offers another approach. HAp nanoparticles can be deposited within gelatin microspheres through gravitational sedimentation, with the penetration depth controlled by the preheating time and temperature.[158] In addition, single-axis stretching of polyvinyl alcohol/amorphous calcium phosphate films aligns polyvinyl

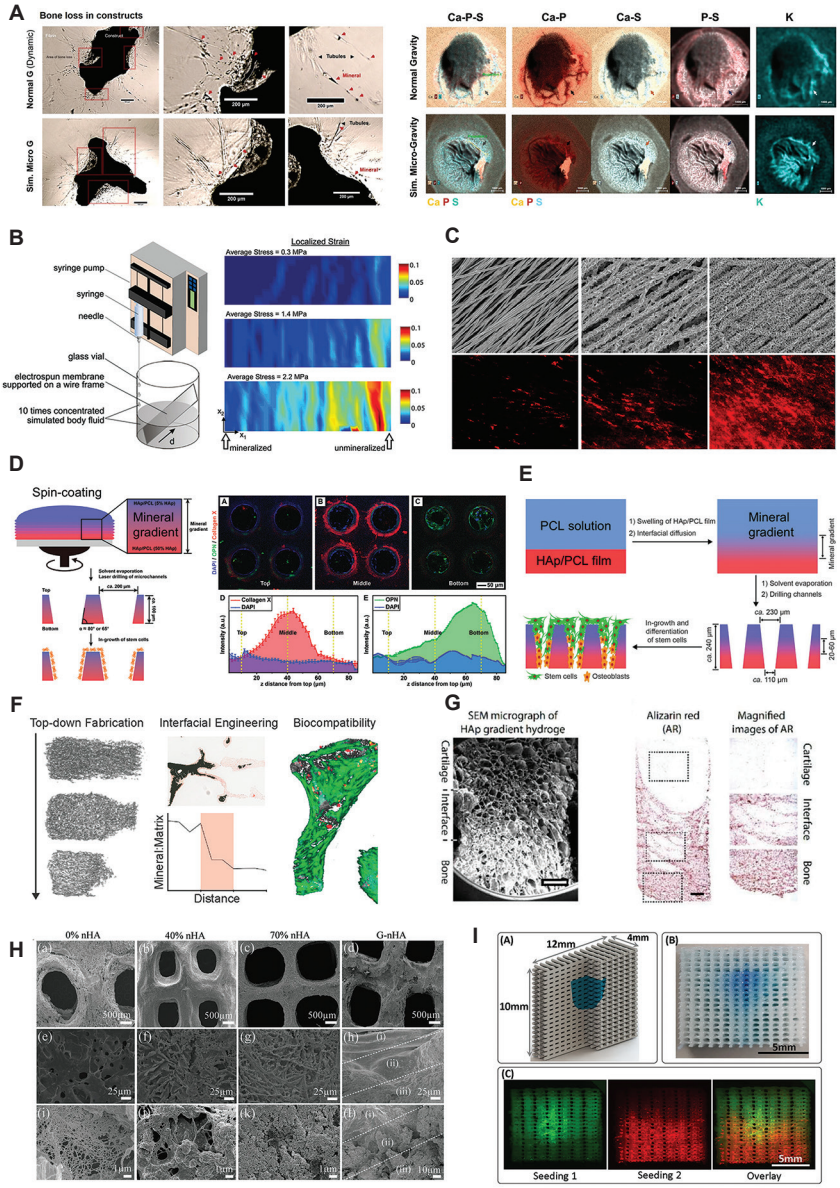

Figure 3. Bone organoids for modeling osteoporosis and strategies for generating gradient-mineralized materials. (A) Brightfield imaging and elemental mapping of trabecular bone organoids reveal mineralized tubular projections. Scale bars: Left panel: 200 μm; right panel: 1,000 μm. Reprinted from Iordachescu *et al.*[124] (B) A graded calcium-phosphate coating on electrospun nanofibers produces a mechanical gradient along the scaffold, enabling spatially graded mineralization. Reprinted with permission from Li *et al.*[148] Copyright © 2009, American Chemical Society. (C) SEM of plasma-treated PLGA nanofibers showing gradient calcium phosphate deposition with corresponding gradient in stained osteogenic markers. Scale bar: 1 μm. Reprinted with permission from Liu *et al.*[149] Copyright © 2014, American Chemical Society. (D) Schematic of a layer-by-layer PCL/HAp coating designed to create an osteogenic to chondrogenic differentiation gradient, with validation of human MSC chondrogenic and osteogenic outcomes in scaffolds with HAp gradient. Scale bar: 50 μm. Reprinted from Chen *et al.*[150] (E) Schematic of forming a biomimetic mineral gradient by swelling-induced interfacial diffusion in a PCL–HAp composite film. Reprinted with permission from Qiu *et al.*[151] Copyright © 2021, Wiley-VCH GmbH. (F) Top-down fabrication of interfacial, mineral-gradient scaffolds by spatially controlled demineralization of trabecular bone using a chelating solution for interfacial tissue engineering. Scale bars: 2 mm. Reprinted with permission from Boys *et al.*[152] Copyright © 2019, American Chemical Society. (G) Photocrosslinked carboxymethyl cellulose–sericin hydrogels bearing dual biochemical gradients that drive graded mineralization. Scale bar: 1 mm. Reprinted with permission from Mahajan *et al.*[153] Copyright © 2024, IOP Publishing Ltd. (H) SEM images of 3D-printed scaffolds with varying nano-HAp/hydrogel ratios illustrating an architecture with a mineral gradient. Scale bar: (a, b, c, d) 500 μm, (e, f, g, h) 25 μm, (i, j, k) 1 μm, (l) 10 μm. Reprinted with permission from Zhang *et al.*[154] Copyright © 2020, Wiley-VCH GmbH. (I) A hybrid additive manufacturing approach to generate bulk and surface composition gradients, with plasma treatment used to pattern two cell populations and produce spatially graded cellular organization. Scale bar: 5 mm. Reprinted from Sinha *et al.*[155] Collectively, these panels illustrate complementary top-down and bottom-up strategies for creating and validating mineral gradients relevant to osteoporosis modeling.

Abbreviations: 3D: Three-dimensional; HAp: Hydroxyapatite; MSC: Mesenchymal stem cell; PCL: Polycaprolactone; PLGA: Poly(lactic-co-glycolic acid); SEM: Scanning electron microscopy.

alcohol chains and enhances crystallinity, with amorphous calcium phosphate nanoparticles confined in oriented regions through nanoconfinement, resulting in a gradient structure.[159]

Biotechnology offers innovative pathways to construct gradient mineralization materials. For instance, controlling immersion time and depth in a demineralization solution, such as ethylenediaminetetraacetic acid, facilitates a gradient transition from fully mineralized to demineralized tissue (Figure 3F).[152] Similarly, creating mineralization gradients can be achieved through segmental immersion, which allows HAp at varying concentrations to be incorporated into hydrogels to form a HAp gradient.[160] Furthermore, by engineering dual pro-regeneration biochemical gradients through chemical modification of carboxymethyl cellulose and sericin for photo-crosslinking, researchers have developed hydrogels with distinct gradients targeted for cartilage and bone regeneration, thus advancing precision in tissue engineering (Figure 3G).[153]

Advanced manufacturing techniques enable the construction of gradient mineralization materials with precise control over material distribution and structure. For example, 3D printing was used to create a gradient nHA/hydrogel scaffold that accurately mimics the natural architecture of osteochondral tissue (Figure 3H).[154] In addition, continuous variation in material composition can be achieved by adjusting the dosage and mixing of two materials through a dual-material nozzle and controlling the gas pressure ratio. When combined with atmospheric pressure plasma jet technology, this method facilitated precise, localized surface modifications (Figure 3I).[155] Researchers have recently developed a mineralized bone model using 3D-bioprinting of human MSC–laden alginate–gelatin–graphene oxide hydrogels and culturing them under dynamic loading.[161] This combination of primary cells, biofabrication, and mechanical conditioning reproducibly yielded mineralized, cell-laden scaffolds suitable for modeling osteoporosis.

These approaches effectively replicate natural tissue mineralization gradients by precisely regulating various parameters during mineralization, offering strong support for tissue engineering and regenerative medicine. The produced structural and compositional gradients can result in corresponding variations in mechanical properties, providing cells with localized biochemical and mechanical cues. These gradient mineralization materials hold great promise for advancing tissue regeneration applications and modeling human osteoporosis and bone aging *in vitro*.

4.1.3. Role of scaffold materials

Biomaterial scaffolds that provide pathologically relevant 3D architecture and tunable niche signals are crucial to constructing osteoporosis-mimicking organoids. Rational scaffold design can be implemented to precisely recapitulate the diseased microenvironment by reproducing altered mechanical properties, including a reduced elastic modulus and modified viscoelastic and stress–relaxation behaviors of osteoporotic bone.[162] For example, by decellularizing and demineralizing scapular bone while reconstructing its intrinsic vessels, researchers generated a biomimetic 3D bone organoid that retained both porous trabecular and compact cortical microanatomy.[163] This provides an optimal niche for cell attachment and functional differentiation, recapitulates bone remodeling *in vitro*, and supports mineralization and vascularization, making it a valuable experimental platform for studying osteoporosis.

In parallel, scaffolds can be functionalized with pathological biochemical cues, such as SASP components and proteins in aged ECM, to emulate the molecular milieu of aged bone and thereby steer osteoblastic, osteoclastic, and stromal responses.[164,165] Engineering spatial gradients of HAp to create regions of graded mineralization reproduces the heterogeneous mechanical and biochemical cues found in osteoporotic bone; by varying HAp concentration and distribution within a scaffold, one can mimic focal areas of hypermineralization and demineralization, thereby modeling localized differences in stiffness, cell adhesion, and osteoclast/osteoblast activity that drive remodeling imbalances.[158]

Scaffolds provide a tunable platform to recapitulate pathological structural topography. Additive manufacturing techniques, including extrusion-based 3D printing, stereolithography, and electrospinning, enable precise control over pore size, strut thickness, and spatial anisotropy, allowing for the production of osteoporotic features such as gradient mineralization, reduced trabecular thickness, loss of connectivity, and altered porosity.[166] For example, 3D printing was used to create a gradient nHA/hydrogel scaffold that accurately mimics the natural architecture of osteochondral tissue.[154] Architected scaffolds could permit the systematic interrogation of how microstructural deterioration affects load distribution, cell–matrix interactions, and matrix remodeling. They can be combined with graded material properties or targeted biofunctionalization to emulate heterogeneous patterns of bone loss.[167]

4.1.4. Functional assessment

Validation of osteoporosis models requires a multi-tiered, quantitative assessment system. Mineralization deficits can be measured by analyzing calcified nodule area using alizarin red staining and bone volume fraction through μCT, including longitudinal μCT workflows to monitor mineralization changes and remodeling kinetics in the same constructs over time.[168] Expression of osteogenic

markers (e.g., osteocalcin, runt-related transcription factor 2) and resorption-related markers (e.g., Tartrate-resistant acid phosphatase, C-telopeptide-1) analyzed through ELISA or quantitative polymerase chain reaction can reveal imbalances in RANKL/osteoprotegerin signaling. Cellular senescence within MSC and osteoblast populations can be identified with SA-β-Gal staining and p16/p21 immunofluorescence.[169] Collectively, this integrated validation system can be utilized to ensure the faithful recapitulation of key aspects of osteoporotic pathology using an *in vitro* system for dissecting cellular interactions involved in skeletal aging and screening anti-osteoporotic therapeutics.

Aging bone organoid models combine biomaterials, defined cell populations, and biochemical cues to reconstruct the pathological 3D niche of aged bone. Beyond modeling osteoporosis, these platforms can be readily adapted to mimic other clinically important skeletal disorders. For example, organoid-based models of avascular necrosis of the femoral head can be established by inducing osteocyte death and impaired angiogenesis in vascularized bone organoids through hypoxia or exposure to excessive glucocorticoid to evaluate pro-angiogenic and regenerative therapies.[170,171] In addition, non-union fracture models could be produced by introducing senescent cells or SASP cocktails into scaffolds that mimic the callus environment to examine how chronic inflammation disrupts healing and test senolytic and pro-remodeling interventions. Thus, organoid models of bone aging and bone cell senescence are poised to rapidly extend beyond osteoporosis research, increasingly encompassing investigations into osteonecrosis, OA, non-union fractures, and bone–muscle comorbidities, offering physiologically relevant platforms for dissecting mechanisms underlying skeletal aging and for preclinical evaluation of novel therapeutics.

4.2. Modeling joint aging using OA

4.2.1. Pathological hallmarks

Joint aging can manifest as cartilage degradation, osteophyte formation, reduced synovial fluid quantity and quality, and progressive loss of tendon and ligament elasticity. Central to this pathophysiology is the accumulation of senescent resident cells within joint tissues (chondrocytes, synovial fibroblasts, and osteoblasts), which drives matrix catabolism, chronic inflammation, and impaired tissue regeneration. As joint cells age, they enter irreversible cell cycle arrest and secrete increased SASP factors, including proinflammatory cytokines such as IL-1 and IL-6, as well as ADAMTS5 and MMPs, notably MMP13. This secretion induces chronic inflammation, ECM degradation, and osteophyte formation, driving the onset and progression of degenerative conditions such as OA.[18,19,50]

At the cellular level, senescent chondrocytes show increased expression of $p16^{INK4a}$, p21, and p53, along with permanent cell cycle arrest caused by hypophosphorylation of the retinoblastoma protein.[172] In addition, mitochondrial dysfunction contributes to elevated levels of ROS, telomere shortening, cumulative DNA damage, and chromatin remodeling, resulting in senescence-associated heterochromatin formation.[18,50] Even under cell cycle arrest, senescent chondrocytes remain metabolically active and continue to secrete SASP factors. They also enhance local inflammation by transmitting senescence signals to nearby cells via extracellular vesicles that are rich in miR-34a and deficient in miR-140.[50]

Cellular senescence in the joint is enacted through multiple, interconnected pathways. Genotoxic stress activates DNA damage response through the ATM/ATR–p53–p21 axis, imposing cell cycle arrest that can become entrenched in a senescent state. Mitochondrial dysfunction and accumulated mtDNA mutations increase ROS, which in turn exacerbate genomic damage and reinforce senescence signaling. Oxidative stress engages stress-activated kinases and inflammatory transcription factors, notably p38, MAPK, and NF-κB, to drive SASP expression. Mechanical perturbation of cells also provokes senescence. Aberrant loading stimulates Piezo1- and transient receptor potential cation channel subfamily V member 4-dependent calcium influx, activating c-Jun N-terminal kinase signaling and ROS production. Finally, pro-inflammatory cytokines such as IL-1β and IL-6 sustain and amplify senescent phenotypes through signal transducer and activator of transcription 3-mediated positive feedback, potentiating SASP expression and paracrine propagation of dysfunction.[18,19,50]

Specific markers of joint cell senescence include $p16^{INK4a}$, which increases with age and is commonly used to identify senescent chondrocytes; however, it is considered more of a marker than a driver in OA development. SA-β-Gal activity increases in senescent chondrocytes; however, its expression is influenced by autophagy and lysosomal function, necessitating validation with additional markers. The urokinase receptor is expressed on the surface of OA chondrocytes. In addition, dipeptidyl peptidase 4 is upregulated in senescent chondrocytes and shows a positive correlation with p16 expression and SASP secretion.[19,50]

Animal studies have confirmed a causal link between joint cell senescence and OA. Transplantation of senescent cells into the knee induced leg pain, reduced mobility, and radiographic and histological features consistent with OA.[173] Conversely, selectively removing p16-positive senescent chondrocytes using genetic models, such as INK-ATTAC mice, or pharmacological agents, such as UBX0101 and Navitoclax, significantly reduces OA severity and alleviates pain behaviors.[18,174] Therefore, joint cell senescence acts as a central mechanism that integrates various age-related

stresses, including genomic instability, mitochondrial dysfunction, and oxidative stress, thereby promoting OA progression through SASP-driven chronic inflammation and ECM breakdown.

In vitro models of OA commonly employ combined inflammatory and biomechanical insults, such as co-treatment with IL-1β, TNF-α, and mechanical overloading, to recapitulate the principal inflammatory and mechanical drivers of disease and elicit OA-like ECM degradation.

4.2.2. Construction strategies

Cytokines play a central role in joint homeostasis and chronic inflammation and are the most commonly employed induction agents in OA models due to their cost-effectiveness and ease of manipulation. An inflammatory factor-induced model was established by adding IL-1β to 3D cultures of human bone marrow MSCs (Figure 4A). This approach successfully generated chondrogenic organoids exhibiting OA features, including reduced expression of aggrecan and collagen Type II alpha 1, along with significant increases in inflammatory mediators such as IL-6, TNF-α, C–C motif chemokine ligand 2, and C–X–C motif chemokine ligand 1, all achieved without the use of a scaffold, relying solely on suspension culture.[126] Furthermore, when cultured in chondrogenic medium supplemented with a "triple inflammatory cytokine" cocktail of IL-1β, IL-6, and TNF-α, the constructs exhibited a typical OA gene signature after 3 weeks (Figure 4B).[38]

Excessive mechanical loading is a key factor driving the initiation and progression of OA, contributing to cartilage degeneration and joint deterioration.[176] Using a MACH-1 mechanical testing device, spherical neocartilage constructs were subjected to supra-physiological mechanical loading at a rate of 5 Hz, with a 20% sinusoidal strain over 10 min, while cylindrical constructs were loaded with a custom device under identical parameters.[177] Altered gene expression of *CD44*, *ITGA5*, and *CAV1*, markers linked to morphogenesis and wound healing, was observed, indicating that excessive mechanical stress induced detrimental phenotypic changes in chondrocytes, which can potentially initiate OA.[177]

Microenvironment modulation approaches were employed to construct OA models through different stimuli. An oxidative stress-induced model was developed using 3D bioprinting, where rat articular chondrocytes encapsulated in a gelatin–sodium alginate hydrogel were exposed to 200 μM hydrogen peroxide for 48 h.[33] This induced senescence phenotypes characterized by increased β-galactosidase activity, elevated p16 and p21 expression, and reduced Safranin O staining (Figure 4C). The scaffold's high porosity ensured uniform hydrogen peroxide permeation and senescence induction.[33] In addition, a radiation-induced aging model was established using primary articular chondrocytes from OA patients. *In vitro*, chondrocyte-derived chondrogenic organoids were subjected to a single dose of 10 or 20 Gy of ionizing radiation, leading to accelerated aging, as evidenced by the upregulation of SASP-related genes, including *CDKN1A*, *IL6*, *IL8*, and *SERPINE1* (Figure 4D). By culturing cartilage organoids in 96-well plates, this approach enables high-throughput drug screening.[127]

Gene editing techniques, such as clustered regularly interspaced short palindromic repeats-Cas9, enable the precise introduction of OA-related mutations into cartilage organoids. For example, cartilage tissue differentiated from human iPSCs with a *COL6A3* mutation could model OA pathology.[178] In addition, transcription factors like hypoxia-inducible factor 2α and enzymes such as ECM-degrading catabolic enzymes can promote OA-like features.[179,180] For instance, elevated levels of hypoxia-inducible factor 2α increase apoptosis pathways in chondrocytes. In addition, agents like monosodium iodoacetate induce chondrocyte death, which is similarly observed in OA.[181] However, these approaches have been used primarily in 2D systems and *in vivo* models, and require further development for effective integration into 3D organoid models.

While cartilage-only models cannot fully replicate the complex interactions present in native tissues, advanced bioreactor and microfluidic systems have been developed to support long-term, multi-tissue culture that mimics OA-associated tissue crosstalk.[176] For example, a miniature joint system (miniJoint) incorporating osteochondral, adipose, and fibrous tissues was created, where distinct media maintained each tissue type, and a universal flow was simulated to mimic synovial fluid, facilitating tissue interactions (Figure 4E). After 28 days, the synovial-like fibrous tissue was stimulated with IL-1β, and therapeutic agents—such as oligodeoxynucleotides and bone morphogenetic protein 7—were applied to evaluate their treatment efficacy, demonstrating miniJoint potential for screening disease-modifying OA drugs.[175] To construct complex tissue morphologies and interfaces, an "assembloid" strategy can be employed. This involves co-culturing pre-formed chondrocyte organoids (derived from OA patient chondrocytes or iPSC-differentiated chondrocytes) with synovial organoids on a microfluidic platform.[182,183] Precise 3D positioning fosters the formation of anatomically relevant interfaces between the synovial organoid and an underlying mineralized, bone-like tissue, creating an integrated, multi-tissue model.[184] This comprehensive, multiscale organoid platform enabled a detailed investigation into OA pathogenesis and offers a promising tool for testing targeted therapies aimed at halting or reversing joint degeneration.

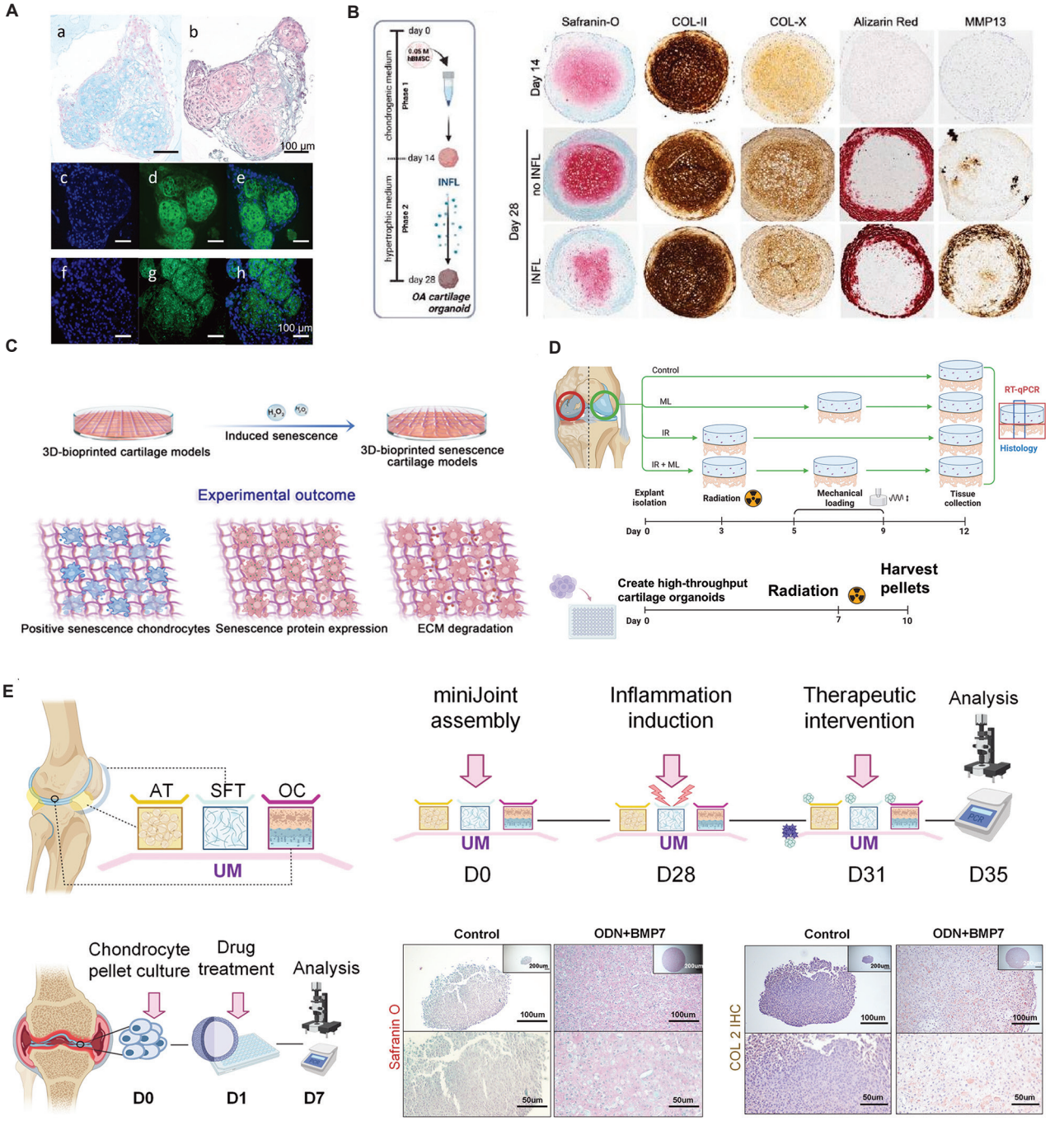

Figure 4. Joint organoids recapitulating OA. (A) Histological and immunofluorescence characterization of cartilaginous organoids. Top: Alcian blue (a) and Safranin O–Fast Green (b) staining showing a proteoglycan-rich extracellular matrix. Bottom: immunofluorescence for ACAN (c-e) and COL2A1 (f-h) with DAPI nuclear counterstain; merged images demonstrate co-localization of matrix markers with cell nuclei. Scale bars: 100 μm. Reprinted from Zhang *et al.*[126] (B) Generation and validation of OA cartilage organoids: histology at days 14 and 28 demonstrated proteoglycan distribution (Safranin O–Fast Green), COL2 and COLX expression, matrix mineralization (Alizarin red), and MMP13 presence. INFL refers to the inflammatory cytokine cocktail-treated group. Scale bars: 100 μm. Reprinted from Donges *et al.*[38] (C) 3D-bioprinted cartilage constructs exposed to hydrogen peroxide (H_2O_2) to induce senescence-like phenotypes. Reprinted from Qin *et al.*[33] (D) Timeline for the assembly and analysis of a radiation-induced aging cartilage model. Reprinted from Boone *et al.*[127] (E) A microphysiological system for modeling joint inflammation and testing therapeutics: schematic of the miniJoint integrating osteochondral, adipose, and synovial-like fibrous tissues, and complementary pellet studies assessing the effects of ODN⁺BMP7 on human OA chondrocytes. Scale bars: Upper row: 100 μm; lower row: 50 μm. Reprinted from Makarczyk *et al.*[175]

Abbreviations: 3D: Three-dimensional; ACAN: Aggrecan; BMP7: Bone morphogenetic protein 7; COL2A1: Collagen type II alpha 1; COLX: Collagen type X alpha 1; DAPI, 4',6-diamidino-2-phenylindole; MMP13: Matrix metallopeptidase 13; OA: Osteoarthritis; ODN: Oligodeoxynucleotides.

4.2.3. Role of scaffold materials

Scaffold materials can be used to create a pathomimetic niche that replicates the initial pathological environment of OA joints, providing cells with a physiologically relevant disease microenvironment that serves as a foundational platform for constructing disease models. The native biomechanical signals can be simulated by tuning hydrogel stiffness and viscoelasticity to replicate OA-related mechanical changes in cartilage and subchondral bone ECM. Such tuning can bias cells toward disease-relevant phenotypes and differentiation trajectories. For instance, by modifying crosslink density to mimic the stiffness of late-stage OA cartilage, silk fibroin–DNA hydrogel-based microspheres can enhance matrix degradation phenotypes of organoids.[38]

Controlling ECM composition through material design involves precisely adjusting the ratios of collagen types as well as modulating GAG content to replicate ECM degradation during OA progression. This approach creates a biochemical environment where cells receive aberrant signals. Declining collagen Type II expression reflects cartilage matrix breakdown, while increased collagen Type I expression indicates a shift toward a fibroblastic phenotype, collectively illustrating the structural disruption and imbalance between degradation and repair characteristics of OA.[185,186] In modeling the collagen composition shifts characteristic of OA, three principal scaffold strategies have been employed to replicate the spatial and compositional alterations observed in disease progression. One approach involves fabricating hydrogels with a gradient of collagen Type II content—ranging from 0% to 100%—to simulate the gradual decrease of collagen Type II during OA, with combined second harmonic generation-residual networks imaging enabling real-time quantitative assessment of fiber structure changes as the ratio shifts.[187] Another strategy maintains a fixed total collagen concentration but varies the collagen Type I to collagen Type II ratio across multiple groups, such as 1:0, 3:1, 1:1, 1:3, and 0:1, reflecting late-stage OA phenotypes with elevated collagen Type I levels. Measurements of mechanical properties, porosity, and protein retention have revealed how such ratio deviations weaken matrix integrity and cause structural loosening.[188] Another approach constructed layered or segmented scaffolds with a spatial gradient along the joint interface, such as a bottom layer predominantly composed of collagen Type I (~70%) and an upper layer rich in collagen Type II (~30%). This configuration effectively models the initial surface loss of collagen Type II, followed by deeper collagen type I replacement, mirroring the pathological progression of OA cartilage, where surface degeneration precedes deeper structural remodeling.[189] These methodologies collectively enable the *in vitro* recreation of the dynamic and spatial heterogeneity of collagen alterations that underpin OA pathogenesis.

Enhancing the reproducibility and reliability of disease modeling requires active, controlled modulation of the microenvironment to induce and accelerate pathogenesis in a standardized manner. One key approach involves stimulus homogenization, whereby biomaterials function as carriers to achieve uniform distribution of bioactive factors, mechanical loads, or oxidative stress stimuli within 3D constructs. This ensures consistent treatment exposure across all cells within organoids, minimizing experimental bias caused by stimulus gradients inherent in traditional culture systems. For example, materials like gelatin–alginate with uniform pore structures facilitate even distribution of hydrogen peroxide or radiation doses, thereby improving model reproducibility.[33,127] Complementarily, the material itself can be designed as a "smart" system responsive to external triggers such as light or enzymes, or capable of sustained release or removal of factors like SASP molecules and therapeutic agents, thereby allowing dynamic modulation of the microenvironment. Such systems enable the simulation of different disease stages and the testing of intervention effects. For instance, hyaluronic acid-sulfobetaine methacrylate hydrogels possess intrinsic anti-inflammatory properties, neutralizing factors like TNF-α to establish a stable baseline for evaluating anti-inflammatory drugs.[190]

The pivotal importance of scaffold materials in disease modeling extends to their capacity to support advanced, high-throughput research applications, thereby increasing the complexity and scalability of models to meet modern biomedical requirements for elucidating mechanisms and screening therapeutics. As 3D platforms, these materials facilitate the co-culture of multiple cell types while precisely controlling their spatial arrangement, providing a structural basis for exploring the complex cellular crosstalk underlying OA. For instance, fibrin–methacrylated gelatin composite hydrogels support long-term co-culture systems that mimic immune cell–matrix interactions by spatially organizing fibroblasts, endothelial cells, and macrophages.[191] Furthermore, biofabrication techniques like bioprinting enable the parallel production of numerous uniform, structurally standardized micro-organoids in high-throughput formats such as microtiter plates, aligning with the demand for large-scale drug screening. This is exemplified by collagen hydrogel films that are compatible with 96-well arrays for radiation-induced aging studies.[127]

4.2.4. Functional assessment

A comprehensive, multi-parameter assessment system is crucial for validating disease models and monitoring disease progression. This includes histological staining (e.g., Alcian blue and Safranin O staining) and immunostaining (e.g.,

collagen Type II staining) to quantify the loss of GAG and collagen Type II, as well as ELISA assays for biochemical quantification. Rheological tests measure viscoelastic properties (G', G", and G"/G') to evaluate matrix degradation, while friction testing characterizes joint lubrication. Cytokine profiles assessed via multiplex assays (e.g., IL-6, IL-8, monocyte chemoattractant protein-1) reveal changes in the inflammatory microenvironment. Protein analyses, such as Western blot or immunofluorescence (e.g., cartilage oligomeric matrix protein[192] and fibulin-3[193]), provide insights into tissue wear. In addition, innovative techniques can be employed for deeper analysis. Fluorescence resonance energy transfer-based sensors can be used to monitor real-time MMP13 activity and cartilage breakdown.[194] Rheological measurements can be conducted to identify impaired mechanics indicative of tissue weakening. Moreover, scRNA-seq of immune cells, such as C–C chemokine receptor Type 2^+ macrophages and Th17 cells, provides a systems-level understanding of immune–matrix interactions driving OA progression.

In summary, aging joint organoid models have utility beyond OA, and are increasingly applied to investigate the pathogenesis and therapeutic strategies for rheumatoid arthritis, post-traumatic arthritis, and focal cartilage defects.[195] By precisely controlling cell composition, biomaterial properties, mechanical loading, and biochemical milieu, these platforms can recapitulate disease-specific pathological states and enable mechanistic dissection of joint aging, offering translationally relevant testbeds for novel interventions.

4.3. Modeling skeletal muscle aging using sarcopenia

4.3.1. Pathological hallmarks

Sarcopenia, the age-related loss of skeletal muscle mass and function, is driven by complex etiologies involving inflammation, nutrient deficiencies, and inactivity and impairs muscular, vascular, and metabolic functions. There is an urgent need for advanced models to better understand the molecular mechanisms of sarcopenia and improve its clinical management for aging populations.[196-198] Aging skeletal muscle undergoes significant structural and cellular alterations, including reduced cross-sectional area, thickening of connective tissues, increased fibrosis, and decreased capillary density.[199-201] These changes lead to a shift in fiber composition toward more slow-twitch (Type I) fibers, coupled with atrophy of fast-twitch (Type II) fibers, which are closely linked to declines in muscle mass and strength.[202,203] This aging process not only limits muscle contraction capacity but also increases the risk of falls, fractures, and mortality in older adults by affecting systemic metabolic homeostasis.

Cellular alterations include adipose infiltration, loss of motor units, and compromised satellite cell functions, marked by reduced progenitor pools, limited myogenic potential, and increased senescence, as well as adipogenic differentiation, which contribute to impaired muscle regeneration and force generation.[204,205] In addition, changes in the immune and vascular environments, such as excessive M2 macrophages and increased endothelial apoptosis, contribute to systemic muscle decline, highlighting the need to understand the molecular underpinnings of these complex aging processes.[206,207]

The structural and cellular changes in aging muscle, notably a decline in regenerative capacity largely due to reduced satellite cell populations and decreased differentiation potential, are inseparably linked with molecular alterations.[208,209] Satellite cell activation is governed by myogenic regulatory factors, such as myogenin, myoblast determination protein, myogenic factor 5, and myogenic regulatory factor 4.[210] While rat studies show increased myoblast determination protein and myogenin expression with age, suggesting a compensatory mechanism, human studies indicate decreases in these factors, highlighting species differences and the need for accurate human muscle models.[211-213] Myostatin, a member of the TGF-β superfamily, inhibits satellite cell proliferation and activation, contributing to muscle atrophy by interacting with glucocorticoid pathways, and its upregulation in aging contributes to muscle mass loss.[214] In addition, mitochondrial dysfunction and oxidative stress are key contributors to aged muscle, where disrupted mitochondrial bioenergetics lead to accumulated ROS, reduced quality control mechanisms like mitophagy and apoptosis, creating a cycle of mitochondrial damage and muscle degeneration.[215]

Age-associated accumulation of mtDNA damage elevates ROS, which activates cell cycle inhibitors, such as $p16^{INK4a}$ and $p21^{CIP1}$, to enforce arrest and foster SASP. Concurrent declines in NAD^+ impair SIRT1-dependent deacetylation, amplifying inflammatory signaling and proteostatic pathways, thereby reinforcing senescence and muscle catabolism.[21] With aging, gut microbiota dysbiosis reduces short-chain fatty acid production, perturbing muscle metabolism and inflammatory tone through the gut–muscle axis. This establishes a microbiota–metabolism–inflammation circuit that promotes skeletal muscle senescence and functional decline.[22]

Metabolomic analyses of aged skeletal muscle reveal significantly decreased plasma levels of branched-chain amino acids, including leucine, isoleucine, and valine, and reduced tryptophan concentrations, coupled with an increased exophthalmic acid/tryptophan ratio, indicating activation of the tryptophan metabolic pathway. Elevated levels of acylcarnitines, such as C4, C6, C8, and C10, suggest impaired fatty acid oxidation, while reductions in

polyamines like spermine and spermidine are associated with diminished cellular proliferation and autophagy, marking key metabolic alterations characteristic of skeletal muscle aging.[22] From an immunological standpoint, reduced levels of myokines such as IL-15 and IL-7 serve as functional markers of skeletal muscle aging, with their concentrations positively associated with muscle mass and immune cell activity.[216]

Muscle fiber atrophy and mitochondrial dysfunction are primary drivers of sarcopenia, leading to reductions in muscle strength and functional capacity.[22] In addition, diminished expression of myogenic factors impairs immunoregulatory functions, creating a vicious cycle of muscle wasting, immune dysregulation, and regenerative failure—further accelerating sarcopenia progression.[216] Clinically, skeletal muscle aging not only underlies age-related sarcopenia but also contributes to secondary muscle wasting associated with comorbidities like diabetes and heart failure, indicating that targeting muscle aging itself may be a crucial strategy for preventing and treating sarcopenia.[21]

4.3.2. Construction strategies

To study sarcopenia using skeletal muscle organoids, researchers can induce aging phenotypes by carefully selecting and manipulating cell sources, administering specific soluble factors, designing microenvironments that incorporate mechanical and electrical stimulation, and using multicellular or pathological co-culture systems that mimic the complex tissue environment.

Cell source control for modeling sarcopenia involves the direct use of senescent or replication-aged cells. For instance, Wang *et al.*[135] embedded myogenic progenitor cells (MPCs) derived from the hindlimb skeletal muscle of 21–23-month-old male C57BL/6 mice into a fibrin–Matrigel hydrogel, which formed 3D muscle bundles exhibiting intrinsic sarcopenic features (a 24% reduction in myotube diameter and approximately 30% decrease in contractile force) even without injury (Figure 5A). Following cardiotoxin-induced damage, these aged muscle constructs demonstrated impaired regenerative capacity, faithfully recapitulating age-related decline without requiring exogenous stimuli.[135] Similarly, Rajabian *et al.*[35] induced replicative senescence in human primary myoblasts after more than 10 passages and embedded them in collagen Type I–Matrigel hydrogels. These senescent cells showed elevated SA-β-Gal activity, decreased myotube fusion, and almost complete loss of contractile response to electrical stimulation, establishing a reliable model of sarcopenia driven solely by cellular aging (Figure 5B).[35] Tendon has a close anatomical and functional connection to skeletal muscle. Self-assembled 3D tendon organoids derived from tendon stem/progenitor cells from aged or degenerative human Achilles tendons exhibit significant functional and molecular deficits characterized by fragility, reduced cellularity, and increased cellular senescence and apoptosis.[217]

Soluble factor-based disease induction typically recapitulates muscle atrophy by exposing it to inflammatory cytokines. Mestre *et al.*[136] applied a single dose of 20–80 ng/mL TNF-α for 24 h to mature human myoblasts within Matrigel–fibrin scaffolds, resulting in myofibrillar atrophy, decreased contractile force, and extended relaxation times (Figure 5C). Building on this, Park *et al.*[134] established a chronic myopathy model by continuously administering 10 ng/mL TNF-α to human pluripotent stem cell-derived skeletal muscle organoids (Figure 5D), thereby enabling the concurrent evaluation of therapeutic interventions on myofibrillar cross-sectional area and neuromuscular junction density.

Aged ECM was incorporated into microenvironments that provided oxidative cues together with mechanical and electrical stimulation to recapitulate key features of tissue aging. For example, Tan *et al.*[117] created a "dECM ink" by blending aged mouse tibialis anterior muscle decellularized ECM (dECM) with collagen Type I, which, through pH-triggered shear extrusion, produced highly aligned nanofibers (Figure 5E). This aged ECM-based environment delivered dual mechanical and biochemical signals that prompted young C2C12 cells to exhibit morphological features typical of aged myotubes.[117] In addition, Mondrinos *et al.*[137] employed surface-directed patterning technology to engineer anisotropic human skeletal muscle strips, which, under sustained longitudinal tension and hydrogen peroxide treatment, rapidly developed an acute injury phenotype characterized by oxidative stress, mitochondrial dysfunction, and poly (ADP-ribose) polymerase overactivation, effectively modeling key aspects of oxidative damage associated with aging muscle (Figure 5F). Extending this approach, they explored a multicellular, pathological microenvironment by co-culturing chemotherapy-resistant tumor cells with 3D-oriented muscle tissues within a surface-guided polydimethylsiloxane-collagen microfluidic system. This setup established a lung cancer cachexia model. Although it does not represent classical sarcopenia, the muscle tissue exhibited early atrophic features, including ROS bursts, reduced citrate synthase activity, NF-κB activation, and disrupted F-actin organization, offering a valuable *in vitro* platform for studying tumor-induced muscle wasting.[137]

4.3.3. Role of scaffold materials

Integrating cells with 3D scaffolds provides a powerful approach to *in vitro* modeling of sarcopenia. By embedding aged primary myogenic cells, replicative senescent cells, or

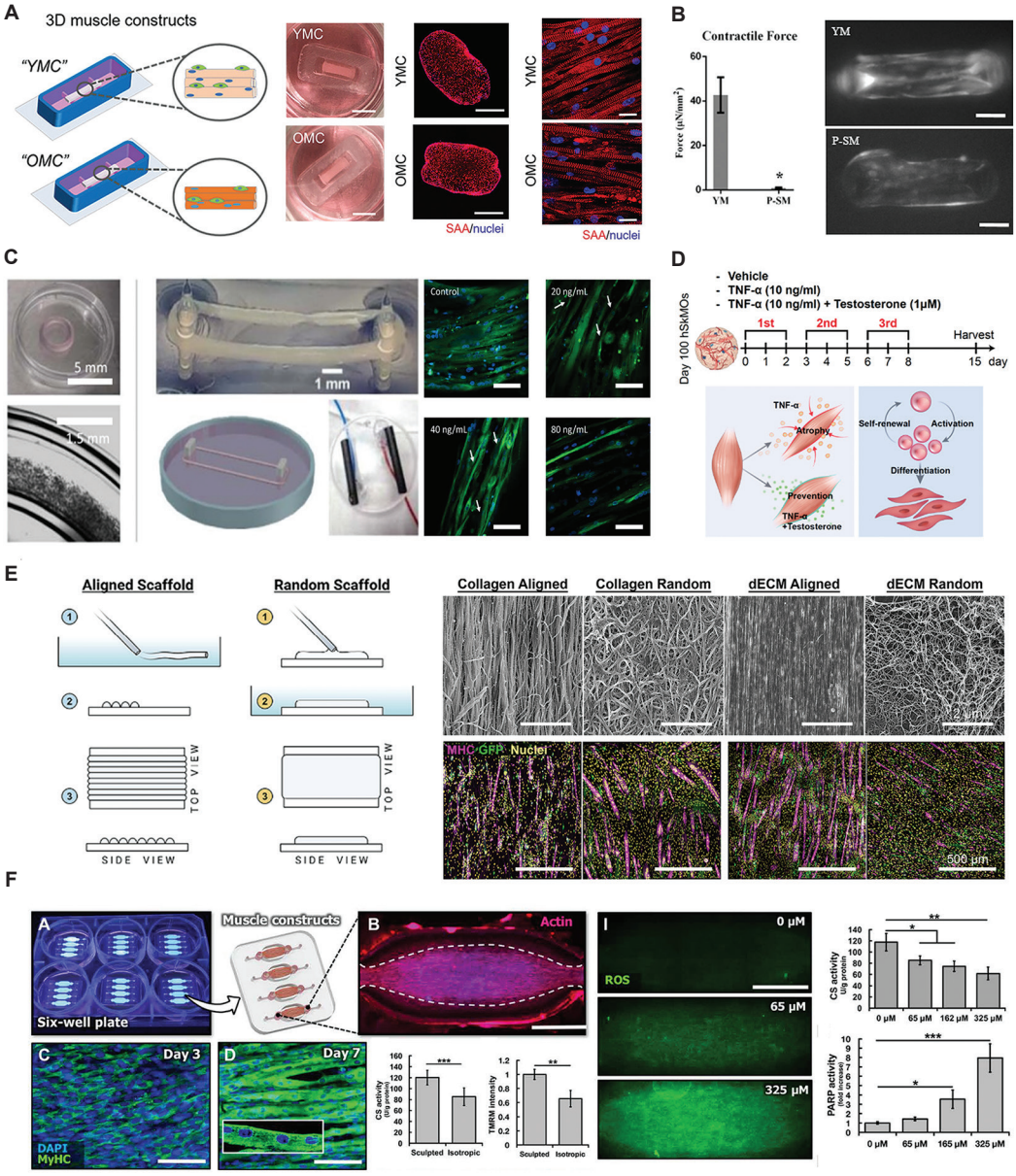

Figure 5. Skeletal muscle organoids for modeling sarcopenia. (A) Muscle constructs engineered from progenitor cells of young or aged mice differentiated over 14 days to form aligned myotubes, as shown by gross images, cross-sectional SAA (red) and nuclear (blue) staining, and confocal imaging of myotube alignment. Scale bars: Left: 5 mm; middle: 500 μm; right: 25 μm. Reprinted from Wang *et al.*[135] (B) Human skeletal muscle microtissues derived from pre-senescent myoblasts exhibited markedly reduced electrically evoked contractile force and attenuated intracellular calcium transients, shown by representative traces and quantification. Scale bars: 100 μm. Reprinted with permission from Rajabian *et al.*[35] Copyright © 2021, Mary Ann Liebert, Inc. (C) 3D human muscle constructs, cast in 3D-printed molds, mounted on PDMS posts and electrically stimulated with carbon electrodes, displayed dose-dependent myotube thinning and morphological disruption after TNF-α treatment, visualized by MyHC (green) and nuclear (blue) staining. Scale bars: Top-left: 5 mm; bottom-left: 1.5 mm; top-right: 1 mm; microscopic images: 80 μm. Reprinted with permission from Mestre *et al.*[136] Copyright © 2021, IOP Publishing Ltd. (D) Timeline of chronic TNF-α treatment of day-100 human skeletal muscle organoids with concurrent testosterone administration to assess attenuation of inflammation-induced wasting. Reprinted from Park *et al.*[134] (E) Aligned scaffolds produced by shear-induced fibrillogenesis through ink extrusion are assembled into multi-stranded constructs, whereas random scaffolds form without shear; SEM confirmed distinct topographies, aligned substrates promoted organized myogenesis of GFP⁺ C2C12 cells, and aged ECM environments induced aged-like myoblast morphologies. Scale bars: Upper row: 2 μm; lower row: 500 μm. Reprinted with permission from Tan *et al.*[117] Copyright © 2025, Wiley-VCH GmbH. (F) Sculpted human muscle tissues matured in six-well plate inserts showed F-actin organization (Scale bar: 2 mm), MyHC-positive myotubes (Scale bars: 50 μm), and myoblast fusion, and baseline mitochondrial function (citrate synthase [CS] activity and TMRM) that were disrupted by 24-h hydrogen peroxide exposure, as indicated by increased cell-ROX, reduced CS activity, and elevated PARP activity. Reprinted from Mondrinos *et al.*[137]

Abbreviations: C2C12: Immortalized mouse myoblast cell line; ECM: Extracellular matrix; GFP: Green fluorescent protein; MyHC: Myosin heavy chain; PARP: Poly (ADP-ribose) polymerase; PDMS: Polydimethylsiloxane; SAA: Serum amyloid A; SEM: Scanning electron microscopy; TMRM: Tetramethylrhodamine methyl ester; TNF-α: Tumor necrosis factor alpha.

inflammation-induced senescent cells into scaffolds with tailored biochemical and mechanical properties, these models facilitate the differentiation and assembly of muscle fibers under controlled conditions.

Scaffold materials can effectively convey signals of the aging microenvironment. For instance, Tan *et al.*[117] developed a "dECM ink" by combining dECM from the tibialis anterior muscle of aged mice with collagen Type I in a 1:1 ratio. This dECM preserved age-related proteins, such as fibromodulin and decorin, which induced partial senescence phenotypes in young C2C12 cells, including increased myotube length but limited nuclear fusion, without the need for external stimuli.[117] Similarly, Wang *et al.*[135] encapsulated muscle progenitor cells from 21–23-month-old mice within a fibrin–Matrigel composite hydrogel, observing a 24% reduction in myotube diameter and approximately a 30% decrease in contractile function, thereby directly modeling age-related muscle decline.[135]

Scaffold materials can function as controllable platforms for delivering inflammatory stimuli. Mestre *et al.*[136] used a Matrigel–fibrin composite ring scaffold, which, after tissue maturation, was exposed to 20–80 ng/mL TNF-α for 24 h. This stimulation induced myofibrillar atrophy, reduced contractile force, and extended relaxation time, demonstrating that the hydrogel's composition allowed for the diffusion of inflammatory mediators within the scaffold environment.[136]

Scaffold materials can also provide mechanical and topological cues to enhance tissue organization. For example, Lee *et al.*[218] developed a "micro-nano" multiscale scaffold by integrating methacrylated porcine skeletal muscle dECM with microfibrillated poly(lactic-co-glycolic acid) pillars. These pillars created unidirectional microgrooves, while the methacrylated porcine skeletal muscle dECM nanofibers supplied muscle-specific biochemical signals. Human MPCs cultured on this scaffold displayed high orientation and formed neatly arranged, mature myotubes, illustrating that a synergistic biomechanical and biochemical microenvironment markedly promoted myogenesis and tissue organization in adult MPCs.[218]

Scaffold materials ensure reproducibility and quantitative assessment through precisely engineered parameters, such as topological structure, stiffness, and surface ligands that systematically modulate key functional features like muscle fiber diameter and regenerative ability. In addition, its mechanical support and spatial constraints establish a stable microenvironment for cell–matrix interactions, enabling sustained, targeted biochemical signaling that influences myocyte fate and overall tissue function.

4.3.4. Functional assessment

Functional and metabolic assessments of skeletal muscle organoids necessitate a comprehensive and dynamic approach. Laser diffraction tracking measures the amplitude and rate of sarcomere shortening, with aging models showing over 30% reductions in contractile performance. Cantilever deflection, post deflection, and force transducers are commonly used configurations for measuring contractile force in 3D models.[219] A microelectrode array can be employed to evaluate neuromuscular junction transmission efficiency, with aged muscle typically showing a twofold increase in synaptic latency. Mitochondrial function can be accessed through Seahorse assays, which typically reveal a 40% decrease in basal respiration and adenosine triphosphate production, alongside increased proton leakage, in aged muscle. Collectively, these multidimensional, quantitative analyses span from cellular metabolism to tissue-level contraction, offering valuable insights into sarcopenia mechanisms and the effects of potential therapeutics targeting muscle degradation and mitochondrial dysfunction.

While existing aging skeletal muscle organoid models have largely been deployed to study sarcopenia, their applicability can be extended far beyond this condition. These biomimetic platforms are increasingly used to interrogate neuromuscular junction disorders, impaired muscle regeneration, sarcopenic obesity, and diverse secondary atrophies. They provide tractable systems for dissecting the complex mechanisms of muscle aging and evaluating candidate therapeutic strategies.[220]

5. Challenges and future prospects

Despite significant advancements in the development of biomaterial-based aging MSK organoid models, several critical challenges persist, presenting avenues for future breakthroughs. A primary concern is the limited long-term stability of these organoids, which constrains our ability to modulate tissue aging rates and sustain aging-related phenotypes over extended periods, ranging from months to years. Furthermore, existing models inadequately capture the heterogeneity of aging, particularly the differential aging rates of various cell types, such as osteoblasts and osteoclasts, within the *in vivo* environment (Figure 6A). Regarding methods used to model aging, several aspects merit scrutiny. A key issue is physiological relevance. Many *in vitro* protocols induce accelerated aging, characterized by high oxidative stress (for example, hydrogen peroxide treatment) or genetic perturbations. It is essential to assess how accurately these acute stress models recapitulate the slow, cumulative aging that occurs in humans. In addition, variability in cell sources (donor age, tissue origin), biological materials (for example, batch-to-batch

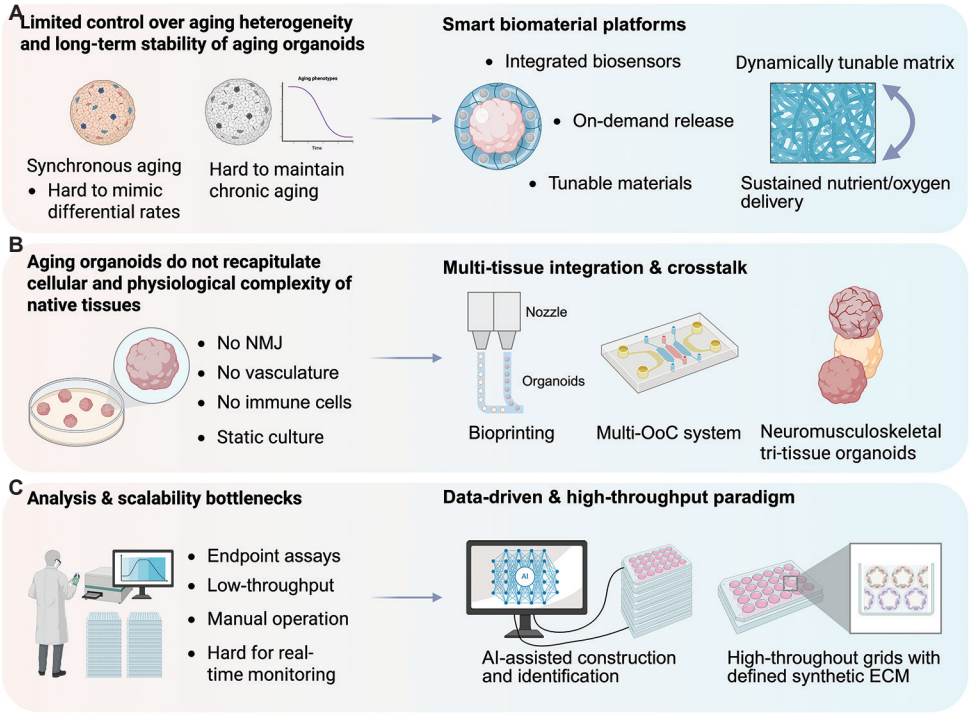

Figure 6. Current limitations in aging organoid culture and strategies to address the challenges. (A) Control over heterogeneity and long-term stability is limited (left), but can be improved with smart biomaterials featuring tunable properties, integrated biosensors, and on-demand release systems (right). (B) Lack of cellular and physiological complexity (left) can be overcome through multi-tissue integration, bioprinting of multi-organ, neuromusculoskeletal systems, and facilitating tissue crosstalk (right). (C) Bottlenecks in analysis and scalability (left), such as low throughput and limited real-time monitoring, can be addressed through data-driven approaches, AI-assisted construction and identification, and high-throughput platforms that employ synthetic ECM grids (right). Created in BioRender. ZENG, Q. (2026) https://BioRender.com/rlbzwk4.
Abbreviations: AI: Artificial intelligence; ECM: Extracellular matrix; NMJ: Neuromuscular junction; OoC: Organ-on-a-chip.

differences in Matrigel), and culture protocols across laboratories hampers direct comparison and replication of findings, slowing progress in the field.

Moreover, technical bottlenecks continue to impede the integration of systemic components, including immune cells, neural networks, and vascular systems, into organoid constructs. This limitation restricts the capacity of these models to accurately replicate the intricate, systemic microenvironments characteristic of aging tissues *in vivo*. As a result, they constrain investigations into key aging mechanisms, including inflammation, nutrient delivery, and neuromodulation (Figure 6B). In native tissues, cell types are arranged in defined proportions and spatial architectures. It remains a major challenge to precisely control these parameters during organoid construction and accurately recapitulate age-related structural changes, such as trabecular remodeling and alterations in muscle fiber bundles. Furthermore, aging not only entails cellular alterations but also progressive changes in the ECM, including its composition and physical properties such as stiffness and viscoelasticity. Most available materials still provide static environments, making it difficult to emulate the dynamic, aging-associated degradation process.

In addition, there are significant challenges related to analysis and scalability, which are predominantly manifested in endpoint assays, limited throughput, manual operations, and difficulties in real-time monitoring (Figure 6C). Most studies rely on endpoint readouts, such as SA-β-Gal staining and specific protein levels. These static snapshots poorly capture the dynamic progression of aging and its real-time effects on functional outputs, such as muscle contractility and bone matrix mineralization rates. A key challenge is proving that molecular or cellular changes observed in organoids (for example, the upregulation of a specific gene) translate into physiologically meaningful functional phenotypes, such as reduced muscle contractility or increased bone fragility, and are directly linked to tissue functional decline in aged individuals.

Looking forward, emerging technologies offer promising routes to address these limitations. Advances in smart biomaterial platforms are enabling tunable, dynamic systems with programmable, on-demand release profiles,[221,222] in which mechanical stiffness, degradability, and biochemical ligand presentation can be modulated temporally or in response to external stimuli to emulate the progression of tissue aging. Coupling these materials with integrated biosensors and closed-loop control schemes

will permit real-time readouts and adaptive modulation of the microenvironment, thereby improving fidelity in recapitulating the temporal and spatial complexity of aging processes and enabling longitudinal mechanistic and therapeutic studies (Figure 6A).

Multi-organoid platforms that functionally couple muscle, bone, and joint will enable the investigation of long-range aging signals and their propagation across tissue compartments. Emerging bioengineering approaches—organoid bioprinting,[223] organ-on-a-chip systems,[224] and the construction of integrated neuro-MSK models[225]—offer particular promise for recapitulating native tissue complexity, including vascularization, innervation, and biomechanical connectivity (Figure 6B). By combining precise spatial patterning, controlled mechanical loading, and physiologically relevant fluidics, these platforms can enhance physiological fidelity, facilitate longitudinal interrogation of inter-tissue crosstalk, and provide scalable testbeds for mechanistic studies and therapeutic screening.

Notably, these biomaterial-driven MSK aging organoids hold substantial translational potential. For instance, patient-derived organoids can serve as personalized preclinical platforms for drug screening, senolytic efficacy testing, and evaluation of targeted therapies, while standardized organoid systems may streamline regulatory validation by providing reproducible, human-relevant data to complement clinical trial outcomes. Such integration bridges in vitro mechanistic insights with clinical applications, facilitating more efficient translation of interventions for preserving MSK function in older adults.

Artificial intelligence can accelerate development and deployment of aging organoid platforms by optimizing biomaterial design,[226] scaffold construction,[227,228] and culture protocols, enabling real-time, automated identification of senescence phenotypes, and supporting data-informed, streamlined experimental design. Complementary efforts to establish open data repositories and standardized reference materials will enhance reproducibility and facilitate cross-laboratory comparability. The integration of biosensor-equipped hydrogels capable of providing continuous readouts of aging biomarkers, combined with closed-loop control algorithms, can permit adaptive modulation of the microenvironment. Implementing these approaches in multi-organ co-cultures and high-throughput platforms built on synthetic, tailored ECM can further increase the level of biomimicry and facilitate the interrogation of functional inter-tissue signaling.

6. Conclusion

In this review, we provided an overview of aging MSK organoids and organoid-like models. Biomaterial-driven aging organoids have the potential to revolutionize research on MSK aging. They offer powerful platforms for deciphering aging mechanisms, screening therapeutic agents, and progressing toward personalized medicine. Realizing this potential will require sustained interdisciplinary collaboration among materials scientists, bioengineers, geriatricians, and clinicians, together with standardized materials and assays, shared data infrastructures, and translational pipelines that connect *in vitro* findings to clinical evaluation. Such coordinated efforts can enhance the translational impact of aging organoid models, thereby accelerating the discovery of interventions that preserve MSK function in older adults.

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Conflict of interest

The authors declare that they have no competing interests.

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