

REVIEW ARTICLE

Mechano-encoding hydrogel microspheres for extracellular and pericellular matrix remodeling in musculoskeletal organoids

Xingkuan Wang^{1,2,3†}, Jinping Chen^{1,2,3†}, Jianye Tan^{1,2,3}, Shengwen Cheng^{1,2,3}, Lifu Jia⁴, Charlotte A. E. Hauser^{5,6*}, Tiziano Serra^{7*}, Wei Huang^{1,2,3*}, Yiting Lei^{1,2,3,8*}, and Pengcheng Xiao^{1,2,3*}

¹Department of Orthopaedic Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

²Chongqing Municipal Health Commission Key Laboratory of Musculoskeletal Regeneration and Translational Medicine, Chongqing Medical University, Chongqing, China

³Orthopaedic Research Laboratory of Chongqing Medical University, Chongqing Medical University, Chongqing, China

⁴Department of Orthopaedics, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China

⁵Institute of Health Care Engineering with European Testing Center of Medical Devices, Graz University of Technology, Graz, Austria

⁶Laboratory for Nanomedicine, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Saudi Arabia

⁷AO Research Institute Davos, Davos, Switzerland

⁸Department of Biomedical Engineering, Faculty of Engineering, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

*Corresponding authors: Charlotte A. E. Hauser (charlotte.hauser-funke@tugraz.at); Tiziano Serra (tiziano.serra@aofoundation.org); Wei Huang (huangw511@163.com); Yiting Lei (leiyyt614@163.com); Pengcheng Xiao (Xiaopc95@hotmail.com)

Citation: Wang X, Chen J, Tan J, Cheng S, *et al.* Mechano-encoding hydrogel microspheres for extracellular and pericellular matrix remodeling in musculoskeletal organoids. *Organoid Res.* 2026;2(3):026090013. doi: 10.36922/OR026090013

Received: February 28, 2026

Revised: May 21, 2026

Accepted: May 25, 2026

Published online: July 24, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, which provided that the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Organoids—three-dimensional, self-organizing living micro-tissue entities—are fundamentally reshaping the outcome of modern biomedical research through their remarkable capacity to recapitulate the topographical complexity and physiological fidelity of native tissues. Additionally, organoids are able to recapitulate the genetic and epigenetic signature of individual human beings, which is a crucial stepping stone for all personalized and regenerative medicine approaches. However, despite their ability to express selected lineage-specific markers, existing organoid culture platforms exhibit fundamental deficiencies in the spatiotemporal precision of extracellular matrix (ECM) and pericellular matrix (PCM) organization—a vexating limitation rooted in the absence of sophisticated spatiotemporal mechano-coding. Consequently, organoids frequently remain arrested in immature phenotypic states, severely constraining their translational efficacy in regenerative medicine. Addressing this critical bottleneck, hydrogel microsphere-mediated engineering strategies have emerged as a transformative paradigm. This review centers on innovative approaches with a focus on osteomuscular organoid models, elucidating their rational biomaterial design principles, unique mechano-coding advantages, and the core mechanisms by which they orchestrate the secretion and microstructural remodeling of endogenous ECM/PCM components. We conclude by prospectively exploring the potential of these microsphere-mediated systems to enhance their physiological accuracy and clinical translatability of *in vitro* musculoskeletal models.

Keywords: Hydrogel microspheres; Osteomuscular organoid; Mechano-coding; Extracellular/pericellular matrix; Tissue regeneration; Biomechanotransduction; Matrix remodeling

1. Introduction

The bioengineering of musculoskeletal organoids is shifting from morphological mimicry to functional maturation. Current culture systems successfully replicate the anatomical architecture of native tissues.¹ However, engineering complex tissues with physiological mechanical responsiveness and metabolic competence remains a major challenge in regenerative medicine.² The deficiency of current functional *in vitro* models to replicate the precise spatiotemporal mechanical and biochemical gradients inherent to the native microenvironment is a crucial bottleneck that needs to be overcome. Functional attainment in native tissues is intrinsically contingent upon the structural anisotropy exhibited by the extracellular matrix (ECM) and the pericellular matrix (PCM).³ Beyond functioning as structural scaffolds, the ECM and PCM transduce critical mechanical cues via specific fibrillar alignment and topological architectures, thereby rigorously orchestrating the establishment of cellular polarity, directed migration, and spatial patterning.⁴ Such highly organized matrix–cell reciprocity serves as the fundamental prerequisite for endowing muscle tissues with contractility and supports tendon tissues with force transmission capabilities.⁵ Consequently, the dynamic remodeling and anisotropic engineering of the ECM/PCM in organoid formation constitute the rate-limiting determinants governing organoid maturation, the reconstitution of functional networks, and ultimately tissue regeneration.

To mitigate any microenvironmental deficits, historically, conventional organoid strategies have predominantly relied upon the exogenous supplementation of ECM proteins or growth factors.⁶ However, this additive engineering pattern encounters huge translational barriers: the cumulative dosage of growth factors not only incurs prohibitive costs but also introduces significant batch-to-batch heterogeneity and causes remarkable standardization challenges.^{7,8} Furthermore, the non-negligible risk of immunogenicity associated with exogenous proteins severely constrains their clinical utility.

In light of these challenges, this review focuses on a robust engineering strategy: leveraging “injectable, degradable, and mechanically tunable” hydrogel microspheres (HMs) to construct intelligent “ECM-like scaffolds.”⁹ Unlike traditional dense bulk hydrogels, granular scaffolds formed via the packed assembly of these microspheres possess inherent interstitial porosity. These open micron-scale channels effectively circumvent physical constraints on nutrient transport and metabolic waste clearance, while providing physical latitude for unimpeded cellular migration and the deep infiltration of nascent ECM.^{10–12} Crucially, capitalizing on the superior modularity of HMs, researchers can precisely engineer anisotropic

or gradient microenvironments that recapitulate native musculoskeletal interfaces by assembling microspheres with distinct mechanical stiffness, degradation rates, or stress relaxation profiles.¹³

This review systematically delineates material design and mechanical encoding strategies for HMs. We then elucidate the core mechanisms driving HMs as active microenvironmental modulators to prime host or organoid niches for endogenous ECM/PCM remodeling (Figure 1). Finally, we summarize how this structural encoding propels musculoskeletal organoids from mere morphological resemblance toward profound functional maturation, outlining the translational landscape for musculoskeletal tissue regeneration and repair.

2. Mechanical encoding strategies for hydrogel microspheres

Hydrogel microspheres, defined as three-dimensionally cross-linked polymeric networks engineered at the micrometer scale, have assumed a pivotal role in contemporary regenerative medicine, precision drug delivery, and tissue engineering. Their prominence is attributed to their exceptional hydration capacity, superior biocompatibility, and highly tunable mechanical properties.^{14–16} The distinct high surface-to-volume ratio of these spherical micro-architectures not only optimizes interaction efficiency with cellular components and biofluids but has also driven a paradigm shift in the research trajectory: evolving from rudimentary structural scaffolds for passive cell encapsulation toward “intelligent” and systemic mechanical encoding frameworks capable of sensing microenvironmental fluctuations, autonomously modulating therapeutic release kinetics, and orchestrating complex tissue regeneration.^{17,18}

2.1 Matrix selection and cross-linking chemistry of hydrogel microspheres

2.1.1. Natural polymeric materials

Hydrogel microspheres fabricated from natural polymers comprise three-dimensionally cross-linked networks capable of substantial aqueous absorption while maintaining structural integrity. These matrices are characterized by innate biocompatibility and biodegradability.¹⁴ Prominent natural substrates include gelatin, collagen, alginate, chitosan, hyaluronic acid (HA), silk fibroin (SF), cellulose, agarose, and decellularized ECM (dECM).¹⁹ The drawback of using these materials in the clinical context is their non-negligible risk of immunogenicity and their batch-to-batch variations. Owing to their abundant sources, cost-effectiveness, and ease of chemical modification, these materials can be processed via diverse fabrication methodologies.²⁰ Furthermore, their superior biological

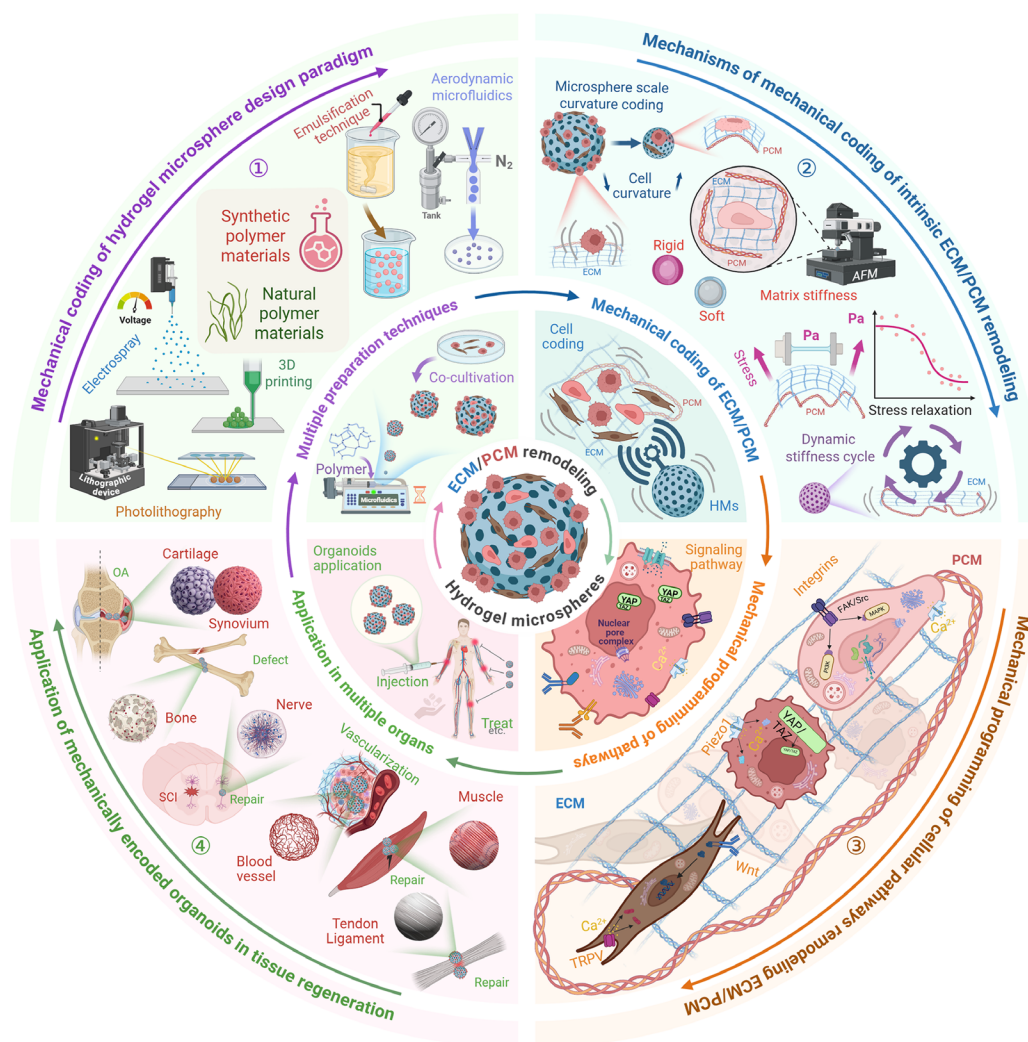


Figure 1. Schematic of mechanical encoding in hydrogel microsphere-mediated ECM/PCM remodeling and skeletal muscle organoid regeneration. (i) Design strategies for mechanically encoded hydrogel microspheres: Aerodynamic microfluidics, 3D bioprinting, lithography, and microfluidics. (ii) Mechanical encoding of ECM/PCM remodeling via hydrogel microspheres: Key parameters include dynamic stiffness, microsphere scale and curvature, stress relaxation, and matrix rigidity. (iii) Mechanotransduction signaling pathways in microsphere-mediated remodeling: Regulation via TRPV channels and nuclear pore complexes, Wnt/ β -catenin signaling, Piezo- Ca^{2+} signaling, the YAP/TAZ axis, and the integrin-FAK axis. (iv) Applications of mechanically encoded organoids in tissue regeneration: organoids of cartilage, synovium, bone, vasculature, and skeletal muscle. Created in BioRender. Lei, Y. (2026) <https://BioRender.com/v73b7iq>

Abbreviations: 3D: Three-dimensional; ECM: Extracellular matrix; FAK: Focal adhesion kinase; HMs: Hydrogel microspheres; PCM: Pericellular matrix; SCI: Spinal cord injury; TRPV: Transient receptor potential vanilloid; Wnt: Wingless-related integration site; YAP/TAZ: Yes-associated protein/transcriptional co-activator with PDZ-binding motif.

profile, tunable mechanical properties of several components, but not all, e.g., gelatin, collagen, alginate, and functional versatility, have facilitated their extensive implementation in tissue engineering, precision drug delivery, and various biomedical applications²¹ (Table 1).

(a) Gelatin and collagen

Gelatin methacryloyl (GelMA) allows for precise tailorability of mechanical strength via photo-cross-linking

while inherently retaining arginine-glycine-aspartic acid (RGD) sequences that augment cellular adhesion.^{22,23} In the context of intervertebral disc degeneration therapy, microfluidic-synthesized GelMA HMs modified with a Wnt5a mimetic peptide (Foxy5, which directs lineage-specific differentiation) and antioxidant peptides (GFA, which scavenges reactive oxygen species) can efficiently guide bone marrow mesenchymal stem cells (BMSCs) toward nucleus pulposus (NP)-like differentiation

Table 1. Material classifications, fabrication modalities, and applications of hydrogel microspheres in musculoskeletal and neural regeneration

Material matrix	Representative macromolecules	Fabrication modalities	Applications
Natural protein polymers	Gelatin (gelatin/gelatin methacryloyl), collagen, silk fibroin	Microfluidics, aerodynamic microfluidics, three-dimensional (3D) bioprinting	Construction of vascularized ossification organoids, reconstitution of tendon stem/progenitor cell niches, and induction of anisotropic skeletal myofiber alignment.
Natural polysaccharides	Hyaluronic acid, alginate (alginate/alginate methacryloyl), chitosan	Mechanical fragmentation, batch emulsion, microfluidics	<i>In situ</i> injectable porous scaffolds for cartilage defects, micro-to-nano cross-scale targeted delivery in osteoarthritis joints, and dynamic assembly of woven bone organoids.
Decellularized extracellular matrix (dECM) systems	Cartilage-derived dECM, bone-derived dECM, muscle-derived dECM	Extrusion bioprinting, physical suspension assembly	Accelerating motor neuron–muscle crosstalk (neuromuscular junction formation), maintaining spatial heterogeneity and long-term phenotypic stability in synovial organoids.
Biodegradable synthetic polymers	Poly(lactide-co-glycolide), poly(ethylene glycol), poly(vinyl alcohol)	Coaxial electrospraying, lithography/masking	Long-term sustained release and protection of sensitive macromolecules (e.g., plasmid DNA, nerve growth factor), microchannel guidance, and topological support for spinal cord injury repair.
Organic–inorganic hybrid networks	Nano-hydroxyapatite doping, gadolinium cross-linked networks, conductive graphene composites	Contactless acoustic assembly, <i>in situ</i> mineralizing microfluidics	Spontaneous biomimetic mineralization for critical-sized calvarial defects, neuro-vascular synergistic regeneration in ischemic limbs, and real-time 3D magnetic resonance imaging tracking of <i>in situ</i> organoid degradation.

and modulate ECM regeneration without exogenous growth factor supplementation.²⁴ Collagen constitutes the predominant structural backbone of the native cartilaginous ECM (primarily type II) and the PCM, where type VI collagen acts as a primary mechanotransductive coupler.^{25,26} Microfluidic-optimized fish collagen composite microspheres loaded with vancomycin deliver localized antimicrobial efficacy against osteomyelitis while accelerating bone defect repair.²⁷ Concurrently, an amniotic-derived collagen hydrogel encapsulating stromal cell-derived factor-1 α (SDF-1 α), a potent stem cell chemoattractant, significantly accelerates angiogenic efficiency and full-thickness wound healing.²⁸ Integrating poly(ethylene glycol) diacrylate via photo-cross-linking substantially augments the structural stability and proteolytic resilience of these natural matrices.^{29,30}

(b) Alginate

Sodium alginate utilizes rapid ionic cross-linking to serve as a versatile encapsulation matrix for musculoskeletal tissue engineering.^{31,32} Addressing the need for precise bioactive cue presentation, Qin *et al.*³³ fabricated a biphasic sustained-release platform utilizing small intestinal submucosa hydrogel/sodium alginate HMs to co-deliver SDF-1 α and bone morphogenetic protein 12, providing a coordinated spatiotemporal delivery framework that enhances cargo stability and bioactivity for tendon

regeneration. To remedy the inherent mechanical fragility and high swelling of pure alginate, current workflows implement strategic hybridization networks, such as blending with gelatin to improve toughness, incorporating nanoclays to suppress swelling, or applying polydopamine coatings to substantially augment interfacial bioadhesion.³⁴

(c) Chitosan

Chitosan is a cationic polysaccharide derived from the deacetylation of chitin, primarily sourced from the exoskeletons of crustaceans.^{35,36} Chitosan offers intrinsic pH-responsiveness and cationic antimicrobial properties optimal for tissue engineering scaffolds.^{37–41} To manage infected tissue niches, HMs encapsulating the antimicrobial peptide LL37 shield the cargo from proteolytic degradation, facilitating targeted toxin clearance and accelerated healing.⁴² Nevertheless, the clinical translation of pure chitosan is hampered by its rapid degradation in neutral environments, limited solubility restricted to weakly acidic media, and suboptimal mechanical robustness.⁴³ To resolve these limitations, hybridization with two-dimensional (2D) graphene oxide (GO) nanosheets establishes a highly stable network via abundant interfacial interactions, markedly reinforcing structural integrity and cargo-loading capacity. Crucially, the embedded GO architecture imparts electro-responsive properties to the HMs, permitting precision-triggered therapeutic delivery under external electric field stimulation to augment regenerative efficiency.⁴⁴

(d) Hyaluronic acid

Hyaluronic acid is a prominent glycosaminoglycan composed of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid, ubiquitously distributed throughout the synovial fluid and cutaneous tissues.^{45–47} HA HMs cross-linked via dynamic covalent chemistries, such as Schiff base linkages, permit precision-controlled cargo liberation to facilitate osteochondral repair.^{48,49} For instance, Lin *et al.*⁵⁰ engineered a sequential factor-delivery microgel system using methacrylated HA and heparinized microparticles (HAMA@HEPMA) to co-deliver IL-4 and TGF- β 1; this platform effectively suppressed early-stage TH17/IL-17 inflammatory cascades and sequentially drove BMSC chondrogenesis, successfully transitioning the pro-inflammatory microenvironment into a pro-regenerative milieu while mitigating cartilage degradation and osteoclastogenesis.⁵⁰ To remedy the suboptimal mechanics of native HA, Hou *et al.*⁵¹ utilized microfluidics to develop metformin-loaded, sulfobetaine-modified HA HMs that exhibit excellent boundary lubricity and sustained-release profiles, successfully ameliorating osteoarthritis (OA) pathogenesis in aged mice by suppressing chondrocyte senescence via the iNOS/ONOO[−]/P53 pathway.⁵¹

(e) Silk fibroin

Silk fibroin exhibits exceptional mechanical strength and structural stability owing to its highly organized β -sheet crystalline domains.⁵² For infected skeletal lesions, Ma *et al.*⁵³ developed a mussel-inspired SF/hydroxyapatite hybrid HM platform that synergistically eradicates drug-resistant bacteria via photothermal therapy while enhancing osteoinductive activity and structural stability to drive bone defect regeneration.⁵³ To remedy the inherent hydrophobicity and suboptimal cell adhesion of pristine SF, strategies such as RGD peptide modification or plasticization with hydrophilic polyols (e.g., glycerol) are widely implemented to reduce the water contact angle, promote inter-chain diffusion, and substantially enhance cell-matrix interactions.⁵⁴ These strategic modifications preserve the intrinsic merits of SF while substantially expanding its functional repertoire in regenerative medicine.

(f) Cellulose

Cellulose variants, including bacterial cellulose and cellulose nanocrystals (CNCs), are increasingly integrated into tissue engineering scaffolds due to their high crystallinity and structural stability.^{55,56} To modulate microenvironmental architectures, CNCs have been hybridized with sodium alginate via electro-processing to yield stimuli-responsive delivery vehicles,⁵⁷ while directional freezing approaches generate cellulose-based cryogel HMs with distinct nanoscale porosity (up to 95%) that significantly enhances

cell adhesion efficiency.⁵⁸ Recognizing that pristine cellulose frequently suffers from low intrinsic porosity and restricted loading capacities, contemporary workflows utilize Pickering emulsion templating or ionic liquid-assisted dissolution for functional hybridization with chitosan.⁵⁹ These modifications substantially optimize structural integrity and functional performance, tailor-making cellulose-based matrices for advanced musculoskeletal regenerative applications.

(g) Agarose

Agarose possesses unique thermo-reversible gelation and uniform porosity, rendering it highly suitable for microfluidic fabrication and three-dimensional (3D) bioprinting workflows.^{60,61} Highlighting its regenerative potential, Huang *et al.*⁶² underscored agarose-based matrices as innovative solutions for enhancing cellular adhesion and biological activity in bone tissue repair.⁶² Although thermo-responsive agarose platforms enable controlled, stimulus-triggered multi-agent delivery,⁶³ the clinical translation of pristine agarose is hindered by biological inertness, low mechanical strength (<50 kPa), and poor long-term stability. To satisfy the mechanical demands of musculoskeletal scaffolds, current strategies employ functional modifications, such as hybridizing agarose with sodium alginate to establish dual-cross-linked networks or methacrylation combined with photo-curing to augment matrix stiffness and structural integrity.⁶⁴

(h) Decellularized extracellular matrix

Decellularized extracellular matrix represents the biomimetic “gold standard” for engineering scaffolds due to its native biochemical and structural signatures.⁶⁵ Processing bulk dECM into monodisperse, injectable HMs via microfluidics or electrospraying enhances nutrient mass transfer and clinical delivery.^{66,67} For chondrogenic regeneration, Chen *et al.*⁶⁸ engineered a dECM microsystem that drives BMSC chondrogenesis and suppresses inflammation in OA,⁶⁸ while Luo *et al.*⁶⁹ utilized bone-derived dECM microgels to accelerate osseous repair by mimicking native osteogenic niches.⁶⁹ Beyond expanding into visceral and cutaneous environments,⁷⁰ dECM matrices are increasingly optimized for multi-responsive, on-demand drug delivery in arthritic joints.⁷¹ Nevertheless, manufacturing standardization and mechanical fragility remain key translational bottlenecks, requiring strategic chemical modifications and material hybridization to construct next-generation, mechanically-adapted intelligent HMs⁶⁶.

2.1.2. Synthetic polymeric materials

Synthetic polymeric HMs—principally aliphatic polyesters (e.g., poly[lactic-co-glycolic acid]; PLGA) and polyethers (e.g., polyethylene glycol [PEG]) or their copolymeric

networks—offer superior mechanical robustness, highly tailorable degradation, and scalability for standardized production. Frequently hybridized with natural substrates to optimize cell–matrix interactions, these injectable platforms facilitate minimally invasive orthopedic interventions. Consequently, integrating these structurally stable synthetic architectures with cellular therapies holds immense clinical promise for orchestrating bone defect repair, spinal fusion, and complex craniofacial or periodontal reconstruction.^{72,73}

(a) Polyethylene glycol

Polyethylene glycol features high chemical versatility and biocompatibility, amenable to precise covalent functionalization,⁷⁴ allowing PEG HMs to serve as sophisticated therapeutic delivery platforms,^{75,76} provided that sterilization workflows are optimized against free-radical-induced cargo degradation.⁷⁷ To overcome the inherent biological inertness of pristine PEG,⁷⁸ contemporary musculoskeletal strategies hybridize branched, multi-arm PEG architectures with bioactive macromolecules, such as chondroitin sulfate. These composite microgels substantially enhance cellular affinity while optimizing the mechanical resilience and sustained degradation profiles essential for articular cartilage repair.^{78,79}

(b) Polyvinyl alcohol

Polyvinyl alcohol (PVA) offers superior mechanical robustness and tunable structural properties, establishing it as a premier candidate for artificial NP replacements and targeted therapeutic delivery in degenerative spinal disorders.^{80,81} Despite these merits, the clinical utility of pristine PVA is often constrained by its limited thermal stability and a lack of intrinsic bioactive motifs,^{82,83} strategic modifications such as bio-nanocellulose (bacterial nanocellulose/PVA) hybridization have been deployed to markedly augment crystallinity and thermal resilience.⁸⁴ Beyond spinal applications, the PVA backbone is highly adaptable for fabricating multi-component therapeutic platforms, including bilayer antimicrobial matrices⁸⁵ and glucose-responsive networks cross-linked via dynamic boronate ester bonds; these advanced configurations optimize soft-tissue microenvironments by suppressing inflammatory cascades and accelerating neovascularization during wound healing.⁸⁶

(c) Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) HMs offer highly tailorable degradation kinetics suitable for musculoskeletal drug delivery and scaffolding.⁸⁷ However, pristine PLGA suffers from inherent brittleness and the accumulation of acidic degradation by-products that can incite localized

inflammation.⁸⁸ To mitigate these limitations, contemporary strategies utilize surface coatings (e.g., chitosan) to neutralize acidity, or hybridization with hydroxyapatite to enhance mechanical resilience.^{89–91} In osteoarthritic management, embedding PLGA nanoparticles or microspheres within hydrogel matrices creates hierarchical networks that achieve sequential payload release⁹²; for instance, a single intra-articular injection of triamcinolone-acetonide-loaded PLGA nanoparticles effectively suppresses inflammation and drives cartilage repair. Furthermore, optimizing polymer concentrations yields fentanyl-loaded PLGA microspheres with quasi-zero-order release kinetics, providing sustained analgesia for up to 15 days to alleviate mechanical joint pain.⁹³

2.1.3. Combined natural and synthetic polymers

A new class of polymer materials is composed of short to ultrashort peptides containing no more than 3–7 natural amino acids.^{94–96} Characterized by their synthetic purity, these matrices circumvent batch variability, pathogen risks, and immunogenicity associated with animal-derived substrates. Notably, lysine-rich peptide bioinks permit instantaneous cell-laden gelation under true physiological salt conditions, entirely bypassing harsh chemical cross-linkers, temperature shifts, or ultraviolet (UV) irradiation.^{97,98} When processed into HMs, these self-assembling architectures efficiently deliver endothelial cells to drive branched macrovascular network morphogenesis.⁹⁹ This paradigm underscores the profound potential of smart peptide hydrogels to serve as completely xeno-free, chemically defined platforms for high-fidelity musculoskeletal organoid fabrication and tissue regeneration.

2.2. Fabrication methodologies

The synthesis of HMs encompasses a diverse array of fabrication modalities, each tailored to meet specific downstream requirements. Prevailing methodologies include microfluidics, air-flow-driven techniques, electro-spraying, photolithography, 3D bioprinting, bulk emulsification, and mechanical fragmentation. The selection of a particular technique is predicated upon a strategic balance between desired microsphere dimensions, monodispersity, targeting capabilities, encapsulation efficiency, and degradation kinetics. As HMs increasingly serve as sophisticated biomimetic vehicles, the horizon of this field is defined by the synergistic integration of novel functional materials with organ-on-a-chip technologies.¹⁰⁰ The convergence of micro-nanofabrication and tissue engineering is driving the development of next-generation HMs capable of recapitulating the structural and functional complexity of native physiological environments.¹⁰⁰

2.2.1. Microfluidics

Microfluidic technology enables precise manipulation of micro-scale fluid dynamics to generate highly monodisperse HMs with structural complexity,^{101–103} though constrained by high instrumentation costs and limited throughput.¹⁰⁴ In OA management, this precision facilitates advanced mechanobiological and immunomodulatory interventions. For instance, Lei *et al.*¹⁰⁵ fabricated RAPA@Lipo@HMs that continuously expose liposomes under joint friction to establish a self-renewable lubrication layer while activating chondrocyte autophagy¹⁰⁵ (Figure 2A). To modulate the arthritic microenvironment, Xiao *et al.*¹⁰⁶ developed antibody-functionalized HMs that efficiently reprogram pro-inflammatory M1 macrophages into a pro-regenerative M2 phenotype, successfully suppressing synovial inflammation and matrix degradation.¹⁰⁶ Additionally, Liu *et al.*¹⁰⁷ engineered aldehyde-functionalized HMs leveraging Schiff base chemistry for micro-scale cartilage adhesion and CD44-mediated nano-scale targeting, effectively scavenging reactive oxygen species to restore mitochondrial bioenergetics.¹⁰⁷

2.2.2. Aerodynamic microfluidics

Aerodynamic microfluidics leverages high-velocity gas streams to achieve rapid, high-throughput aerosolization of monodisperse droplets,¹⁰⁸ effectively circumventing the throughput limitations of traditional capillary networks.¹⁰⁹ In skeletal regeneration, Xiao *et al.*¹⁰⁶ utilized this high-throughput modality to fabricate mineralized GelMA/alginate methacryloyl (AlgMA) HMs encapsulating Jagged1-functionalized osteocyte membrane vesicles; this platform established a biomimetic osteogenic niche that activated the Notch pathway, accelerating critical-sized bone defect repair.¹⁰⁶ Furthermore, Chen *et al.*¹¹⁰ employed gadolinium ions as active cross-linkers to synthesize double-network microgels (G-AlgMA HMs), providing inherent magnetic resonance imaging visibility without signal attenuation.¹¹⁰ The controlled erosion of these HMs systematically alters local water relaxivity to enable non-invasive, real-time radiopaque monitoring of matrix degradation synchronized with peptide-driven localized angiogenesis.

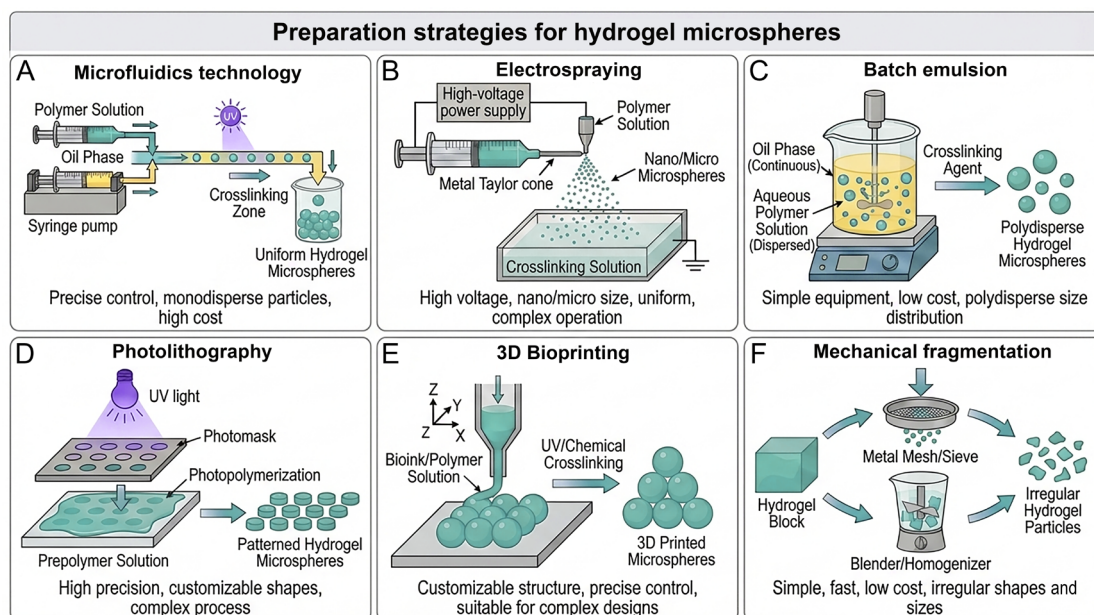


Figure 2. Fabrication strategies for hydrogel microspheres. (A) Microfluidics: Precision generation of monodisperse droplets via micro-scale channels. Coupled with photo-initiated or ionic cross-linking, this technique produces highly uniform microspheres with tailor-made dimensions and geometries.¹⁰⁵ (B) Electrospaying: Atomization of polymer solutions into charged microdroplets under a high-voltage electrostatic field. This method is proficient at fabricating nano- and micro-scale particles while circumventing the protein denaturation typical of conventional emulsion techniques.¹¹⁵ (C) Batch emulsion: A “bottom-up” approach involving the mechanical dispersion of an aqueous polymer phase into a continuous oil phase. Characterized by minimal instrumentation requirements and cost-effectiveness, though typically resulting in a broader polydispersity.¹³⁸ (D) Lithography: A high-precision patterning technique utilizing ultraviolet irradiation and optical masks to template hydrogel colloids. It enables the fabrication of customized shapes and microarrays with micrometer-level fidelity. (E) Three-dimensional (3D) bioprinting: A computer-aided design-based manufacturing modality utilizing layer-by-layer digital deposition to construct 3D entities. This technology enables complex architectural designs and the integration of heterogeneous functional materials (e.g., cells and therapeutics) into a single structural unit.¹³² (F) Mechanical fragmentation: A “top-down” strategy employing rotary blenders or metallic sieves to physically disrupt bulk hydrogels into micro-scale particles. While rapid, simplistic, and scalable, it yields irregular morphologies with high polydispersity.¹⁴² Created by the authors using Adobe Photoshop with AI-assisted design tools.

2.2.3. Electrospraying

Electrospraying utilizes high-voltage electrostatic fields to atomize polymer solutions into highly uniform microgels, presenting a highly controllable paradigm for fabricating drug-loaded matrices such as PLGA.^{111,112} Although requiring meticulous parameter optimization to mitigate electrical damage to sensitive cargos,¹¹³ this technique streamlines traditional manufacturing workflows by producing porous microspheres with tailored topologies.¹¹⁴ Advancing this paradigm, coaxial electrospraying circumvents the protein denaturation and aggregation common in traditional aqueous-organic emulsion interfaces¹¹⁵ (Figure 2B). This methodology successfully encapsulates sensitive macromolecules—including proteins, enzymes, plasmid DNA, and even viable cells—within biodegradable HMs, maintaining over 90% of native bioactivity and sustaining release kinetics beyond 30 days for precise tissue engineering applications.

2.2.4. Lithography

Lithography offers a precise “top-down” masking paradigm using UV irradiation or digital micromirrors to template photosensitive materials into shape-defined arrays with micrometer-level fidelity.^{116,117} This microfabrication significantly expands the design space for hydrogel particles, enabling tailored internal architectures and controlled aspect ratios for advanced scaffolding.^{118,119} For instance, Palange *et al.*¹²⁰ engineered deformable micro-combinatorial hydrogel particles optimized for multi-agent co-loading by cross-linking short polymeric chains within shape-defined templates.¹²⁰ Despite providing unparalleled control over microstructural complexity essential for high-precision tissue engineering, lithography remains constrained by high operational costs and complex workflows, limiting its scalability for high-throughput industrial manufacturing⁹ (Figure 2D).

2.2.5. Three-dimensional bioprinting

Three-dimensional bioprinting constructs personalized architectural frameworks via layer-by-layer digital deposition,^{121–124} offering superior control over internal morphology, uniform porosity,¹²⁵ and multi-directional payload release kinetics within HMs.^{126,127} In musculoskeletal engineering, airflow-assisted 3D bioprinting enables the one-step fabrication of hierarchical helical microstructures within microspheres,¹²⁸ successfully recapitulating the complex anatomy of helically vascularized ossification organoids.¹²⁹ Additionally, high-throughput “bead-jet” systems facilitate the intraoperative delivery of mesenchymal stem cell (MSC)-laden microgels,

augmenting stem cell functionality to accelerate skeletal muscle regeneration with minimal fibrosis.¹³⁰ Despite translational hurdles regarding long-term structural stability under dynamic physiological loading^{131,132} (Figure 2E), contactless 3D acoustic bioassembly utilizes controlled acoustic fields to pattern microgels without nozzle-induced mechanical shear, offering a gentle, complementary strategy to organize functional HMs into complex musculoskeletal constructs.¹³³

2.2.6. Batch emulsion

Batch emulsion operates as a cost-effective, high-throughput “bottom-up” modality for scaling HM production,¹³⁴ though inherently constrained by high polydispersity and poor morphological control compared to microfluidics.¹³⁵ In regenerative medicine, this technique is widely implemented for mass-fabricating sustained-release platforms and 3D cell carriers.¹³⁶ For instance, Contessi Negrini *et al.*¹³⁷ synthesized cross-linked gelatin microspheres using N,N'-methylene bisacrylamide, yielding dimensionally stable, highly spherical structural vehicles that successfully supported long-term cell culture and tissue assembly.¹³⁷ Furthermore, polysaccharide and PEG-hybrid microgels fabricated via bulk emulsification have been optimized to encapsulate cells, establishing uniform 3D models to resolve complex cell-cell and cell-matrix interactions¹³⁸ (Figure 2C). These configurations underscore the utility of emulsion-derived architectures for high-throughput screening and functional tissue scaffolding.

2.2.7. Mechanical fragmentation

Mechanical fragmentation operates as a rapid, high-throughput “top-down” modality that physically disrupts bulk hydrogels into granular microgels via mechanical shear, though inherently limited by high polydispersity.¹³⁹ Despite its morphological imprecision, this approach offers immense translational utility for low-cost, intraoperative musculoskeletal scaffolding.¹⁴⁰ For instance, Puiggali-Jou *et al.*¹⁴¹ forced bulk methacrylated HA and highly negatively charged sulfated HA hydrogels through meshes to yield microgels that sequestered growth factors (TGF- β 3/PDGF-BB) into bioactive “micro-islands”; subsequent enzymatic annealing generated an injectable, heterogeneously porous scaffold that accelerated cartilage repair.¹⁴¹ Similarly, high-speed blending of bulk hydrogels produces granular gelatin slurries capable of restoring aged tendon stem/progenitor cell functions¹⁴² (Figure 2F). This technical accessibility underscores the value of fragmented microgels for the large-scale assembly of functional tissue niches.

2.3. Construction modalities of microsphere-based organoids

2.3.1. Encapsulation culture

The intrinsic cross-linked network of hydrogels closely mimics the natural ECM, providing essential structural scaffolding for cellular growth, migration, and proliferation in 3D. Within these encapsulation environments, cells can self-assemble into spherical micro-tissues—driven by surface energy minimization—or undergo directed differentiation and spontaneous aggregation to form organoid models that faithfully mirror the architecture and function of parent organs.¹⁴³ Compared to conventional bulk hydrogels, microgels offer distinct technical advantages through miniaturization: they effectively eliminate diffusion barriers for nutrients and oxygen, markedly enhancing mass transfer efficiency, and circumvent the limitations of optical scattering that often hinder high-resolution microscopic visualization in bulk systems.^{144,145} Furthermore, microgel platforms enable the systematic remodeling of the tumor microenvironment or stem cell niches, serving as ideal tools for investigating cellular fate commitment and metabolic kinetics under varying physical constraints^{146–148}. In organoid engineering, HMs leverage their high surface-area-to-volume ratio to optimize solute diffusion kinetics. This significantly mitigates the risk of core necrosis—a common failure mode in traditional models caused by steep oxygen and nutrient gradients.¹⁴⁹ Recent advancements have pivoted toward integrated microfluidic platforms, utilizing negative-pressure-driven systems to achieve high-throughput (>200 uL/h) and monodisperse production. These platforms further utilize core-shell or Janus architectures to precisely arrange diverse cell types, thereby reconstructing complex spatiotemporal heterogeneity within tumor microenvironments.^{12,150} OA poses a clinical challenge due to the avascular nature and limited regenerative capacity of articular cartilage, with existing therapeutic strategies suffering from multiple drawbacks. Yang *et al.*¹⁵¹ prepared a composite bioink comprising type II collagen monoclonal antibody and chondrocytes and constructed engineered cartilage organoids via digital light processing (DLP) 3D bioprinting. The organoids exhibited excellent performance and induced the formation of neocartilage resembling native cartilage in a rat model, providing a novel, biomimetic, and scalable strategy for the treatment of OA.¹⁵¹

2.3.2. Surface culture and interfacial interactions

Surface culture on HMs and the study of interfacial interactions are pivotal for deciphering complex biological signaling pathways. Utilizing droplet microfluidics, microgels enable precise control over the dimensions of cell clusters. This is particularly critical in scenarios where biological function is highly size-dependent, such as in

cancer spheroids characterized by a necrotic core and a proliferative periphery.^{152,153} The microsphere platform further facilitates in-depth investigations of intercellular crosstalk. By employing core-shell architectures or enabling direct surface contact, researchers can probe communication between tumor cells and immune cells, fibroblasts, or stromal components.¹⁵⁴ The core value of surface culture on microspheres lies in providing a microenvironment that closely mimics *in vivo* conditions, serving as an essential tool for exploring the regulatory mechanisms of cellular behavior.¹⁴⁰ This modality offers several distinct advantages: The exceptionally high surface-area-to-volume ratio significantly enhances nutrient and gas exchange. Research indicates that maintaining a microgel radius below 200 µm preserves stable oxygen and nutrient gradients, effectively circumventing the central necrosis common in bulk hydrogel systems.¹⁵⁵ Microfluidically engineered curved interfaces can simulate complex tissue geometries, such as pulmonary alveoli, to guide cells into forming physiologically relevant monolayers.¹⁵⁶ Physical contact at the interface serves as a key regulator of cellular fate, influencing the maintenance of stem cell pluripotency or modulating hepatocyte proliferation.^{157,158} Despite these strengths, several challenges persist. First, the long-term mechanical stability of natural polymer microspheres is often insufficient for extended culture cycles.¹⁵⁹ Second, the single-cell encapsulation process is inherently governed by the Poisson distribution, leading to significant heterogeneity in initial cell loading and affecting experimental reproducibility. Finally, the efficient integration of multifunctional molecules and the standardized, large-scale production of these platforms remain technical bottlenecks.¹⁴ Current research is focused on three strategic directions to address these limitations: Enhancing the mechanical resilience and service life of natural polymers through composite modification and advanced cross-linking chemistries. Developing novel microfluidic strategies to bypass Poisson distribution limits, aiming for high-efficiency, uniform single-cell encapsulation. Establishing automated, large-scale production workflows while optimizing the loading and integration of bioactive moieties. Furthermore, the integration of interdisciplinary technologies, such as machine learning, is enabling researchers to systematically resolve the influence of diverse culture parameters on cellular behavior, providing a theoretical foundation for the precision optimization of culture systems.¹⁶⁰

2.3.3. Organ-on-a-chip culture

Organ-on-a-chip represents a transformative paradigm that integrates microfluidic technologies with tissue engineering, utilizing platforms such as microwell arrays, bifurcated channels, and paired droplet fusion to construct *in vitro* physiological and pathological models with

unprecedented precision.^{161–163} By manipulating fluids at the micrometer scale, this technology recapitulates the intricate microenvironments of human organs, including tissue-tissue interfaces, biochemical gradients, and dynamic mechanical stimuli.^{164,165} Within the realms of drug screening and toxicological assessment, these microphysiological systems exhibit superior potential compared to traditional 2D cultures and animal models, offering high-fidelity predictions of human drug responses and significantly mitigating clinical trial failure rates and developmental costs.¹⁶⁶ As life sciences research transitions from static observation toward dynamic systems modeling, organoids—3D cellular assemblies formed by stem cell self-organization—have emerged as a pivotal bridge between *in vitro* models and *in vivo* physiology.¹⁶⁷ However, conventional organoid cultures, which often rely on static, uncontrolled suspension or Matrigel embedding, suffer from limitations such as size heterogeneity, restricted nutrient transport, and a lack of physical stimulation.¹⁶⁸ To circumvent these bottlenecks, the convergence of microfluidics and organoid technology has given rise to the field of organoids-on-a-chip.^{169,170} By integrating perfusion, mechanical loading, and real-time monitoring, these systems provide a dynamic microenvironment that more faithfully mimics the *in vivo* niche.^{171,172} Despite this potential, HMs technology faces technical trade-offs and bottlenecks. The high shear stress during droplet generation, exposure to free radicals during cross-linking, and interfacial tension within the oil phase can jeopardize fragile primary cells. Research indicates that microchannel dimensions significantly impact cell viability, with narrower channels generating shear forces that drastically reduce survival rates.¹⁷³ Furthermore, while HMs facilitate small-molecule diffusion, the typical nanometer-scale mesh size (10–1,000 nm) of natural hydrogels often hinders the migration of viruses, pathogens, or immune cells, thereby limiting the authenticity of infection models.¹⁷⁴ As a highly heterogeneous tissue regulated by complex biomechanics, its modeling requires exquisite control over the ECM, the PCM, and multiaxial mechanical loading. Native articular cartilage exhibits pronounced zonal heterogeneity: the superficial zone features tangentially aligned type II collagen fibers designed to withstand friction and shear while secreting lubricin (proteoglycan-4) to maintain a lubricated environment.¹⁷⁵ In contrast, the middle zone contains randomly distributed fibers, while the deep zone fibers are oriented perpendicularly to the interface and are enriched with glycosaminoglycans to resist high-intensity compressive loads.¹⁷⁶ Crucially, the PCM, which, alongside the chondrocyte, constitutes the “chondron,” is enriched with type VI collagen and laminin, typically possessing a lower modulus than the surrounding ECM. Emerging evidence suggests the PCM functions as a “mechanical coupler” in mechano-transduction, converting macroscopic

ECM compressive loads into microscopic physical cues that modulate surface ion channel activation and trigger protective metabolic responses. In osteochondral chips, encapsulating cells within HMs provides a preliminary simulation of the PCM's physical confinement. Advanced biofabrication techniques, such as coaxial electrospray, are now being explored to endow HMs with an “artificial PCM” shell of specific stiffness, enabling investigations into the mechanical degradation mechanisms during the progression of OA.⁷⁴

3. Mechanical encoding of extracellular matrix/pericellular matrix remodeling via hydrogel microspheres

3.1. Functional significance of endogenous extracellular matrix/pericellular matrix

The musculoskeletal system relies on a highly coordinated ECM and PCM hierarchy to govern tissue-level biomechanics and cellular homeostasis.¹⁷⁷ While the macro-scale ECM acts as a structural scaffold, driving mechanotransduction and lineage specification,¹⁷⁸ the PCM—a specialized microdomain immediately enveloping individual cells—functions as a biomechanical transducer and selective filter that orchestrates reciprocal cell–matrix communication.^{179,180}

In skeletal muscle, the ECM forms a hierarchical reticular network composed of collagens (types I, III, IV) and proteoglycans structured across multi-scale matrices.¹⁸¹ This framework mediates the lateral transmission of contractile forces, dictates passive compliance, and protects myofibers from contraction-induced sarcolemma injury.¹⁸² Concurrently, ECM–integrin interactions modulate myoblast proliferation, fusion, and neuromuscular junction (NMJ) maturation, whereas aberrant matrix remodeling drives fibrotic degeneration.¹⁸³

In articular cartilage, the avascular ECM comprises a dense type II collagen network interspersed with highly hydrated aggrecan to withstand heavy compressive regimes.¹⁷⁹ Superimposed on this macro-matrix is the PCM, which combines with the sequestered chondrocyte to form the “chondron”—the primary functional unit of cartilage.¹⁸⁴ Enriched with type VI/IX collagens and perlecan,¹⁸⁴ the PCM filters osmotic and fluid dynamics while transducing localized stress-strain fields to maintain anabolic-catabolic equilibrium.¹⁸⁵ Furthermore, PCM components actively sequester and modulate growth factor bioavailability to guide spatiotemporal regenerative repair.^{186,187}

Collectively, the musculoskeletal ECM/PCM hierarchy serves as a dynamic, mechanobiologically active signaling hub indispensable for force transduction, phenotypic stability, and physiological homeostasis.

3.2. Mechanotransductive cues in hydrogel microspheres for extracellular matrix/pericellular matrix remodeling

Extracellular matrix and PCM biomechanics are critical determinants of cellular lineage commitment and tissue remodeling. Due to their modular mechanical tunability and interstitial porosity, HMs and granular hydrogels serve as sophisticated platforms for interrogating the effects of elastic and compressive moduli on stem cell fate.^{143,188} Specifically, we synthesize how matrix rigidity, viscoelastic stress relaxation, curvature-encoded topographies, and temporal stiffness fluctuations collectively modulate endogenous ECM/PCM remodeling.

3.2.1. Matrix rigidity: Impact of localized compressive moduli and microsphere stiffness on extracellular matrix/pericellular matrix remodeling

Tissue engineering and regenerative medicine are predicated on the high-fidelity recapitulation of the native cellular niche to orchestrate specific cellular behaviors.¹⁸⁹ Within endogenous tissues, the ECM functions not merely as a structural scaffold, but as a primary reservoir of biophysical cues; its mechanical attributes—notably stiffness and viscoelasticity—profoundly modulate cellular adhesion, migration, proliferation, and lineage-specific differentiation via sophisticated mechano-transductive signaling pathways.¹⁹⁰ Particularly within the context of articular cartilage engineering, the meticulous calibration of matrix rigidity is paramount, as cartilaginous tissues possess a distinctive viscoelastic profile and a defined range of compressive moduli. While conventional tissue engineering paradigms have frequently prioritized mechanical homogeneity, contemporary strategies, such as gradient hydrogels, enable researchers to simultaneously interrogate a spectrum of mechanical regimes to identify optimal microenvironmental conditions for chondrogenesis.¹⁹¹ Furthermore, the cross-linking density of the hydrogel dictates not only its macroscopic stiffness but also the kinetics of nutrient transport and the spatial distribution of de novo matrix deposition—factors that are indispensable for the functional integrity and long-term stability of engineered constructs.¹⁹² Recent advancements further underscore that, beyond traditional elastic moduli, the viscous modulus and interstitial porosity represent pivotal determinants in fine-tuning cellular metabolic responses.^{193,194}

Recent investigations underscore that, beyond conventional elastic moduli, the viscous modulus and interstitial porosity are pivotal determinants in modulating cellular responses.^{194,195} In *in vitro* models, researchers have demonstrated that precisely tuning the elastic modulus of hydrogel matrices (on the kilopascal scale) can autonomously direct the lineage specification of MSCs in

the absence of exogenous biochemical induction.¹⁹⁶ For instance, compliant matrices (≈ 5.75 kPa) have been shown to favor pro-chondrogenic differentiation.¹⁹⁷ Regarding dedifferentiated chondrocytes, meticulous calibration of hydrogel elasticity facilitates phenotypic rescue and preserves the chondrogenic secretome.¹⁹⁸ Furthermore, the precise mechanical control of HMs (within the kPa range) guides MSC chondrogenesis, while the mechanical mismatch between the PCM and the bulk ECM acts as a biophysical rheostat to regulate specific cellular fate decisions.¹⁹⁹ Specifically, Chen *et al.*²⁰⁰ engineered fibronectin-functionalized GelMA microspheres with programmable elastic moduli (1–10 kPa) and ligand densities (2–10 $\mu\text{g/mL}$). Their findings revealed that low-modulus matrices (2 kPa) combined with sparse ligand density (2 $\mu\text{g/mL}$) promoted NP-like differentiation of intervertebral disc progenitor/stem cells without supplemental growth factors. Mechanistically, fluctuations in matrix rigidity and ECM composition modulate cytoskeletal tension and the nuclear translocation of Yes-associated protein (YAP) through distinct mechano-transductive pathways, thereby dictating differentiation trajectories. Collectively, these insights suggest that optimized matrix stiffness and ligand presentation can effectively “train” stem cells through mechanical conditioning, offering a robust paradigm for advanced tissue engineering and regenerative medicine.

3.2.2. Stress relaxation: Impact of stress relaxation and elastic moduli on extracellular matrix/pericellular matrix remodeling

Mechanotransduction between cells and the ECM constitutes a decisive factor in governing cellular lineage specification and tissue morphogenesis. While conventional biomaterial design has primarily emphasized static stiffness (Young's modulus), native soft tissues—such as skin, muscle, and nerves—exhibit pronounced viscoelasticity, characterized by the coexistence of viscous (time-dependent) and elastic (strain-recovery) properties.^{201,202} This time-dependent behavior is most frequently quantified via stress relaxation—the phenomenon where stress decays over time under constant strain—which has been increasingly recognized as a pivotal biophysical cue. Recent studies underscore the significance of strain-enhanced stress relaxation (SESR), revealing its fundamental role in myogenic development; a deficiency in SESR within the matrix correlates with impaired myogenic differentiation.²⁰³ Furthermore, fibrous hydrogel assemblies often display strain-hardening characteristics—a non-linear behavior that enables cells to effectively remodel and program tissue morphology via contractile forces, rendering such materials particularly advantageous for biofabrication.²⁰⁴ Elucidating and precisely characterizing such stress-dependent relaxation kinetics remains a formidable challenge in advancing the frontiers of mechano-transduction research.²⁰⁵

Pioneering studies utilizing alginate-based hydrogel systems have established that matrix viscoelasticity—specifically the rate of stress relaxation—serves as a primary physical cue in orchestrating MSC fate.²⁰¹ Rapid stress relaxation has been shown to facilitate MSC spreading, proliferation, and osteogenic commitment.²⁰¹ This dynamic relaxation operates by modulating the clustering of integrin-binding (RGD) ligands and the subsequent nuclear translocation of YAP,^{206,207} effects that significantly transcend the influence of static stiffness alone. Articular cartilage and the intervertebral disc NP are quintessential load-bearing soft tissues, where viscoelasticity is indispensable for shock absorption and functional persistence. In the context of NP regeneration, leveraging viscoelastic hydrogels to recapitulate the unique dynamic mechanical niche of the NP effectively sustains the NP-like phenotype of adipose-derived mesenchymal stem cells, thereby averting fibrotic transdifferentiation.²⁰⁸ Zhao *et al.*²⁰⁹ identified chronic mechanical vibration and endoplasmic reticulum (ER) stress as primary drivers of OA progression, subsequently proposing an innovative “cellular shock-absorption” strategy. By developing viscoelastic HMs with tunable stress-relaxation profiles, they sought to mitigate OA pathology. Their work details the modulation of chemical bonding within the hydrogel network via oxidation and hydrazine ligation, yielding microspheres capable of dissipating impact energy and attenuating mechanical-stimulus-induced ER stress. By calibrating the stress-relaxation time constant to 23.81 seconds—approximating the mechanical signature of the native cartilaginous matrix—these microspheres effectively suppressed chondrocyte apoptosis by alleviating ER stress.

In summary, viscoelasticity has emerged as a universal biophysical signal for modulating cellular fate and tissue remodeling, surpassing the traditional paradigm of static stiffness models. From the lineage specification of MSCs to the complex maturation of neurons,²¹⁰ the stress-relaxation rate constitutes a pivotal design parameter. Future endeavors must prioritize the precise physiological characterization and therapeutic application of non-linear viscoelasticity, including SESR and strain-hardening behaviors.^{203–205}

3.2.3. Microsphere scale and curvature: Impact of microsphere scale and curvature on extracellular matrix/pericellular matrix remodeling

In the realms of tissue engineering and regenerative medicine, the high-fidelity recapitulation of the native cellular niche remains the cornerstone for orchestrating functional regeneration. While conventional biomaterial design has predominantly focused on modulating biochemical cues and matrix rigidity, endogenous cells *in vivo* reside within 3D microenvironments characterized by complex geometries—including the concave surfaces

of trabecular bone, the spherical geometry of chondrocyte lacunae, and the cylindrical architecture of vasculature.²¹¹ Recent investigations have highlighted that the intrinsic surface curvature (κ) of a biomaterial serves as a potent biophysical signal. For spherical microcarriers, this curvature is inversely proportional to the particle diameter (D), defined by the relationship ($\kappa = 2/D$).^{212,213} Consequently, subtle variations in microsphere scale can profoundly alter the physical curvature perceived by the cell. This “curvature encoding” modulates cellular spreading and cytoskeletal tension, thereby driving remodeling of the endogenous ECM and PCM via distinct mechano-transductive pathways.

Convex geometry represents the most distinctive architectural feature of microspheres and microcarriers. Ample evidence suggests that such geometries impose intrinsic physical constraints on cellular spreading, thereby inducing specific “dedifferentiation” or soft-tissue phenotypes. The landmark study by Lee *et al.*²¹⁴ elucidated a positive correlation between microsphere diameter and cellular spreading area: when human BMSCs were seeded onto glass beads with diameters ranging from 0.5 mm to 2 mm, smaller diameters (corresponding to higher convex curvature) resulted in diminished spreading areas and a marked inhibition of actin stress fiber assembly.²¹⁴ This finding established a fundamental paradigm: small diameter $\rightarrow$ high curvature $\rightarrow$ attenuated cytoskeletal tension $\rightarrow$ pro-soft tissue phenotype. Leveraging this theoretical framework, Chen *et al.*²⁰⁰ developed fibronectin-functionalized GelMA HMs specifically for intervertebral disc regeneration. This platform synergistically combined convex geometric constraints with low matrix stiffness (2 kPa) and sparse ligand density. The elevated surface curvature hindered robust cytoskeletal assembly, leading to cytoplasmic sequestration of the mechanosensitive transcription factor YAP and preventing its nuclear translocation. The retention of YAP in the cytoplasm serves as a critical biochemical switch that initiates NP-like differentiation. (Figure 3A). This mechanism successfully orchestrated the secretion of a specialized ECM enriched with type II collagen and aggrecan, achieving growth-factor-free “training” of cellular fate.

In stark contrast to the convex surfaces of microcarriers, native chondrocytes reside within concave “lacunae.” Emerging research has begun to employ concave geometries to reverse-engineer this specific microenvironment. Inspired by the native chondrocytic niche, Ding *et al.*²¹⁵ engineered microcarriers with concave microwells, highlighting the unique advantages of concavity for chondrogenesis. While secretomes on convex surfaces are prone to diffusion into the bulk medium, concave architectures provide a semi-enclosed 3D niche. This “bio-confinement” effect facilitates the localized accumulation

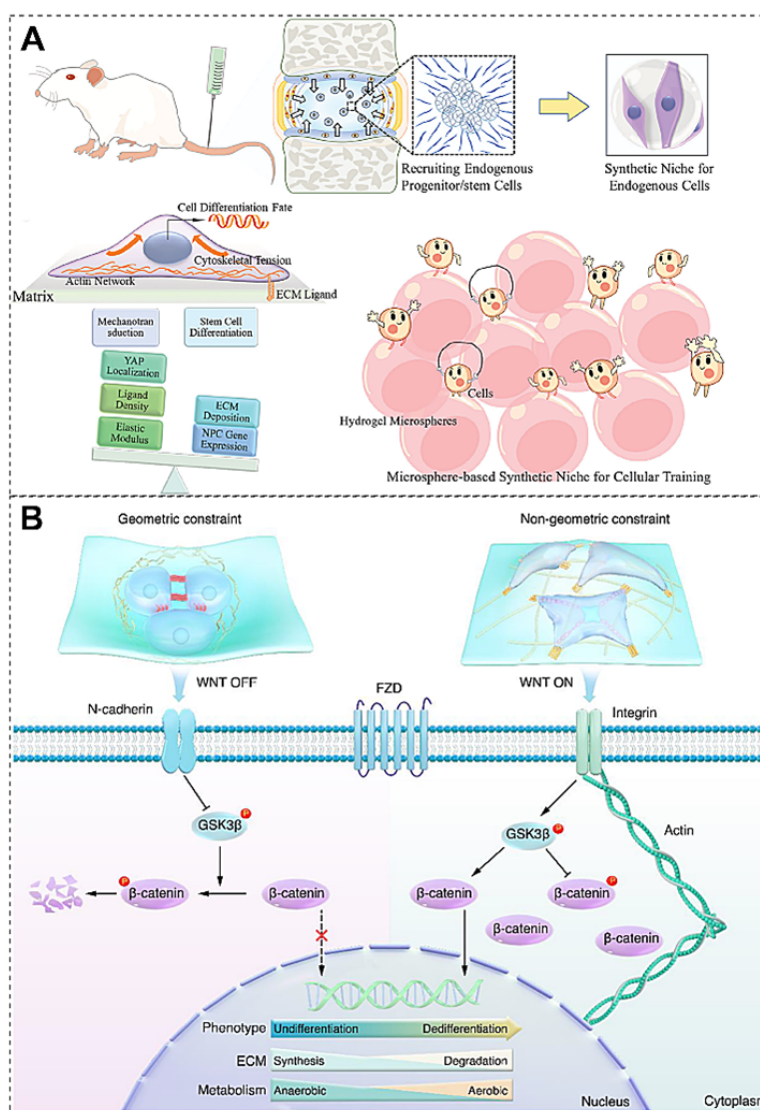


Figure 3. Regulation of extracellular matrix by microsphere mechanical stiffness and curvature. (A) Diagram of mechanical signal-tailored hydrogel microspheres capable of recruiting and training stem cells for directional differentiation to promote nucleus pulposus regeneration. Reprinted with permission from Ref.²⁰⁰ Copyright © 2023 Wiley-VCH GmbH. (B) Chondrocytes are subjected to geometric constraints and interactions with surrounding cells in lacunar hyaluronic acid microcarriers, leading to resistance to chondrocyte dedifferentiation. Reprinted with permission from Ref.²¹⁵ Copyright © 2023 Wiley-VCH GmbH. Abbreviations: ECM: Extracellular matrix; FZD: Frizzled receptor; GSK3 β : Glycogen synthase kinase-3 beta; WNT: Wingless-related integration site; YAP: Yes-associated protein.

of secreted matrix proteins (e.g., type II collagen and Aggrecan) at high concentrations around the cell, driving the assembly of a structurally dense PCM (Figure 3B). Compared to smooth-surfaced microspheres, concave microcarriers significantly enhance hyaline cartilage regeneration and effectively circumvent common fibrotic outcomes. Consequently, future biomaterial design should evolve from rudimentary “stiffness modulation” toward sophisticated topological engineering, utilizing precise control over microsphere scale and surface topography to

achieve programmable physical regulation of stem cell fate and tissue morphogenesis.

3.2.4. Dynamic stiffness: Impact of dynamic stiffness cycling on extracellular matrix/pericellular matrix remodeling

Native articular cartilage is a quintessential viscoelastic tissue that undergoes continuous, periodic dynamic compression and shear loading during physiological locomotion. Conventional engineered scaffolds have

predominantly focused on the optimization of static mechanical stiffness; however, they often neglect the intrinsic viscoelasticity and dynamic loading environments inherent to physiological joints, frequently resulting in phenotypic instability and suboptimal biosynthetic efficiency. Recent investigations demonstrate that the dynamic mechanical environment governs more than nutrient mass transfer; crucially, it directly modulates the equilibrium between chondrocyte anabolic and catabolic activities through mechano-transductive pathways.²¹⁶ Evidence suggests that rapid stress relaxation effectively alleviates “mechanical sequestration,” thereby promoting matrix assembly. Furthermore, specific dynamic compressive loading and real-time conformational shifts in scaffold architecture significantly upregulate chondrogenic gene expression and matrix accumulation.^{217,218}

Early research successfully delineated the divergent metabolic responses elicited by static versus dynamic loading. In a landmark study, Davisson *et al.*²¹⁹ demonstrated that high-amplitude (50%) static compression exerts a profound inhibitory effect on the synthesis of sulfated glycosaminoglycans and total proteins within engineered cartilage, manifesting a pro-catabolic trajectory. Conversely, tailored dynamic compressive loading exerts a potent stimulatory effect. Dynamic compression facilitates anabolic processes by inducing interstitial fluid flow, shear stress, and periodic fluctuations in cellular volume. Nevertheless, this stimulatory response is not universal; it is highly contingent upon the loading frequency, static offset, and the macroscopic architecture of the scaffold. Using finite element modeling and experimental validation, Mesallati *et al.*²²⁰ elucidated the decisive role of scaffold geometry (e.g., channeled architectures) in modulating chondrocyte mechanosensitivity. Optimizing scaffold design enables precise regulation of the interstitial fluid environment generated by dynamic compression, thereby enhancing chondrocyte synthetic activity. More recently, Farcasanu *et al.*²¹⁸ further substantiated the superiority of dynamic compression in *in vitro* models. Their findings revealed that specific mechanical stimulation effectively recapitulates the physiological joint environment, significantly upregulating cartilage-specific gene expression (e.g., *COL2A1* and *ACAN*) while promoting the accumulation and homogeneous distribution of the ECM within hydrogel scaffolds.²¹⁸ These insights underscore that dynamic loading constitutes a pivotal physiological signal for the induction and maintenance of the chondrogenic phenotype.

Recent advances have elucidated the instrumental role of scaffold dynamics in tissue regeneration. A seminal study by Zhao *et al.*²²¹ introduced the paradigm of “dynamic material signaling.” Utilizing SF hydrogels, they leveraged the spontaneous protein conformational transition—

specifically from random coils to stable β structures—following chemical cross-linking. This real-time, progressive “proteomorphosis” triggers a synchronized evolution of both mechanical properties and interstitial pore architecture. Their findings demonstrate that such intrinsic dynamic reconfigurations serve as potent biophysical stimuli, significantly augmenting the proliferation and chondrogenic lineage commitment of encapsulated progenitor cells. This concept of endogenous dynamic signaling paves the way for the development of “four-dimensional” biomaterials that exhibit time-dependent mechanical modulation. Furthermore, the interplay between neo-tissue growth and scaffold degradation constitutes a tightly coupled dynamic process. Schneider *et al.*²²² employed matrix metalloproteinase-sensitive PEG hydrogels to interrogate tissue growth kinetics under extrinsic dynamic compressive loading. They observed that dynamic loading not only accelerates the convective transport of nutrients but also facilitates programmed scaffold degradation and matrix reorganization. This research underscores that cartilage development is a sophisticated spatiotemporal coupling process: to achieve high-fidelity tissue regeneration, the scaffold degradation rate must be precisely synchronized with the rate of *de novo* matrix biosynthesis. Premature degradation results in the catastrophic loss of mechanical integrity, whereas protracted degradation impedes cellular remodeling and the volumetric expansion of nascent tissue. Within this equilibrium, dynamic loading serves as both a catalyst and a homeostatic regulator.²²³

4. Cellular signaling pathways in hydrogel microsphere-mediated extracellular matrix/pericellular matrix remodeling

4.1. The integrin–focal adhesion kinase axis

The integrin–focal adhesion kinase (FAK) signaling axis serves as a quintessential molecular bridge, transducing biophysical cues from the ECM into cellular responses. Pathways are initiated when $\alpha\beta$ integrins perceive matrix rigidity or tensile strain, triggering FAK autophosphorylation at Tyr397. This Src recruitment establishes a signaling complex that activates downstream phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), wingless-related integration site (Wnt)/ β -catenin, and mitogen-activated protein kinase cascades, collectively regulating MSC lineage specification and tissue repair.^{224–226} Engineering dynamic ECM microenvironments can autonomously drive this pathway, thereby guiding endogenous matrix remodeling.²²⁷ Specifically, Shi *et al.*²²⁸ developed a dynamic hydrogel cross-linked via reversible acyl hydrazone bonds that triggered early-stage mechanotransduction via the integrin–FAK axis, accelerating nascent protein deposition to guide sustained cell migration. Furthermore, Dai *et*

*al.*²²⁹ immobilized exosomes onto zinc–calcium phosphate coatings to activate the integrin–FAK pathway, markedly enhancing human mesenchymal stem cell adhesion, osteogenic differentiation, and immunomodulatory reprogramming of M1 macrophages into a pro-healing M2 phenotype. Strategically calibrating the spatiotemporal evolution of matrix mechanics within complex *in vivo* niches remains a critical bioengineering frontier.²³⁰

4.2. The Yes-associated protein/PDZ-binding motif signaling axis

Yes-associated protein and transcriptional coactivator with PDZ-binding motif (TAZ) function as downstream effectors of the Hippo cascade, acting as intrinsic mechanosensors that integrate matrix rigidity and viscoelastic profiles.²³¹ Under mechanical quiescence or on compliant substrates, Hippo pathway activation drives mammalian STE20-like protein kinases 1/2 and large tumor suppressor kinases 1/2 to phosphorylate YAP/TAZ, inducing cytoplasmic retention or degradation.²³² Conversely, elevated matrix stiffness or cytoskeletal tension inhibits the Hippo pathway, facilitating the nuclear translocation of unphosphorylated YAP/TAZ to interact with TEA domain transcription factors for lineage specification.²³² Within 3D microgels, cells exhibit refined sensitivity to their local mechanical niche. For instance, tuning GelMA microspheroid stiffness to an optimal modulus of 2 kPa directs nucleus pulposus-like differentiation via regulated YAP translocation.²⁰⁰ To harness temporal dynamics, Li *et al.*²³³ developed a soft-to-stiff temporal hydrogel system, demonstrating that dynamic stiffening profiles amplify YAP/TAZ nuclear localization more efficiently than statically stiff matrices to accelerate osteogenesis.²³³ At the single-cell level, Iyisan *et al.*²³⁴ engineered a 3D-printed compression device applying 200 kPa external pressure to single stem cells in microgels, confirming that localized physical stress transiently induces integrin-dependent YAP nuclear translocation via rapid cytoskeletal remodeling.²³⁴

4.3. The Piezo channel–Ca²⁺ signaling pathway

The Piezo channel family (Piezo1 and Piezo2) delineates a more direct and rapid mechanosensation mechanism compared to the cytoskeleton-dependent integrin–FAK complex.^{235,236} Piezo1 directly mediates the rapid influx of extracellular Ca²⁺ via mechanically induced conformational shifts.²³⁷ Its robust expression in osteocytes, chondrocytes, and MSCs establishes Piezo1 as a primary nexus perceiving ECM stiffness to sustain tissue homeostasis.^{236,238} The resulting Ca²⁺ transients propagate through three therapeutic axes to orchestrate matrix remodeling: the Piezo1–Ca²⁺–calcineurin/nuclear factor of activated T cells pathway regulating neurogenesis; the Piezo1–Ca²⁺–AMP-activated protein kinase (AMPK)/autophagy axis, where Wu *et al.*²³⁹ proved that 3D high-stiffness GelMA matrices

trigger Piezo1-mediated AMPK phosphorylation and autophagic flux to autonomously drive MSC osteogenesis²³⁹; and the Piezo1–Ca²⁺–YAP/TAZ axis controlling osteoblast–osteoclast crosstalk and type II/IX collagen expression.²³⁶ Translating these mechanisms, Chen *et al.*²⁴⁰ engineered a sequential delivery system releasing nerve growth factor to initialize early vascularization, followed by the sustained release of the Piezo1 agonist Yoda1 to drive late-stage osteoblast differentiation and mineralized bone matrix deposition.²⁴⁰

4.4. The wingless-related integration site/β-catenin signaling pathway

The canonical Wnt/β-catenin pathway acts as a master regulator governing musculoskeletal embryonic ontogeny and tissue homeostasis.^{241,242} In the absence of Wnt ligands, cytosolic β-catenin undergoes targeted degradation by a destruction complex comprising axin, adenomatous polyposis coli, glycogen synthase kinase-3 beta, and casein kinase 1.²⁴² Ligand binding to Frizzled and low-density lipoprotein receptor-related protein 5/6 receptors inactivates this complex, enabling stable, hypophosphorylated β-catenin to accumulate and translocate into the nucleus, where it complexes with T-cell factor/lymphoid enhancer factor transcription factors to drive cellular myelocytomatosis oncogene, cyclin D1, and osteogenic gene transcription.²⁴³ Within self-assembling 3D bone organoids, the canonical Wnt cascade is the primary target for biomaterial-driven bone remodeling.²⁴⁴ Highlighting its therapeutic value, Deng *et al.*²⁴⁵ pioneered a microfluidic HMs strategy to assemble bone organoid units capable of oxygen supply and immunomodulation.²⁴⁵ By encapsulating calcium peroxide and hydroxyapatite nanoparticles, these microspheres sustained cellular viability and autonomous biomineralization; their synergistic coupling with canonical Wnt signaling markedly accelerated *de novo* osteogenesis and vascular integration in murine critical-sized calvarial defects.

4.5. Alternative mechanotransduction pathways: Transient receptor potential vanilloid channels and nuclear pore complexes

Frontier mechanobiology reveals that cellular perception of matrix mechanics relies on a sophisticated cascade spanning from plasma membrane ion channels to nuclear envelope gateways.^{246,247} At the cell surface, transient receptor potential vanilloid 4 (TRPV4) acts as a pivotal integrator of ECM/PCM rigidity.²⁴⁸ TRPV4 perceives stiffness fluctuations to prompt Ca²⁺ influx and activate the Ras homolog family member/Rho-associated protein kinase cascade, establishing a feedback loop that dictates matrix remodeling and contractility.²⁴⁸ Utilizing layer-by-layer single-cell microgels to mimic PCM mechanics, Yang *et al.*²⁴⁹ activated TRPV4 channels to drive MSC

chondrogenesis via downstream YAP/TAZ and PI3K–AKT signaling.²⁴⁹ TRPV-mediated Ca^{2+} transients stimulate actomyosin contractility to generate cellular tension,²⁵⁰ which is physically coupled to the nuclear envelope via the linker of nucleoskeleton and cytoskeleton complex, inducing nuclear flattening and anisotropic deformation under high-stiffness regimes.²⁵¹ The nuclear pore complex (NPC), serving as the macromolecular transport gateway, is highly sensitive to such structural alterations.²⁵¹ Leveraging this, Li *et al.*²⁵² developed microfluidic anisotropic MicroRod and MicroSphere 3D microgels, proving that tailored geometric topographies apply cytoskeletal-nuclear forces that gate the NPC to activate YAP/TAZ, while simultaneously preserving nuclear envelope integrity and suppressing the cyclic GMP–AMP synthase/stimulator of interferon genes pathway to retard cell senescence.^{252,253} Modulating TRPV activation and NPC mechanical gating via topological engineering represents a potent strategy to counteract musculoskeletal tissue aging.²⁵⁴

5. Applications of mechanically encoded organoids in tissue regeneration

In HM-based musculoskeletal organoids (comprising

bone, cartilage, tendon/ligament, and skeletal muscle), “mechanical encoding” embeds developmental and regenerative biophysical cues within the 3D niche to drive functional fidelity.^{255–257} This paradigm incorporates critical physiological parameters: interstitial porosity, anisotropic alignment, and tissue-specific dynamic regimes—such as periodic compression for cartilage, tensile strain for tendons, electromechanical coupling for muscle, and fluid shear for vascular networks.^{257–259} Unlike transient biochemical stimuli, this structural encoding acts as a sustained regulatory framework; cells continuously adapt cytoskeletal tension to dictate lineage specification and patterned ECM deposition, ultimately yielding mature load-bearing capacity, contractility, and seamless interfacial integration with host tissues²⁶⁰ (Table 2).

5.1. Cartilage organoids

Contemporary cartilage organoids have evolved from simple spheroids into scalable, phenotype-stable functional units for osteochondral repair.^{261,262} Fabricated via bioreactor-mediated fusion²⁶³ or *in situ* coalescence within injectable hydrogels, these micro-tissues outperform cell suspensions by unifying high-density cells with autocrine

Table 2. Fabrication strategies, biochemical cues, and seed cell populations in musculoskeletal organoid engineering

Organoid type	Fabrication strategy	Biochemical factors	Seed cells
Cartilage organoids	Bioreactor-mediated micro-tissue fusion; <i>in situ</i> coalescence within injectable hydrogels; digital light processing bioprinting with pre-tethered micro-networks; structural silk fibroin-DNA hydrogel microspheres	Pro-chondrogenic small molecules; articular cartilage decellularized extracellular matrix (dECM)-derived microparticles; hyaluronic acid-modified selenium nanoparticles	Articular chondrocytes (including osteoarthritis patient-derived); articular chondroprogenitor cells; bone marrow mesenchymal stem cells
Bone organoids	Woven bone dynamic dual-networks (DNA/gelatin methacryloyl [GelMA]); <i>in situ</i> injectable biomimetic micro-cryogels; callus models with encoded silk fibroin micro-architectures	Nerve growth factor ; Yoda1 (Piezo1 agonist)/wingless-related integration site activators; calcium peroxide oxygen-generating particles	Urine-derived stem cells; bone marrow mesenchymal stem cells; multipotent osteogenic progenitor cells
Skeletal muscle organoids	Three-dimensional (3D) suspension self-assembly within viscoelastic/stress-relaxing matrices; microfluidic porous co-delivery microspheres (adipose-derived mesenchymal stem cells/ FGF19@μsphere); electromechanical coupling dynamic loading platforms	Fibroblast growth factor 19; skeletal muscle dECM and arginine–glycine–aspartic acid adhesive ligands; contraction-induced “training” secretome	Human induced pluripotent stem cells; paired box protein Pax-7 skeletal muscle satellite cells/myogenic progenitors; adipose-derived stem cells (including tri-culture with motor neurons and endothelial cells)
Neural/spinal cord organoids	Microchannel collagen scaffold topological guidance; laminin-modified porous GelMA microspheres; microfluidic-directed neurite bundle harvesting	Laminin-derived modification motifs; decellularized placenta extracellular matrix extracts	Neural stem cells; human induced pluripotent stem cell-derived motor and sensory neurons
Synovial organoids	High-throughput monodisperse droplet encapsulation via microfluidics; spatially stratified assembly via 3D bioprinting; microfluidic interconnected multi-tissue “joint-on-a-chip” platforms	(Physiological maintenance) Lubricin; (pathomimetic modeling) macrophage-specific chemokines and pro-inflammatory cytokines	Fibroblast-like synoviocytes; macrophage-like synoviocytes; synovium-derived mesenchymal stem cells

matrices.^{257,264} Here, biomaterial-based HMs are critical to arrest phenotypic drift, optimize 3D diffusion gradients, and facilitate stable interfacial integration.²⁶⁵ Illustrating this, Sun *et al.*²⁶⁵ integrated cartilage-dECM microparticles within adhesive, anti-inflammatory hydrogels to promote chondrogenesis while modulating arthritic niches.^{265,257} Additionally, tethering pro-chondrogenic small molecules into hydrogels via DLP permits precise assessment of how culture duration impacts hyaline stability,²⁶³ while Shen *et al.*²⁶⁴ deployed structural SF-DNA HMs as organoid

precursors to accelerate structural defect repair²⁶⁴ (Figure 4).

5.2. Bone organoids

Bone organoids unify autonomous cellular organization, autocrine matrix deposition, and biomineralization into modular building blocks for translational bone repair.²⁶⁶ Diverse construction modalities—including woven bone, rapid self-mineralizing, callus-mimetic, and injectable configurations²⁶⁷ (Figure 5)—leverage distinct mechanical

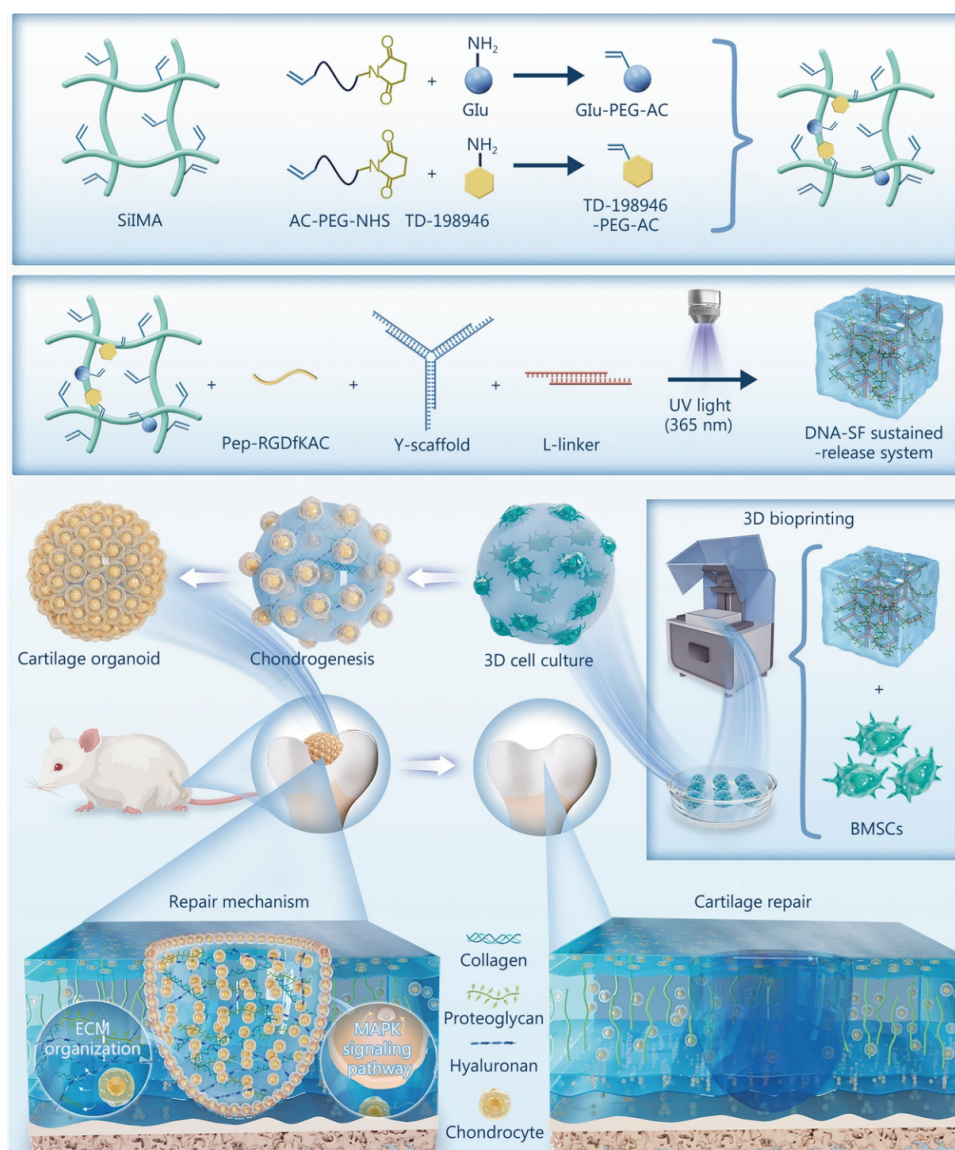


Figure 4. Preparation of cartilage organoids from hydrogel microspheres. DNA-SF sustained-release system synthesis and its mechanism for cartilage repair. Reprinted with modifications from Ref.²⁶⁴

Abbreviations: 3D: Three-dimensional; AC-PEG-NHS: Acrylic acid-polyethylene glycol-N-hydroxysuccinimide; BMSCs: Bone marrow mesenchymal stem cells; DNA-SF: DNA-silk fibroin; ECM: Extracellular matrix; Glu: Glucosamine; Glu-PEG-AC: Glucosamine-polyethylene glycol-acrylic acid; MAPK: Mitogen-activated protein kinase; NH₂: Amino group; Pep-RGDfKAC: RGD-containing peptide modified with D-Phe-Lys and an AC functional group; SiIMA: Silk fibroin methacrylate; UV: Ultraviolet.

encoding principles. To mimic early-stage osteogenesis, dynamic DNA/GelMA dual-networks demonstrate that matrix reconfigurability, rather than high static moduli, is crucial to support cellular migration and sequenced mineralization during 3D culture.²⁵⁵ Alternatively, pre-activating intracellular mechanosensitive pathways (e.g., via Piezo1/Wnt signaling) drives rapid scaffold-free self-assembly and accelerated self-mineralization, truncating manufacturing cycles without external loading apparatuses.²⁶⁸ For structural refinement, encoding fibrous SF architectures into callus organoids modulates cellular crosstalk and oxidative stress to dictate maturation kinetics.²⁶⁹ Clinically, injectable biomimetic organoids and modular micro-cryogels enable *in situ* self-assembly within critical-sized calvarial defects or complex osteochondral interfaces, simultaneously regenerating cartilage and

subchondral bone to achieve seamless interfacial repair.^{256,270}

5.3. Muscle organoids

Standardized 3D skeletal muscle organoid assembly utilizes fibrin or collagen matrices integrated with automated mechanical loading platforms.²⁷¹ To sustain long-term reparative capacity, contemporary 3D culture and suspension workflows prioritize the preservation of a functional, uncommitted paired box protein Pax-7 (PAX7⁺) satellite cell pool capable of replenishing endogenous niches and restoring contractility across successive injury cycles.²⁷² This progenitor fate is tightly governed by matrix viscoelasticity; specifically, hydrogels with rapid stress relaxation maintain Pax7 expression and arrest premature terminal differentiation through modulated RGD ligand density and YAP/TAZ mechanotransduction.²⁷³

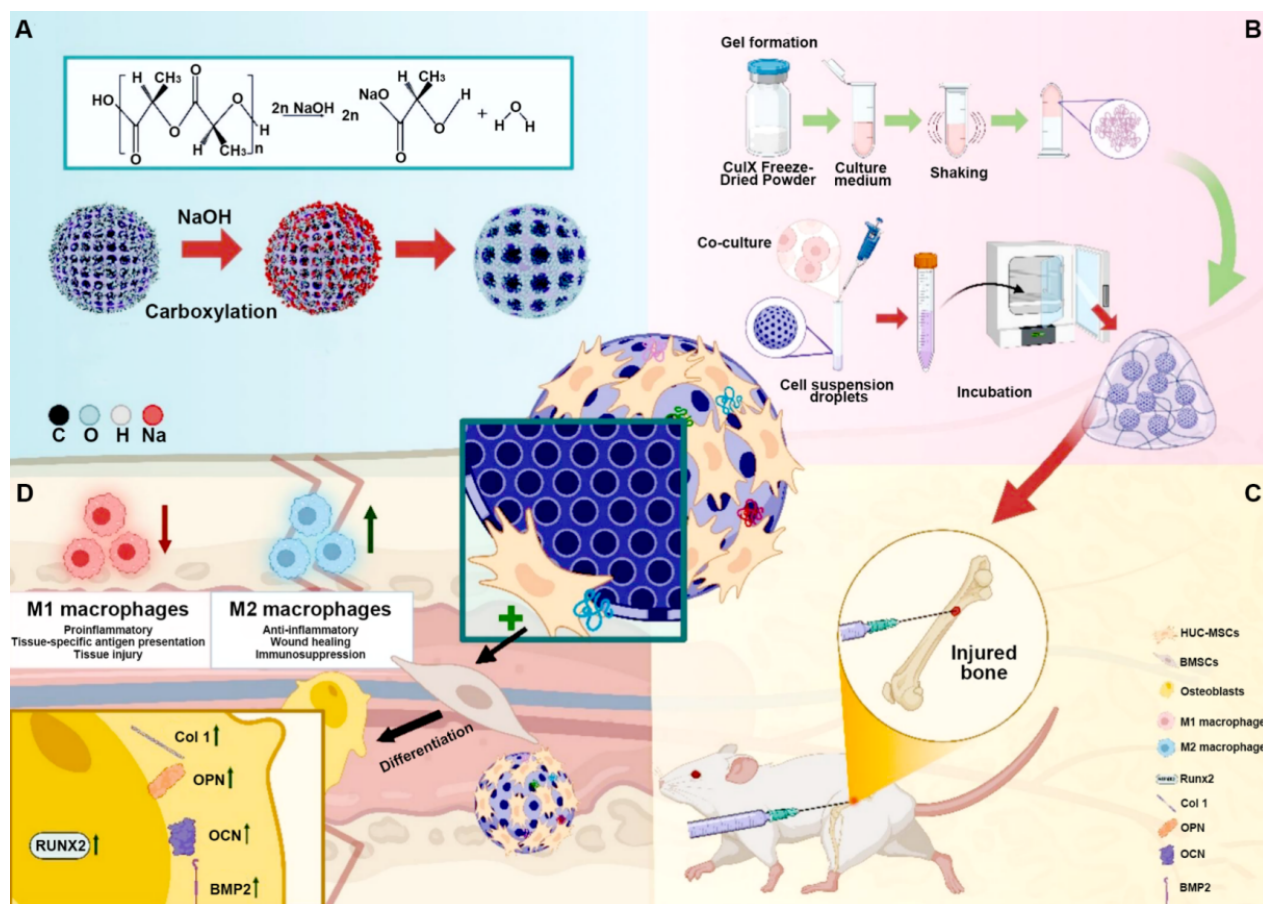


Figure 5. Preparation of bone organoids using hydrogel microspheres. (A) Schematic diagram of Cull X II@PDLLA@HUC-MSCs (CPH) synthesis pathway and bone defect repair after transplantation. Schematic representation of the carboxylation mechanism of PDLLA microspheres. (B) CPH synthetic pathway. (C) The CPH were transplanted into a bone defect model. (D) Mechanism of bone defect repair by CPH. Reprinted from Ref.²⁶⁷ Abbreviations: BMP2: Bone morphogenetic protein 2; BMSCs: Bone marrow mesenchymal stem cells; C: Carbon; CH₃: Methyl group; Col 1: Type 1 collagen; H: Hydrogen; HO: Hydroxyl group; HUC-MSCs: Human umbilical cord-derived mesenchymal stem cells; Na: Sodium; NaO: Sodium oxide fragment; NaOH: Sodium hydroxide; O: Oxygen; OCN: Osteocalcin; OPN: Osteopontin; PDLLA: Poly(D,L-lactic acid); RUNX2: Runt-related transcription factor 2.

Consequently, muscle organoid scaffolding revolves around three mechanical pillars: structural anisotropy to align myofibers and maximize effective contractility; viscoelastic stress relaxation to control stem cell kinetics; and dynamic electromechanical loading to accelerate contractile maturation and stimulate motor neuron axonal outgrowth via a contraction-induced training secretome. To reconstitute the native neurovascular niche, tri-cultures with motor neurons and endothelial cells are deployed to drive NMJ maturation.²⁷⁴ This interface can be augmented by embedding muscle-derived dECM within conductive graphene-PLGA structural scaffolds to accelerate functional axonal networking.²⁷⁵ Clinically, Wang *et al.*²⁷⁶

engineered a microfluidic adipose-derived stem cells/fibroblast growth factor 19 porous microsphere system that co-delivers cellular and biochemical cues to synergistically drive angiogenesis and myogenesis for ischemic limb repair²⁷⁶ (Figure 6).

5.4. Tendon and ligament organoids

The primary objective of tendon and ligament organoids is to reconstruct tissue characteristics tailored for mechanical load bearing, specifically focusing on high structural anisotropy, a collagen type I-dominant ECM, and parallel cellular alignment, all while preserving the regenerative potential necessary for injury repair or mechanistic

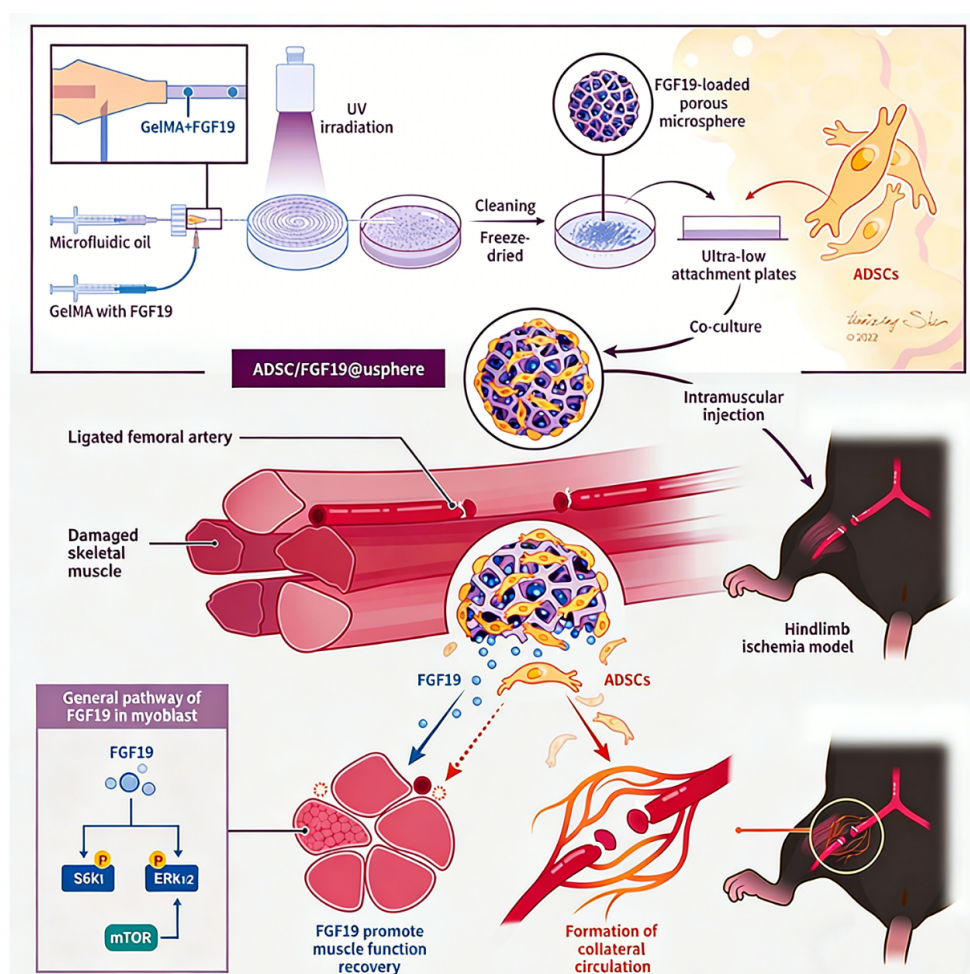


Figure 6. Preparation of skeletal muscle organoids using hydrogel microspheres. Schematic illustration of the synthetic process of ADSC/FGF19@μspheres and ischemic limb restoration. Uniform droplets of GelMA hydrogel with FGF19 were first prepared with the assistance of microfluidic technology, followed by rapid light cross-linking and freeze-drying to obtain FGF19-loaded porous microspheres. ADSCs were then loaded into the microspheres by absorption to construct the ADSC/FGF19@μsphere. The resultant ADSC/FGF19@μsphere was injected into the ischemic hindlimb, wherein it realized highly efficient ADSC delivery and sustained release of FGF19 to facilitate the simultaneous neovascularization and skeletal muscle restoration. Reprinted from Ref.²⁷⁶

Abbreviations: ADSC/FGF19: Adipose-derived stem cells/fibroblast growth factor 19; ERK12: Extracellular signal-regulated kinase 1/extracellular signal-regulated kinase 2; GelMA: Gelatin methacryloyl; mTOR: Mammalian target of rapamycin; S6K1: Ribosomal protein S6 kinase beta-1.

pharmacology. A hallmark advancement in this field is the development of “scaled” tendon organoids. By meticulously mimicking the native ECM and optimizing inductive signaling, researchers have successfully fabricated centimeter-scale (>3 cm) transplantable tendon organoids characterized by robust tenogenic lineage phenotypes and dense matrix deposition, demonstrating significant reparative potential in animal injury models.²⁷⁷ In terms of “operational standardization,” more engineering-centric protocols have emerged. For example, methods based on self-assembling cell sheets can generate manually handleable “rod-shaped tendon/ligament-like organoids.” The advantage of this architecture is the ease with which static or dynamic tensile strain can be applied, thereby directly integrating “mechanical encoding” into the maturation process.²⁷⁸ It is crucial to emphasize that the most significant clinical challenge lies in the repair of interfacial tissues, such as the enthesis (the tendon-to-bone interface). This region is defined by a complex continuous gradient between soft and hard tissues and pronounced anisotropy. While not strictly categorized as pure organoid research, current studies utilizing programmable hydrogel systems to promote enthesis regeneration suggest a vital future direction: “encoding” these interfacial gradients directly into tendon/ligament organoids or their delivery vehicles to better align with the translational requirements of sports medicine.²⁷⁹

5.5. Neural organoids

Neural organoids serve as modular structural units to drive functional reconnection following spinal cord or peripheral nerve injuries.²⁸⁰ Shifting beyond basic cell culture, structural encoding utilizes channelized collagen scaffolds to direct axonal outgrowth in human spinal cord organoids (hSCOs), effectively suppressing glial scarring and restoring motor function.²⁸¹ For peripheral nerve reconstruction, microfluidic devices harvest aligned neurite bundles from human induced pluripotent stem cell-derived motor and sensory organoids to accelerate structural regeneration, while decellularized placenta ECM hydrogels provide inductive biochemical cues that accelerate hSCO maturation.²⁸² Addressing spinal cord injury niches, laminin-modified porous GelMA microspheres activate the PI3K–AKT pathway to enhance neural stem cell survival and directed differentiation, mitigating localized inflammation while potently triggering endogenous neurogenesis to restore locomotor function *in vivo*.²⁸³ (Figure 7).

5.6. Vascular organoids

The central challenge in vascular organoid engineering has shifted from the mere formation of capillary-like sprouts to the attainment of mature vascular hierarchies (such as arteriole-like structures) and the stability of

these networks under physiological shear stress and perfusion post-transplantation.²⁸⁴ Modern biomaterial strategies increasingly emphasize the role of the “mechanical microenvironment” in guiding vascular lineage commitment. A study highly congruent with the theme of “mechanical encoding” demonstrated that matrix viscoelasticity regulates the differentiation of vascular organoids from predominantly capillary-like structures toward arteriole-like architectures containing smooth muscle-like cells.²⁸⁵ In myocardial infarction models, these viscoelastic matrices promoted neovascular reconstruction and functional recovery, suggesting that a “dynamic/stress-relaxing matrix” is more pivotal than simple stiffness for vascular maturation. Furthermore, vascular organoids serve as a sophisticated “cell source reservoir.” Researchers have isolated organoid-derived endothelial cells and pericyte-like cells to construct perfusable macro-vascular scaffold models, subsequently validating their responsiveness to fluid shear stress and the establishment of robust barrier functions, such as trans-endothelial electrical resistance.²⁸⁶ Vascular organoids are often accompanied by the synergistic repair of other organoids. While bone organoids effectively simulate the osseous microenvironment, the inadequate vascularization of large-scale aggregates remains a significant barrier to clinical translation. Addressing this, Lou *et al.*²⁸⁷ engineered a spatiotemporally graded hydrogel system that integrates cell-laden and dimethylolallylglycine-encapsulated GelMA matrices with nano-hydroxyapatite-loaded HMs. By leveraging the gradient degradation of these materials, they achieved sequential angiogenic-osteogenic coupling. This approach prioritizes the formation of a vascular network prior to promoting osteogenic maturation, thereby overcoming the vascularization bottleneck and significantly enhancing bone regeneration in critical-sized calvarial defect models. This provides a new paradigm for the engineering of pre-vascularized bone organoids²⁸⁷ (Figure 8).

5.7. Synovial organoids

Synovial organoids replicate the spatial heterogeneity (intima and subintima) and paracrine dynamics of native synovium, preventing the rapid phenotypic loss seen in monolayer cultures.^{288–290} Utilizing monodisperse HMs optimizes nutrient mass transfer while permitting precise tuning of elastic moduli (2.43–68.2 kPa) to recapitulate regional mechanical niches.²⁹¹ However, conventional static hydrogel models remain limited by a lack of vascularization and neural networks, hindering the simulation of leukocyte infiltration and chronic arthritic pain,^{291,292} alongside the absence of continuous shear stress and compression essential for synoviocyte homeostasis and lubricin secretion.²⁹³ To resolve these translational bottlenecks, contemporary workflows employ 3D bioprinting to construct spatially stratified tissue interfaces, or engineer

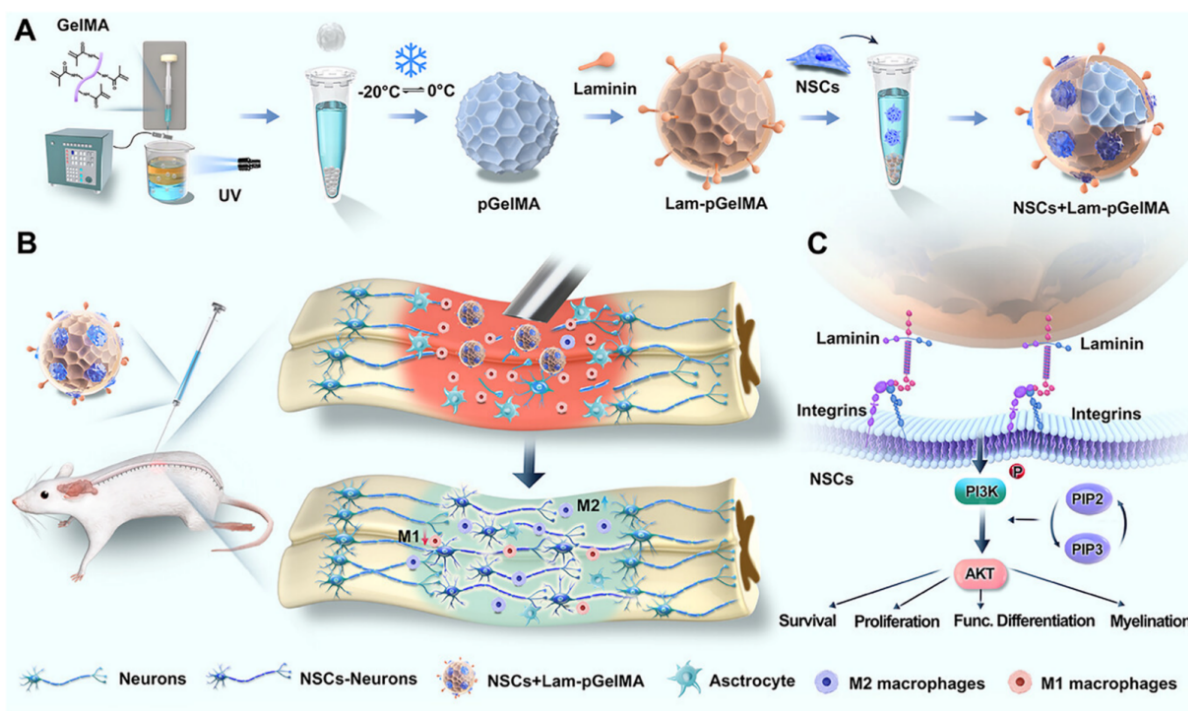


Figure 7. Preparation of nerve organoids using hydrogel microspheres. Schematic illustrating the fabrication of Lam-pGelMA microspheres and the therapeutic effect of NSCs loaded on the functionalized microspheres for spinal cord injury repair. (A) The workflow depicts the fabrication process of Lam-pGelMA microspheres and their co-culture with NSCs. (B) In a rat SCI model, NSC-loaded Lam-pGelMA microspheres were injected into the lesion site after injury, which reduced tissue distortion, attenuated neuroinflammation, and promoted neurogenesis and neuronal differentiation of the transplanted NSCs. (C) The underlying mechanism of the protective effect might involve the interactions between laminin and integrins and the subsequent activation of the PI3K–AKT pathway. Reprinted with permission from Ref.²⁸³ Copyright © 2025, Wiley-VCH GmbH.

Abbreviations: Lam-pGelMA: Laminin-modified porous GelMA microspheres; NSC: Neural stem cell; pGelMA: Photo-cross-linkable gelatin methacryloyl; PI3K–AKT: Phosphoinositide 3-kinase–protein kinase B; PIP₂: Phosphatidylinositol 4,5-bisphosphate; PIP₃: Phosphatidylinositol 3,4,5-trisphosphate; SCI: Spinal cord injury; UV: Ultraviolet.

microfluidic multi-tissue “joint-on-a-chip” platforms to track the inflammatory crosstalk driving chondrocyte senescence.²⁹³ Ultimately, human-derived synovial biochips prove that mechanical encoding via matrix stiffness directly dictates fibrotic and inflammatory phenotypes.¹⁸

6. Challenges and future perspectives

While musculoskeletal organoids fabricated from mechanically encoded HMs show great regenerative potential, transitioning from passive cell carriers to functional grafts requires addressing complex hurdles in materials science, biomechanics, and clinical translation.

6.1. Challenges

A primary constraint is the lack of a quantitative consensus on defining microenvironmental mechanical cues. This deficit hinders our understanding of “mechanobiological” coupling. Current characterization typically relies on bulk rheology (macroscopic storage modulus, G') or atomic

force microscopy for surface stiffness. However, granular scaffolds formed from packed microspheres possess intricate contact networks. Consequently, bulk measurements fail to reflect the local stiffness sensed by cells within interstitial voids, nor do they capture the dynamic viscoelasticity that dictates cell migration.

A critical challenge is the “spatiotemporal handover”—balancing HM degradation with the secretion of endogenous ECM. Ideally, scaffold degradation should mirror the rate at which nascent tissue gains mechanical integrity. However, current HM degradation is largely governed by fixed hydrolytic or enzymatic rates, remaining decoupled from the real-time metabolic demands and remodeling activity of resident cells.

6.2. Future perspectives

Next-generation HMs must evolve from passive scaffolds into stimuli-responsive intelligent systems. Integrating single-cell mechanomics with artificial intelligence (AI) will enable:

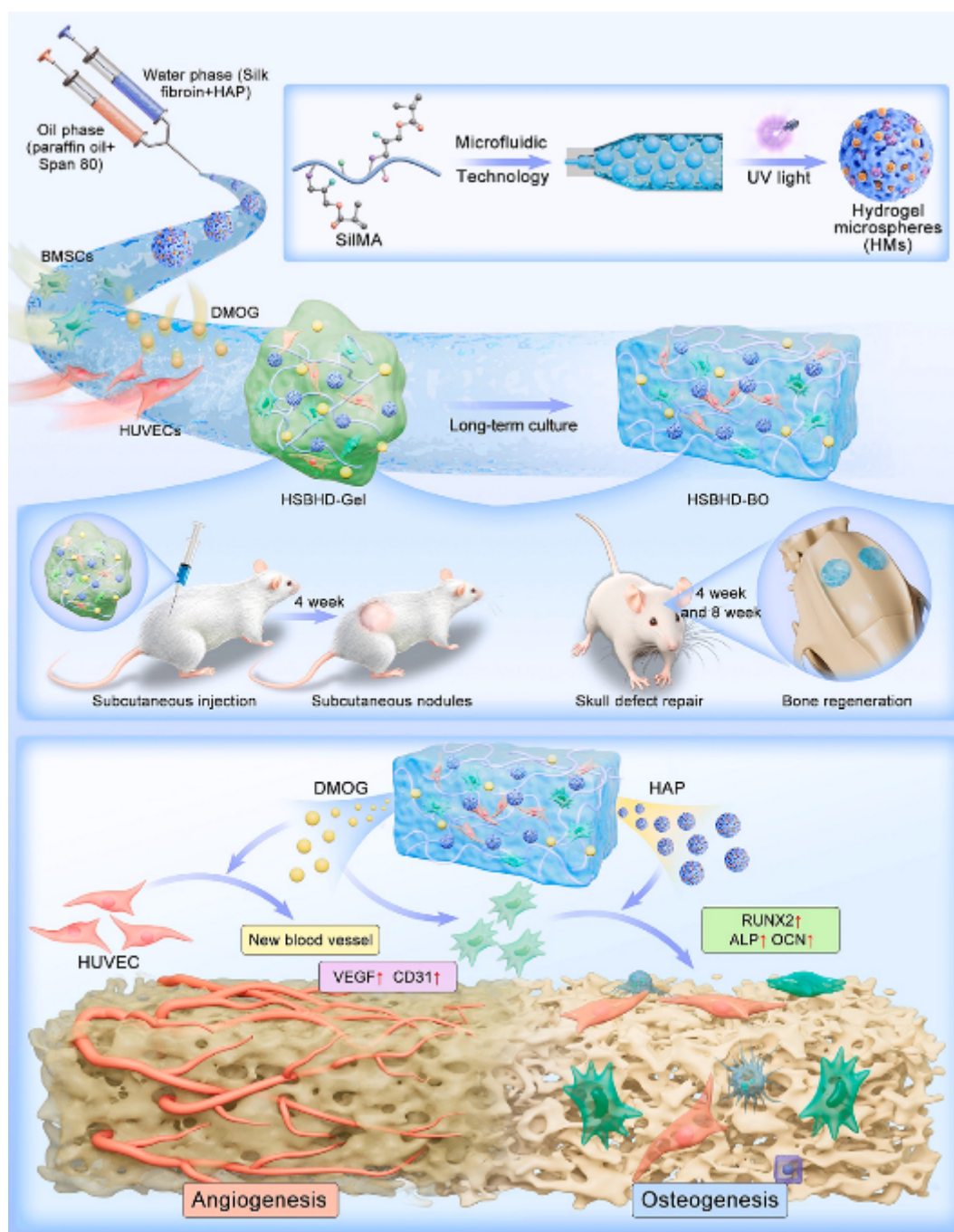


Figure 8. Preparation of vascular and bone organoids using hydrogel microspheres. Schematic diagram of the design and application of a hydrogel system integrating angiogenic and osteogenic components for vascularized bone organoid construction and bone defect repair.²⁸⁷ Reprinted with permission from Ref.²⁸⁷ Copyright © 2025 Elsevier Ltd.

Abbreviations: ALP: Alkaline phosphatase; BMSCs: Bone marrow-derived mesenchymal stem cells; CD31: Cluster of differentiation 31; DMOG: Dimethylxalylglycine; HAP: Hydroxyapatite; HSBHD: Human stem cell-derived bone and hematopoietic defect; HSBHD-BO: Human stem cell-derived bone and hematopoietic defect bone organoid; HUVECs: Human umbilical vein endothelial cells; OCN: Osteocalcin; RUNX2: Runt-related transcription factor 2; SilMA: Silicate methacrylate; UV: Ultraviolet; VEGF: Vascular endothelial growth factor.

- (a) Machine learning can analyze large datasets of cell-material interactions to identify the mechanical parameters needed for complex tissues, such as the enthesis. AI will guide molecular architecture, deducing material structure from biological requirements.
- (b) HMs could act as sensors that detect pathological shifts. For instance, they may autonomously alter local mechanics to protect tissue upon sensing inflammatory cytokines like IL-1 β or TNF- α .
- (c) Precision engineering will allow for universal, modular organoid precursors. This transition from personalized customization to mass-produced, off-the-shelf grafts will enable immediate functional restoration of musculoskeletal systems.

7. Conclusion

This review underscores recent progress in musculoskeletal organoid engineering, reflecting a shift from simple morphological replication toward functional maturation through mechanically encoded HMs. Integrating artificial intelligence and machine learning with single-cell mechanomics may enable the high-throughput engineering of modular organoids capable of adapting in vivo and promoting rapid functional repair.

Acknowledgments

The authors acknowledge the support from the Institute of Rheumatic Immunology Affiliated Hospital of North Sichuan Medical College. The authors thank BioRender (BioRender.com) for providing the tools and granting the publication rights for [Figure 1](#) and the Graphical Abstract.

Funding

This work was supported by the Chongqing City Postdoctoral Innovative Talent Support Plan (CQBX202411); the Postdoctoral Fellowship Program of CPSF (GZC20251501), the Postdoctoral Fellowship Program (2025MD784153); Hong Kong Scholars Program (XJ2024017); Project of North Sichuan Medical College (CBY23-QNA01) (2024MPZK013); and Sichuan Science and Technology Program (2025ZNSFSC0650).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Pengcheng Xiao, Yiting Lei, Wei Huang
Visualization: Jianye Tan, Shengwen Cheng, Lifu Jia
Writing—original draft: Xingkuan Wang, Jinping Chen
Writing—review & editing: Charlotte A. E. Hauser, Tiziano Serra, Yiting Lei

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

- Zhang J, Wu Y, Shen T, *et al.* Organoid research: new concepts and new technologies. *Burn Trauma* 2026;14:tkag015. doi: 10.1093/burnst/tkag015
- Zeng Q, Xie D, Wang D, *et al.* Bioengineered materials-driven construction of musculoskeletal organoids in aging research: Strategies, applications, and future perspectives. *OR*. 2025;1(4):025450033. doi: 10.36922/OR025450033
- Yin Y, Zhou W, Zhu J, *et al.* Generation of self-organized neuromusculoskeletal tri-tissue organoids from human pluripotent stem cells. *Cell Stem Cell*. 2025;32(1):157-171.e8. doi: 10.1016/j.stem.2024.11.005
- Mostert D, van der Putten C, Sahlgren CM, Kurniawan NA, Bouten CVC. Bioengineering structural anisotropy in living tissues. *Nat Rev Bioeng*. 2025;3(9):727-741. doi: 10.1038/s44222-025-00338-x
- Gao Y, Zhang Z, Yao Y, *et al.* Engineering of tissue in microphysiological systems demonstrated by modelling skeletal muscle. *Regen Biomater*. 2025;12:rbaf059. doi: 10.1093/rb/rbaf059
- Shan B-H, Wu F-G. Hydrogel-Based Growth Factor Delivery Platforms: Strategies and Recent Advances. *Adv Mater*. 2024;36(5):e2210707. doi: 10.1002/adma.202210707
- Caballero Aguilar LM, Silva SM, Moulton SE. Growth factor delivery: Defining the next generation platforms for tissue engineering. *J Control Release*. 2019;306:40-58. doi: 10.1016/j.jconrel.2019.05.028
- Wang X, Lei Y, Jiang K, *et al.* Mito-battery: Micro-nanohydrogel microspheres for targeted regulation of cellular mitochondrial respiratory chain. *Nano Today*. 2023;49:101820. doi: 10.1016/j.nantod.2023.101820
- Daly AC, Riley L, Segura T, Burdick JA. Hydrogel microparticles for biomedical applications. *Nat Rev Mater*. 2020;5(1):20-43. doi: 10.1038/s41578-019-0148-6

10. Zhang D, Liu W, Feng L, *et al.* Innovative Advances in Droplet Microfluidics. *Research*. 2025;8:0856.
doi: 10.34133/research.0856
11. Xiao P, Yuan H, Lei Y, *et al.* Lubrication Barriers Hydrogel Microspheres Improve Dynamic Tissue Repair via the Blockade of Inflammatory Communication. *Adv Funct Mater*. 2026;36(6):e15734.
doi: 10.1002/adfm.202515734
12. Zhang L, Luo Z, Chen H, Wu X, Zhao Y. Glycyrrhizic Acid Hydrogel Microparticles Encapsulated with Mesenchymal Stem Cell Exosomes for Wound Healing. *Research*. 2024;7:0496.
doi: 10.34133/research.0496
13. Liu H, Chen Z, Lin Q, *et al.* A multicellular self-organized probiotic platform for oral delivery enhances intestinal colonization. *Nat Commun*. 2025;16(1):7060.
doi: 10.1038/s41467-025-62349-x
14. Lei Y, Santos HA, Cui W. Advances and challenges in hydrogel microspheres for biomedical applications. *Biomater Transl*. 2024;5(3):203-204.
doi: 10.12336/biomatertransl.2024.03.001
15. Wang X, He J, Hong Y, *et al.* Spermidine-encapsulated chondroitin sulfate methacryloyl hydrogel delivery system for remodeling bone homeostasis in rheumatoid arthritis. *Mater Today Bio*. 2025;35:102531.
doi: 10.1016/j.mtbio.2025.102531
16. Chen Z, Lv Z, Zhang Z, *et al.* Advanced microfluidic devices for fabricating multi-structural hydrogel microsphere. *Exploration*. 2021;1(3):20210036.
doi: 10.1002/EXP.20210036
17. Hu X, Zhang MX, Xu FW, *et al.* Preparation Methods of Hydrogel Microspheres and Recent Advances in Their Application for Treating Diabetic Wounds. *Int J Nanomed*. 2025;20:10645-10666.
doi: 10.2147/IJN.S527650
18. Reihls EI, Stoegner A, Vasconez Martínez MG, *et al.* An Animal-Free Patient-Derived Tissue-Mimetic Biochip Model of the Human Synovial Membrane for Human-Relevant Osteoarthritis Research. *Adv Healthc Mater*. 2025;14(23):e2404799.
doi: 10.1002/adhm.202404799
19. Yuan N, Shao K, Huang S, Chen C. Chitosan, alginate, hyaluronic acid and other novel multifunctional hydrogel dressings for wound healing: A review. *Int J Biol Macromol*. 2023;240:124321.
doi: 10.1016/j.ijbiomac.2023.124321
20. Chi H, Qiu Y, Ye X, Shi J, Li Z. Preparation strategy of hydrogel microsphere and its application in skin repair. *Front Bioeng Biotechnol*. 2023;11:1239183.
doi: 10.3389/fbioe.2023.1239183
21. Chelu M, Calderon Moreno JM, Musuc AM, Popa M. Natural Regenerative Hydrogels for Wound Healing. *Gels*. 2024;10(9):547.
doi: 10.3390/gels10090547
22. Zhang Y, Chen H, Li J. Recent advances on gelatin methacrylate hydrogels with controlled microstructures for tissue engineering. *Int J Biol Macromol*. 2022;221:91-107.
doi: 10.1016/j.ijbiomac.2022.08.171
23. Chen S, Li J, Zheng L, Huang J, Wang M. Biomimicking trilayer scaffolds with controlled estradiol release for uterine tissue regeneration. *Exploration*. 2024;4(5):20230141.
doi: 10.1002/EXP.20230141
24. Chen Y, Yang ZR, Cheng Z, *et al.* Injectable hydrogel microspheres promoting inflammation modulation and nucleus pulposus-like differentiation for intervertebral disc regeneration. *J Control Release*. 2025;380:599-614.
doi: 10.1016/j.jconrel.2025.02.016
25. Ghobadi F, Saadatmand M, Simorgh S, Brouki Milan P. Microfluidic 3D cell culture: potential application of collagen hydrogels with an optimal dose of bioactive glasses. *Sci Rep*. 2025;15(1):569.
doi: 10.1038/s41598-024-84346-8
26. Wang Y, Lv H, Ren S, *et al.* Biological Functions of Macromolecular Protein Hydrogels in Constructing Osteogenic Microenvironment. *ACS Biomater Sci Eng*. Sep 9 2024;10(9):5513-5536.
doi: 10.1021/acsbomaterials.4c00910
27. Hu X, Tang J, Yu H, Yang H, Lu X, Zheng D. Preparation of fish collagen and vancomycin microspheres based on microfluidic technology and its application in osteomyelitis. *Front Bioeng Biotechnol*. 2023;11:1249706.
doi: 10.3389/fbioe.2023.1249706
28. Suliman M, Alissa M, Alghamdi A. Collagen-based hydrogel encapsulated with SDF-1 α microspheres accelerate diabetic wound healing in rats. *Tissue Cell*. 2025;95:102877.
doi: 10.1016/j.tice.2025.102877
29. Hakim Khalili M, Zhang R, Wilson S, Goel S, Impey SA, Aria AI. Additive Manufacturing and Physicomechanical Characteristics of PEGDA Hydrogels: Recent Advances and Perspective for Tissue Engineering. *Polymers*. 2023;15(10):2341.
doi: 10.3390/polym15102341
30. Yan Y, Vladislavljević GT, Lin Z, Yang H, Zhang X, Yuan W. PEGDA hydrogel microspheres with encapsulated salt for versatile control of protein crystallization. *J Colloid Interface Sci*. 2024;660:574-584.
doi: 10.1016/j.jcis.2024.01.125
31. Chen S, Shahar T, Cohen S. Thermo-controlled microfluidic

- generation of monodisperse alginate microspheres based on external gelation. *RSC Adv.* 2024;14(44):32021-32028.
doi: 10.1039/d4ra07049f
32. Zhang R, Lei L, Song Q, Li X. Calcium ion cross-linking alginate/dexamethasone sodium phosphate hybrid hydrogel for extended drug release. *Colloids Surf B Biointerfaces.* 2019;175:569-575.
doi: 10.1016/j.colsurfb.2018.11.083
 33. Qin BQ, Wu SZ, Nie R, *et al.* SDF-1 α /BMP-12 loaded biphasic sustained-release SIS hydrogel/SA microspheres composite for tendon regeneration. *Biomaterials.* 2025;320:123246.
doi: 10.1016/j.biomaterials.2025.123246
 34. Gowda AHJ, Bu Y, Kudina O, *et al.* Design of tunable gelatin-dopamine based bioadhesives. *Int J Biol Macromol.* 2020;164:1384-1391.
doi: 10.1016/j.ijbiomac.2020.07.195
 35. Wu K, Yan Z, Wu Z, *et al.* Recent Advances in the Preparation, Antibacterial Mechanisms, and Applications of Chitosan. *J Funct Biomater.* 2024;15(11):318.
doi: 10.3390/jfb15110318
 36. Upadhyay A, Alimohammadi F, Tehrani R. Engineering Porosity-Tuned Chitosan Beads: Balancing Porosity, Kinetics, and Mechanical Integrity. *ACS Omega.* 2024;9(31):33857-33867.
doi: 10.1021/acsomega.4c03583
 37. Li Y, Li X, Zhu L, Liu T, Huang L. Chitosan-based biomaterials for bone tissue engineering. *Int J Biol Macromol.* 2025;304(Pt 2):140923.
doi: 10.1016/j.ijbiomac.2025.140923
 38. Xin C, Jia P, Zhao Y, *et al.* Antioxidant effects of *Gastrodia elata* polysaccharide-based hydrogels loaded with puerarin/gelatin microspheres for D-galactose-induced aging-skin wound healing. *Int J Biol Macromol.* 2025;296:139809.
doi: 10.1016/j.ijbiomac.2025.139809
 39. Du J, Liu Q, Diao X, Ma W, Liu G. Development of plantaricin RX-8 loaded pectin/4-carboxyphenylboric acid/carboxymethyl chitosan hydrogel microbead: A potential targeted oral delivery system. *Int J Biol Macromol.* 2025;292:139134.
doi: 10.1016/j.ijbiomac.2024.139134
 40. Conzatti G, Nadal C, Berthelot J, *et al.* Chitosan-PNIPAM Thermogel Associated with Hydrogel Microspheres as a Smart Formulation for MSC Injection. *ACS Appl Bio Mater.* 2024;7(5):3033-3040.
doi: 10.1021/acsbm.4c00071
 41. Ressler A. Chitosan-Based Biomaterials for Bone Tissue Engineering Applications: A Short Review. *Polymers.* 2022;14(16):3430.
doi: 10.3390/polym14163430
 42. Lim BY, Azmi F, Ng SF. LL37 Microspheres Loaded on Activated Carbon-chitosan Hydrogel: Anti-bacterial and Anti-toxin Wound Dressing for Chronic Wound Infections. *AAPS PharmSciTech.* 2024;25(5):110.
doi: 10.1208/s12249-024-02826-6
 43. Priya R, Yun Nam S, Cho W-S, Maruthupandy M. Graphene Reinforced Chitosan Nanocomposites for Drug Delivery. In: Swain SK, ed. *Carbon Based Nanomaterials for Drug Delivery.* Singapore: Springer; 2025:443-468.
doi: 10.1007/978-981-97-8086-0_15
 44. Ramezani Farani M, Zare I, Mirshafiei M, *et al.* Graphene oxide-engineered chitosan nanoparticles: Synthesis, properties, and antibacterial activity for tissue engineering and regenerative medicine. *Chem Eng J.* 2025;509:160852.
doi: 10.1016/j.cej.2025.160852
 45. Luo J, Huang H, Jiang J, Zheng W, Chen P, Bai H. Clearance of Intracellular Pathogens with Hyaluronic Acid Nanomicelles Responsive to H(2)S and pH. *Molecules.* 2024;29(24):5971.
doi: 10.3390/molecules29245971
 46. Heydari M, Hosseinzadeh Colagar A, Sabour D, Khorasani HR. Evidence of *Urtica dioica* Agglutinin's Antiproliferative and Anti-migratory Potentials on the Hyaluronic Acid-Overexpressing Prostate Cancer Cells. *Planta Med.* 2024;90(10):774-784.
doi: 10.1055/a-2324-2250
 47. Wang Y-J, Jiang W-J, Zhao H-J, *et al.* A Bioorthogonal and Programmable Bacterial Delivery System for Spatiotemporally Targeted Therapy of Solid Tumors. *Exploration.* 2025;5(6):20240396.
doi: 10.1002/EXP.20240396
 48. Liu J, Chen S, Zhang Z, *et al.* The oxidized hyaluronic acid hydrogels containing paeoniflorin microspheres regulates the polarization of M1/M2 macrophages to promote wound healing. *Int J Biol Macromol.* 2024;282(Pt 5):137107.
doi: 10.1016/j.ijbiomac.2024.137107
 49. Yao Y, Wei G, Ding J, Cui W. [Injectable hydrogel microspheres experimental research for the treatment of osteoarthritis]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.* 2023;37(8):918-928.
doi: 10.7507/1002-1892.202302105
 50. Lin C, Ying C, Xu Y, *et al.* Synergizing adaptive immunity and regenerative signals to enhance osteochondral defects repair. *Bioact Mater.* 2025;46:242-258.
doi: 10.1016/j.bioactmat.2024.12.011
 51. Hou J, Lin Y, Zhu C, *et al.* Zwitterion-Lubricated Hydrogel Microspheres Encapsulated with Metformin Ameliorate Age-Associated Osteoarthritis. *Adv Sci.* 2024;11(30):e2402477.
doi: 10.1002/advs.202402477
 52. Pritchard EM, Kaplan DL. Silk fibroin biomaterials for controlled release drug delivery. *Expert Opin Drug Deliv.*

- 2011;8(6):797-811.
doi: 10.1517/17425247.2011.568936
53. Ma K, Yang L, Li W, *et al.* Mussel-inspired multi-bioactive microsphere scaffolds for bone defect photothermal therapy. *Mater Today Bio.* 2024;29:101363.
doi: 10.1016/j.mtbio.2024.101363
54. Kaewpirom S, Piboonnithikasem S, Sroisroemsap P, Uttoom S, Boonsang S. Tailoring silk fibroin hydrophilicity and physicochemical properties using sugar alcohols for medical device coatings. *Sci Rep.* 2024;14(1):13781.
doi: 10.1038/s41598-024-64450-5
55. Tu H, Zhu M, Duan B, Zhang L. Recent Progress in High-Strength and Robust Regenerated Cellulose Materials. *Adv Mater.* 2021;33(28):e2000682.
doi: 10.1002/adma.202000682
56. Chen Y, Liu X, Zhou R, *et al.* Porous sodium alginate/cellulose nanofiber composite hydrogel microspheres for heavy metal removal in wastewater. *Int J Biol Macromol.* 2024;278(Pt 3):135000.
doi: 10.1016/j.ijbiomac.2024.135000
57. Batta-Mpouma J, Kandhola G, Kaur J, *et al.* Cellulose nanocrystal-based hydrogel microspheres prepared via electrohydrodynamic processes for controlled release of bioactive compounds. *Carbohydr Polym.* 2025;356:123355.
doi: 10.1016/j.carbpol.2025.123355
58. Xu Z, Tian W, Wen C, *et al.* Cellulose-Based Cryogel Microspheres with Nanoporous and Controllable Wrinkled Morphologies for Rapid Hemostasis. *Nano Lett.* 2022;22(15):6350-6358.
doi: 10.1021/acs.nanolett.2c02144
59. Wang R, Fujie T, Itaya H, Wada N, Takahashi K. Force-Induced Alignment of Nanofibrillated Bacterial Cellulose for the Enhancement of Cellulose Composite Macrofibers. *Int J Mol Sci.* 2023;25(1):69.
doi: 10.3390/ijms25010069
60. Su M, Ruan L, Dong X, *et al.* Current state of knowledge on intelligent-response biological and other macromolecular hydrogels in biomedical engineering: A review. *Int J Biol Macromol.* 2023;227:472-492.
doi: 10.1016/j.ijbiomac.2022.12.148
61. Jiang F, Xu XW, Chen FQ, *et al.* Extraction, Modification and Biomedical Application of Agarose Hydrogels: A Review. *Mar Drugs.* 2023;21(5):299.
doi: 10.3390/md21050299
62. Huang Y, Peng S, Chen Y, Chu B. Agarose Hydrogels for Bone Tissue Engineering, from Injectables to Bioprinting. *Gels.* 2025;11(4):255.
doi: 10.3390/gels11040255
63. Zhu S, Shou X, Kuang G, *et al.* Stimuli-responsive hydrogel microspheres encapsulated with tumor-cell-derived microparticles for malignant ascites treatment. *Acta Biomater.* 2025;192:328-339.
doi: 10.1016/j.actbio.2024.11.034
64. Ivanova I, Slavkova M, Popova T, *et al.* Agar Graft Modification with Acrylic and Methacrylic Acid for the Preparation of pH-Sensitive Nanogels for 5-Fluorouracil Delivery. *Gels.* 2024;10(3):165.
doi: 10.3390/gels10030165
65. Pang Q, Chen Z, Li X, *et al.* Cytokine-Activated Mesenchymal-Stem-Cell-Derived Extracellular Matrix Facilitates Cartilage Repair by Enhancing Chondrocyte Homeostasis and Chondrogenesis of Recruited Stem Cells. *Research.* 2025;8:0700.
doi: 10.34133/research.0700
66. Golebiowska AA, Intravaia JT, Sathe VM, Kumbar SG, Nukavarapu SP. Decellularized extracellular matrix biomaterials for regenerative therapies: Advances, challenges and clinical prospects. *Bioact Mater.* 2024;32:98-123.
doi: 10.1016/j.bioactmat.2023.09.017
67. Pang Q, Li X, Tang B, *et al.* Cartilage-anchoring ECM-functionalized microgels targeting miMOMP to suppress senescence and restore cartilage in osteoarthritis. *Chem Eng J.* 2025;525:170093.
doi: 10.1016/j.cej.2025.170093
68. Chen Y, Chen L-F, Wang Y, *et al.* Engineered dECM-based microsystem promotes cartilage regeneration in osteoarthritis by synergistically enhancing chondrogenesis of BMSCs and anti-inflammatory effect. *Compos Part B Eng.* 2025;290:111974.
doi: 10.1016/j.compositesb.2024.111974
69. Luo SC, Li MT, Wang YC, *et al.* Microfluidic GelMA/Bone-Derived Extracellular Matrix Microgels for Enhanced Stem Cell-Based Bone Regeneration. *ACS Appl Bio Mater.* 2026;9(2):906-920.
doi: 10.1021/acsabm.5c01821
70. Chen X, Zhang J, Lin T, *et al.* Bioactive Decellularized Extracellular Matrix Platform Integrating Multifunctional Nanozymes and Cell-Laden Microgels for Acute Liver Failure Treatment. *ACS Nano.* 2025;19(7):6890-6910.
doi: 10.1021/acsnano.4c13709
71. Im P, Shin H, Kim J. Tilapia-Derived Granular Hydrogel as a 3D Scaffold Promoting Rapid Wound Healing. *Biomacromolecules.* 2024;25(2):1153-1161.
doi: 10.1021/acs.biomac.3c01137
72. Dreifke MB, Ebraheim NA, Jayasuriya AC. Investigation of potential injectable polymeric biomaterials for bone regeneration. *J Biomed Mater Res A.* 2013;101A(8):2436-2447.
doi: 10.1002/jbm.a.34521

73. Santerre JP, Woodhouse K, Laroche G, Labow RS. Understanding the biodegradation of polyurethanes: from classical implants to tissue engineering materials. *Biomaterials*. 2005;26(35):7457-7470.
doi: 10.1016/j.biomaterials.2005.05.079
74. Mainardi A, Börsch A, Occhetta P, *et al.* An Organ-on-Chip Platform for Strain-Controlled, Tissue-Specific Compression of Cartilage and Mineralized Osteochondral Interface to Study Mechanical Overloading in Osteoarthritis. *Adv Healthc Mater*. 2025;14(23):e2501588.
doi: 10.1002/adhm.202501588
75. Zhao X, Ma H, Han H, *et al.* Precision medicine strategies for spinal degenerative diseases: Injectable biomaterials with in situ repair and regeneration. *Mater Today Bio*. 2022;16:100336.
doi: 10.1016/j.mtbio.2022.100336
76. Shirbhate U, Bajaj P. Injectable and Self-Invigorating Hydrogel Applications in Dentistry and Periodontal Regeneration: A Literature Review. *Cureus*. 2022;14(9):e29248.
doi: 10.7759/cureus.29248
77. Bédouet L, Beilvert A, Servais E, Moine L. Degradable Hydrogel Microspheres for Drug Delivery: In Vitro Performance and Influence of E-Beam Sterilization. *AAPS PharmSciTech*. 2025;26(5):162.
doi: 10.1208/s12249-025-03144-1
78. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev*. 2016;99(Pt A):28-51.
doi: 10.1016/j.addr.2015.09.012
79. Li X, Xu Q, Johnson M, *et al.* A chondroitin sulfate based injectable hydrogel for delivery of stem cells in cartilage regeneration. *Biomater Sci*. 2021;9(11):4139-4148.
doi: 10.1039/D1BM00482D
80. Liang X, Zhong HJ, Ding H, *et al.* Polyvinyl Alcohol (PVA)-Based Hydrogels: Recent Progress in Fabrication, Properties, and Multifunctional Applications. *Polymers*. 2024;16(19):2755.
doi: 10.3390/polym16192755
81. Allen MJ, Schoonmaker JE, Bauer TW, Williams PF, Higham PA, Yuan HA. Preclinical evaluation of a poly (vinyl alcohol) hydrogel implant as a replacement for the nucleus pulposus. *Spine*. 2004;29(5):515-523.
doi: 10.1097/01.BRS.0000113871.67305.38
82. Tsiptsias C, Fardis D, Ntampou X, Tsivintzelis I, Panayiotou C. Thermal Behavior of Poly(vinyl alcohol) in the Form of Physically Crosslinked Film. *Polymers*. 2023;15(8):1843.
doi: 10.3390/polym15081843
83. Rahman Khan MM, Rumon MMH. Synthesis of PVA-Based Hydrogels for Biomedical Applications: Recent Trends and Advances. *Gels*. 2025;11(2):88.
doi: 10.3390/gels11020088
84. Długa A, Bajer D, Kaczmarek H. Photochemical and Thermal Stability of Bionanocellulose/Poly(Vinyl Alcohol) Blends. *Polymers*. 2022;14(20):4364.
doi: 10.3390/polym14204364
85. Țăin Anastasiu AE, Bircă AC, Naulea AMI, Niculescu AG, Grumezescu AM, Croitoru GA. Alginate/PVA Hydrogel Incorporating HA-Liposomes and Aronia-Derived Silver Nanoparticles for Advanced Wound Management. *Int J Mol Sci*. 2025;26(18):9203.
doi: 10.3390/ijms26189203
86. Liu W, Lei L, Ma F, *et al.* A Dioscorea opposita Polysaccharide-Calcium Carbonate Microsphere-Doped Hydrogel for Accelerated Diabetic Wound Healing via Synergistic Glucose-Responsive Hypoglycemic and Anti-Inflammatory Effects. *ACS Biomater Sci Eng*. 2025;11(1):415-428.
doi: 10.1021/acsbomaterials.4c02090
87. Karam JP, Muscari C, Sindji L, *et al.* Pharmacologically active microcarriers associated with thermosensitive hydrogel as a growth factor releasing biomimetic 3D scaffold for cardiac tissue-engineering. *J Control Release*. 2014;192:82-94.
doi: 10.1016/j.jconrel.2014.06.052
88. Ma S, Feng X, Liu F, Wang B, Zhang H, Niu X. The pro-inflammatory response of macrophages regulated by acid degradation products of poly(lactide-co-glycolide) nanoparticles. *Eng Life Sci*. 2021;21(10):709-720.
doi: 10.1002/elsc.202100040
89. Liu H, Slamovich EB, Webster TJ. Less harmful acidic degradation of poly(lactico-glycolic acid) bone tissue engineering scaffolds through titania nanoparticle addition. *Int J Nanomed*. 2006;1(4):541-545.
doi: 10.2147/nano.2006.1.4.541
90. El Khatib M, Russo V, Prencipe G, *et al.* Amniotic Epithelial Stem Cells Counteract Acidic Degradation By-Products of Electrospun PLGA Scaffold by Improving Their Immunomodulatory Profile In Vitro. *Cells*. 2021;10(11):3221.
doi: 10.3390/cells10113221
91. Yu Z, Ma S, Wu M, *et al.* Self-assembling hydrogel loaded with 5-FU PLGA microspheres as a novel vitreous substitute for proliferative vitreoretinopathy. *J Biomed Mater Res A*. 2020;108(12):2435-2446.
doi: 10.1002/jbm.a.36995
92. Segneanu AE, Bejenaru LE, Bejenaru C, *et al.* Advancements in Hydrogels: A Comprehensive Review of Natural and Synthetic Innovations for Biomedical Applications. *Polymers*. 2025;17(15):2026.
doi: 10.3390/polym17152026
93. Choi HS, Seo SA, Khang G, Rhee JM, Lee HB. Preparation and characterization of fentanyl-loaded PLGA microspheres: in vitro release profiles. *Int J Pharm*. 2002;234(1-2):195-203.
doi: 10.3390/gels11020088

- doi: 10.1016/s0378-5173(01)00968-1
94. Hauser CAE, Deng R, Mishra A, *et al.* Natural tri- to hexapeptides self-assemble in water to amyloid β -type fiber aggregates by unexpected α -helical intermediate structures. *Proc Natl Acad Sci USA*. 2011;108(4):1361-1366.
doi: 10.1073/pnas.1014796108
 95. Mishra A, Loo Y, Deng R, *et al.* Ultrasmall natural peptides self-assemble to strong temperature-resistant helical fibers in scaffolds suitable for tissue engineering. *Nano Today*. 2011;6(3):232-239.
doi: 10.1016/j.nantod.2011.05.001
 96. Susapto HH, Alhattab D, Abdelrahman S, *et al.* Ultrashort Peptide Bioinks Support Automated Printing of Large-Scale Constructs Assuring Long-Term Survival of Printed Tissue Constructs. *Nano Lett*. 2021;21(7):2719-2729.
doi: 10.1021/acs.nanolett.0c04426
 97. Loo Y, Goktas M, Tekinay AB, Guler MO, Hauser CAE, Mittraki A. Self-Assembled Proteins and Peptides as Scaffolds for Tissue Regeneration. *Adv Healthc Mater*. 2015;4(16):2557-2586.
doi: 10.1002/adhm.201500402
 98. Loo Y, Lakshmanan A, Ni M, Toh LL, Wang S, Hauser CAE. Peptide Bioink: Self-Assembling Nanofibrous Scaffolds for Three-Dimensional Organotypic Cultures. *Nano Lett*. 2015;15(10):6919-6925.
doi: 10.1021/acs.nanolett.5b02859
 99. Ramirez-Calderon G, Susapto HH, Hauser CAE. Delivery of Endothelial Cell-Laden Microgel Elicits Angiogenesis in Self-Assembling Ultrashort Peptide Hydrogels In Vitro. *ACS Appl Mater Interfaces*. 2021;13(25):29281-29292.
doi: 10.1021/acsami.1c03787
 100. Liu J, Du C, Chen J, *et al.* Hydrogel Microspheres Empowering Organ-on-a-Chip Systems: Innovations and Applications. *Small*. 2025;21(44):e2504563.
doi: 10.1002/smll.202504563
 101. Tumarkin E, Kumacheva E. Microfluidic generation of microgels from synthetic and natural polymers. *Chem Soc Rev*. 2009;38(8):2161-2168.
doi: 10.1039/b809915b
 102. Cheng S, Yang L, Dong M, *et al.* Metabolism-Regulating Microspheres: Design Principles, Therapeutic Applications Across Multisystem Diseases, and Future Perspectives. *Research*. 2026;9:1106.
doi: 10.34133/research.1106
 103. Cheng S, Luo X, Yang L, *et al.* Design and applications of immunomodulatory microspheres: From material properties to multisystem disease therapies. *Mater Today*. 2026;92:925-951.
doi: 10.1016/j.mattod.2025.12.035
 104. Xia X, Liu Y, Lu Y, *et al.* Retuning Mitochondrial Apoptosis/Mitophagy Balance via SIRT3-Energized and Microenvironment-Modulated Hydrogel Microspheres to Impede Osteoarthritis. *Adv Health Mater*. 2023;12(32):2302475.
doi: 10.1002/adhm.202302475
 105. Lei Y, Wang Y, Shen J, *et al.* Injectable hydrogel microspheres with self-renewable hydration layers alleviate osteoarthritis. *Sci Adv*. 2022;8(5):eabl6449.
doi: 10.1126/sciadv.abl6449
 106. Xiao P, Han X, Huang Y, *et al.* Reprogramming macrophages via immune cell mobilized hydrogel microspheres for osteoarthritis treatments. *Bioact Mater*. 2024;32:242-259.
doi: 10.1016/j.bioactmat.2023.09.010
 107. Liu J, Liu J, Liu S, *et al.* Cascade targeting selenium nanoparticles-loaded hydrogel microspheres for multifaceted antioxidant defense in osteoarthritis. *Biomaterials*. 2025;318:123195.
doi: 10.1016/j.biomaterials.2025.123195
 108. Mahou R, Vlahos AE, Shulman A, Sefton MV. Interpenetrating Alginate-Collagen Polymer Network Microspheres for Modular Tissue Engineering. *ACS Biomater Sci Eng*. 2018;4(11):3704-3712.
doi: 10.1021/acsbiomaterials.7b00356
 109. Li X, Li L, Wang D, *et al.* Fabrication of polymeric microspheres for biomedical applications. *Mater Horiz*. 2024;11(12):2820-2855.
doi: 10.1039/D3MH01641B
 110. Chen T, Cai Z, Zhao X, *et al.* Dynamic monitoring soft tissue healing via visualized Gd-crosslinked double network MRI microspheres. *J Nanobiotechnol*. 2024;22(1):289.
doi: 10.1186/s12951-024-02549-7
 111. Zhang X, Zhu R, Wang X, *et al.* Core-Shell Microspheres Prepared Using Coaxial Electrostatic Spray for Local Chemotherapy of Solid Tumors. *Pharmaceutics*. 2023;16(1):45.
doi: 10.3390/pharmaceutics16010045
 112. Wu Y, Liao IC, Kennedy SJ, *et al.* Electrosprayed core-shell microspheres for protein delivery. *Chem Commun*. 2010;46(26):4743-4745.
doi: 10.1039/c0cc00535e
 113. Yang Y, Sha L, Zhao H, Guo Z, Wu M, Lu P. Recent advances in cellulose microgels: Preparations and functionalized applications. *Adv Colloid Interface Sci*. 2023;311:102815.
doi: 10.1016/j.cis.2022.102815
 114. Zhu L, Li M, Liu X, Jin Y. Drug-Loaded PLGA Electrospraying Porous Microspheres for the Local Therapy of Primary Lung Cancer via Pulmonary Delivery. *ACS Omega*. 2017;2(5):2273-2279.

- doi: 10.1021/acsomega.7b00456
115. Xie J, Ng WJ, Lee LY, Wang CH. Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray. *J Colloid Interface Sci.* 2008;317(2):469-476.
doi: 10.1016/j.jcis.2007.09.082
 116. Helgeson ME, Chapin SC, Doyle PS. Hydrogel microparticles from lithographic processes: novel materials for fundamental and applied colloid science. *Curr Opin Colloid Interface Sci.* 2011;16(2):106-117.
doi: 10.1016/j.cocis.2011.01.005
 117. Stornello DE, Kim J, Chen Z, Heaton K, Qazi TH. Controlling Microparticle Aspect Ratio via Photolithography for Injectable Granular Hydrogel Formation and Cell Delivery. *ACS Biomater Sci Eng.* 2025;11(2):1242-1252.
doi: 10.1021/acsbiomaterials.4c02102
 118. Lee SA, Chung SE, Park W, Lee SH, Kwon S. Three-dimensional fabrication of heterogeneous microstructures using soft membrane deformation and optofluidic maskless lithography. *Lab Chip.* 2009;9(12):1670-1675.
doi: 10.1039/b819999j
 119. Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S, Khademhosseini A. Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials.* 2010;31(21):5536-5544.
doi: 10.1016/j.biomaterials.2010.03.064
 120. Palange AL, Mascolo DD, Ferreira M, *et al.* Boosting the Potential of Chemotherapy in Advanced Breast Cancer Lung Metastasis via Micro-Combinatorial Hydrogel Particles. *Adv Sci.* 2023;10(10):2205223.
doi: 10.1002/advs.202205223
 121. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol.* 2014;32(8):773-785.
doi: 10.1038/nbt.2958
 122. Zhu W, Ma X, Gou M, Mei D, Zhang K, Chen S. 3D printing of functional biomaterials for tissue engineering. *Curr Opin Biotechnol.* 2016;40:103-112.
doi: 10.1016/j.copbio.2016.03.014
 123. Kotani T, Hananouchi T, Sakai S. Enhancing visible light-induced 3D bioprinting: alternating extruded support materials for bioink gelation. *Biomed Mater.* 2025;20(3):035005.
doi: 10.1088/1748-605X/adc0d6
 124. Grottkau BE, Hui Z, Pang Y. Articular Cartilage Regeneration through Bioassembling Spherical Micro-Cartilage Building Blocks. *Cells.* 2022;11(20):3244.
doi: 10.3390/cells11203244
 125. Wu CA, Zhu Y, Venkatesh A, Stark CJ, Lee SH, Woo YJ. Optimization of Freeform Reversible Embedding of Suspended Hydrogel Microspheres for Substantially Improved Three-Dimensional Bioprinting Capabilities. *Tissue Eng Part C Methods.* 2023;29(3):85-94.
doi: 10.1089/ten.tec.2022.0214
 126. De Lorenzi F, Hansen N, Theek B, *et al.* Engineering Mesoscopic 3D Tumor Models with a Self-Organizing Vascularized Matrix. *Adv Mater.* 2024;36(5):e2303196.
doi: 10.1002/adma.202303196
 127. Du C, Huang W, Lei Y. The application and prospects of 3D printable microgel in biomedical science and engineering. *Int J Bioprint.* 2023;9(5):753.
doi: 10.18063/ijb.753
 128. Zhao H, Chen Y, Shao L, *et al.* Airflow-Assisted 3D Bioprinting of Human Heterogeneous Microspheroidal Organoids with Microfluidic Nozzle. *Small.* 2018;14(39):e1802630.
doi: 10.1002/sml.201802630
 129. Zhang S, Li G, Man J, *et al.* Fabrication of Microspheres from High-Viscosity Bioink Using a Novel Microfluidic-Based 3D Bioprinting Nozzle. *Micromachines.* 2020;11(7):681.
doi: 10.3390/mi11070681
 130. Cao Y, Tan J, Zhao H, *et al.* Bead-jet printing enabled sparse mesenchymal stem cell patterning augments skeletal muscle and hair follicle regeneration. *Nat Commun.* 2022;13(1):7463.
doi: 10.1038/s41467-022-35183-8
 131. Clua-Ferré L, De Chiara F, Rodríguez-Comas J, *et al.* Collagen-Tannic Acid Spheroids for β -Cell Encapsulation Fabricated Using a 3D Bioprinter. *Adv Mater Technol.* 2022;7(7):2101696.
doi: 10.1002/admt.202101696
 132. Gao Q, Liu Z, Lin Z, *et al.* 3D Bioprinting of Vessel-like Structures with Multilevel Fluidic Channels. *ACS Biomater Sci Eng.* 2017;3(3):399-408.
doi: 10.1021/acsbiomaterials.6b00643
 133. Rocha Luiz ME, Carreira M, Nadine S, *et al.* Contactless 3D acoustic assembly of liquid capsules for bottom-up tissue engineering. *Biomaterials.* 2026;325:123555.
doi: 10.1016/j.biomaterials.2025.123555
 134. Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *J Control Release.* 2005;102(2):313-332.
doi: 10.1016/j.jconrel.2004.10.015
 135. van der Kooij RS, Steendam R, Frijlink HW, Hinrichs WLJ. An overview of the production methods for core-shell microspheres for parenteral controlled drug delivery. *Eur J Pharm Biopharm.* 2022;170:24-42.
doi: 10.1016/j.ejpb.2021.11.007
 136. Ren Z, Wang Y, Wu H, Cong H, Yu B, Shen Y. Preparation

- and application of hemostatic microspheres containing biological macromolecules and others. *Int J Biol Macromol.* 2024;257(Pt 1):128299.
doi: 10.1016/j.ijbiomac.2023.128299
137. Contessi Negrini N, Lipreri MV, Tanzi MC, Farè S. In vitro cell delivery by gelatin microspheres prepared in water-in-oil emulsion. *J Mater Sci Mater Med.* 2020;31(3):26.
doi: 10.1007/s10856-020-6363-2
 138. Pradhan S, Clary JM, Seliktar D, Lipke EA. A three-dimensional spheroidal cancer model based on PEG-fibrinogen hydrogel microspheres. *Biomaterials.* 2017;115:141-154.
doi: 10.1016/j.biomaterials.2016.10.052
 139. Muir VG, Qazi TH, Shan J, Groll J, Burdick JA. Influence of Microgel Fabrication Technique on Granular Hydrogel Properties. *ACS Biomater Sci Eng.* 2021;7(9):4269-4281.
doi: 10.1021/acsbiomaterials.0c01612
 140. Lin CH, Srioudom JR, Sun W, *et al.* The use of hydrogel microspheres as cell and drug delivery carriers for bone, cartilage, and soft tissue regeneration. *Biomater Transl.* 2024;5(3):236-256.
doi: 10.12336/biomatertransl.2024.03.003
 141. Puiggali-Jou A, Asadikorayem M, Maniura-Weber K, Zenobi-Wong M. Growth factor-loaded sulfated microislands in granular hydrogels promote hMSCs migration and chondrogenic differentiation. *Acta Biomater.* 2023;166:69-84.
doi: 10.1016/j.actbio.2023.03.045
 142. Jin S, Wang Y, Wu X, *et al.* Young Exosome Bio-Nanoparticles Restore Aging-Impaired Tendon Stem/Progenitor Cell Function and Reparative Capacity. *Adv Mater.* 2023;35(18):e2211602.
doi: 10.1002/adma.202211602
 143. Huang G, Shen H, Xu K, *et al.* Single-Cell Microgel Encapsulation Improves the Therapeutic Efficacy of Mesenchymal Stem Cells in Treating Intervertebral Disc Degeneration via Inhibiting Pyroptosis. *Research.* 2024;7:0311.
doi: 10.34133/research.0311
 144. Baker BM, Chen CS. Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. *J Cell Sci.* 2012;125(Pt 13):3015-3024.
doi: 10.1242/jcs.079509
 145. Raghavan S, Shen CJ, Desai RA, Sniadecki NJ, Nelson CM, Chen CS. Decoupling diffusional from dimensional control of signaling in 3D culture reveals a role for myosin in tubulogenesis. *J Cell Sci.* 2010;123(17):2877-2883.
doi: 10.1242/jcs.055079
 146. Özkale B, Lou J, Özelçi E, *et al.* Actuated 3D microgels for single cell mechanobiology. *Lab Chip.* 2022;22(10):1962-1970.
doi: 10.1039/d2lc00203e
 147. Schindler M, Siriwardena D, Kohler TN, *et al.* Agarose microgel culture delineates lumenogenesis in naive and primed human pluripotent stem cells. *Stem Cell Rep.* 2021;16(5):1347-1362.
doi: 10.1016/j.stemcr.2021.04.009
 148. Munger C, Kohler TN, Slatery E, *et al.* Microgel culture and spatial identity mapping elucidate the signalling requirements for primate epiblast and amnion formation. *Development.* 2022;149(20):dev200263.
doi: 10.1242/dev.200263
 149. Lavickova B, Kronabitter H, Cervera-Negueruela M, *et al.* Integrated Microfluidic Platform for High-Throughput Generation of Intestinal Organoids in Hydrogel Droplets. *Adv Sci.* 2026;13(13):e16507.
doi: 10.1002/advs.202516507
 150. Zheng F, Tian R, Lu H, *et al.* Droplet Microfluidics Powered Hydrogel Microparticles for Stem Cell-Mediated Biomedical Applications. *Small.* 2024;20(42):e2401400.
doi: 10.1002/sml.202401400
 151. Yang X, Wang L, Chen X, Wei N, Sun X, Xiao J. Digital light processing 3D bioprinting of type II collagen-based engineered cartilage organoids for enhanced chondrogenic regeneration. *Chem Eng J.* 2025;520:165969.
doi: 10.1016/j.cej.2025.165969
 152. Tevis KM, Colson YL, Grinstaff MW. Embedded Spheroids as Models of the Cancer Microenvironment. *Adv Biosyst.* 2017;1(10):1700083.
doi: 10.1002/adbi.201700083
 153. Fang G, Lu H, Rodriguez de la Fuente L, *et al.* Mammary Tumor Organoid Culture in Non-Adhesive Alginate for Luminal Mechanics and High-Throughput Drug Screening. *Adv Sci.* 2021;8(21):e2102418.
doi: 10.1002/advs.202102418
 154. Sun Q, Tan SH, Chen Q, *et al.* Microfluidic Formation of Coculture Tumor Spheroids with Stromal Cells As a Novel 3D Tumor Model for Drug Testing. *ACS Biomater Sci Eng.* 2018;4(12):4425-4433.
doi: 10.1021/acsbiomaterials.8b00904
 155. Lv C, Li S, Sang M, Cui T, Xie J. Construction of Microsphere Culture System for Human Mesenchymal Stem Cell Aggregates. *Int J Mol Sci.* 2025;26(13):6435.
doi: 10.3390/ijms26136435
 156. Zhou C, Ji J, Fu M, *et al.* Hydrogel Microsphere-Based Alveolar Models for Toxicity Assessment and Pathogen Infection Studies. *Bioengineering.* 2025;13(1):17.

- doi: 10.3390/bioengineering13010017
157. Kohler TN, De Jonghe J, Ellermann AL, *et al.* Plakoglobin is a mechanoresponsive regulator of naive pluripotency. *Nat Commun.* 2023;14(1):4022.
doi: 10.1038/s41467-023-39515-0
 158. Huey DJ, Hu JC, Athanasiou KA. Unlike bone, cartilage regeneration remains elusive. *Science.* 2012;338(6109):917-921.
doi: 10.1126/science.1222454
 159. Ahmed MS, Yun S, Kim HY, Ko S, Islam M, Nam KW. Hydrogels and Microgels: Driving Revolutionary Innovations in Targeted Drug Delivery, Strengthening Infection Management, and Advancing Tissue Repair and Regeneration. *Gels.* 2025;11(3):179.
doi: 10.3390/gels11030179
 160. Ortega Quesada BA, Chauvin C, Martin E, Melvin A. Droplet microfluidics integrated with machine learning reveals how adipose-derived stem cells modulate endocrine response and tumor heterogeneity in ER(+) breast cancer. *Lab Chip.* 2025;25(15):3817-3830.
doi: 10.1039/d5lc00320b
 161. Ochoa A, Gastéum G, Rocha J, Olguin LF. High-throughput bacterial co-encapsulation in microfluidic gel beads for discovery of antibiotic-producing strains. *Analyst.* 2023;148(22):5762-5774.
doi: 10.1039/D3AN01101A
 162. Mulas C, Hodgson AC, Kohler TN, *et al.* Microfluidic platform for 3D cell culture with live imaging and clone retrieval. *Lab Chip.* 2020;20(14):2580-2591.
doi: 10.1039/d0lc00165a
 163. Lei Y, Zhang Y, Zhou L, *et al.* Organoids and organs-on-chips for accelerating R&D and clinical translation in Orthopaedics: Emerging opportunities and regulatory pathways. *J Orthop Translat.* 2026;56:101021.
doi: 10.1016/j.jot.2025.10.013
 164. Zhu Y, Cai L, Chen H, Zhao Y. Developing organs-on-chips for biomedicine. *Sci Bull.* 2022;67(11):1108-1111.
doi: 10.1016/j.scib.2022.04.012
 165. You L, Chen Y, Zhang Z, *et al.* The FLOW Framework: a panvascular-on-a-chip platform to model systemic disease and guide panvascular interventional device suitcordance. *Sci Bull.* 2026;71(3):654-670.
doi: 10.1016/j.scib.2025.12.051
 166. Pierfelice TV, D'Amico E, Petrini M, *et al.* A Systematic Review on Organ-on-a-Chip in PDMS or Hydrogel in Dentistry: An Update of the Literature. *Gels.* 2024;10(2):102.
doi: 10.3390/gels10020102
 167. Geng Q, Xu Y, Hu Y, *et al.* Progress in the Application of Organoids-On-A-Chip in Diseases. *Organogenesis.* 2024;20(1):2386727.
doi: 10.1080/15476278.2024.2386727
 168. Nejati B, Shahhosseini R, Hajiabbasi M, *et al.* Cancer-on-chip: a breakthrough organ-on-a-chip technology in cancer cell modeling. *Med Biol Eng Comput.* 2025;63(2):321-337.
doi: 10.1007/s11517-024-03199-5
 169. Gong X, Yang C, Peng J, *et al.* Vascularized organoid-on-a-chip for centimeter-scale organoid cultivation. *Bio-Des Manuf.* 2025;8(3):410-422.
doi: 10.1631/bdm.2400424
 170. Qian K, Wang Y, Li R, *et al.* Application and Prospects of Organoid-on-a-Chip in Research on the Intestinal Mucosal Barrier. *Burn Trauma.* 2026;tkag029.
doi: 10.1093/burnst/tkag029
 171. Cho Y, You J, Lee JH. Natural Polymer-Based Hydrogel Platforms for Organoid and Microphysiological Systems: Mechanistic Insights and Translational Perspectives. *Polymers.* 2025;17(15):2109.
doi: 10.3390/polym17152109
 172. Qiao L, Chang S, Zou L, Zhang F, Cui C, Huang N. Multiorgan-on-a-chip: an advanced platform for disease modeling, drug toxicity assessment, and therapeutic screening. *Bio-Des Manuf.* 2025;8(6):1035-1062.
doi: 10.1631/bdm.2500076
 173. Yang M, Shi Y, Wang F, *et al.* Hydrogel Microspheres as Versatile Platforms for Biomedical Research: Design, Properties, and Applications. *MedComm.* 2025;6(10):e70423.
doi: 10.1002/mco2.70423
 174. Caires HR, Castillo-Fernández Ó, Sima N, *et al.* Advancing Organ-on-Chip Models With a Sacrificial Granular Hydrogel Strategy for Enhanced Permeability and Biomimicry. *Small Methods.* 2025;9(12):e00652.
doi: 10.1002/smt.202500652
 175. Pye JS, Zhang Y, Lin X, Yousefi M, Wen C, Li JJ. Joint-on-a-Chip: The Future of Preclinical Modelling of Joint Diseases. *Adv Healthc Mater.* 2025:e04487.
doi: 10.1002/adhm.202504487
 176. Conceição F, Meneses J, Lebre F, *et al.* Sex-stratified osteochondral organ-on-chip model reveals sex-specific responses to inflammatory stimulation. *Mater Today Bio.* 2025;32:101728.
doi: 10.1016/j.mtbio.2025.101728
 177. Lei L, Wen Z, Cao M, *et al.* The emerging role of Piezo1 in the musculoskeletal system and disease. *Theranostics.* 2024;14(10):3963-3983.
doi: 10.7150/thno.96959
 178. Li J, Yang Y, Yi Z, *et al.* Microdroplet-Engineered Skeletal

- Muscle Organoids from Primary Tissue Recapitulate Parental Physiology with High Reproducibility. *Research*. 2025;8:0699.
doi: 10.34133/research.0699
179. Pueyo Moliner A, Ito K, Zaucke F, Kelly DJ, de Ruijter M, Malda J. Restoring articular cartilage: insights from structure, composition and development. *Nat Rev Rheumatol*. 2025;21(5):291-308.
doi: 10.1038/s41584-025-01236-7
 180. Wilusz RE, Sanchez-Adams J, Guilak F. The structure and function of the pericellular matrix of articular cartilage. *Matrix Biol*. 2014;39:25-32.
doi: 10.1016/j.matbio.2014.08.009
 181. Zhang W, Liu Y, Zhang H. Extracellular matrix: an important regulator of cell functions and skeletal muscle development. *Cell Biosci*. 2021;11(1):65.
doi: 10.1186/s13578-021-00579-4
 182. Wohlgemuth RP, Brashear SE, Smith LR. Alignment, cross linking, and beyond: a collagen architect's guide to the skeletal muscle extracellular matrix. *Am J Physiol Cell Physiol*. 2023;325(4):C1017-C1030.
doi: 10.1152/ajpcell.00287.2023
 183. Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev*. 2004;84(2):649-698.
 184. Conway R, O'Neill L, Gallagher P, et al. Ustekinumab for refractory giant cell arteritis: A prospective 52-week trial. *Semin Arthritis Rheum*. 2018;48(3):523-528.
doi: 10.1016/j.semarthrit.2018.04.004
 185. Gilbert SJ, Bonnet CS, Blain EJ. Mechanical Cues: Bidirectional Reciprocity in the Extracellular Matrix Drives Mechano-Signalling in Articular Cartilage. *Int J Mol Sci*. 2021;22(24):13595.
doi: 10.3390/ijms222413595
 186. Vincent TL, McClurg O, Troeberg L. The Extracellular Matrix of Articular Cartilage Controls the Bioavailability of Pericellular Matrix-Bound Growth Factors to Drive Tissue Homeostasis and Repair. *Int J Mol Sci*. 2022;23(11):6003.
doi: 10.3390/ijms23116003
 187. Wilusz RE, Defratre LE, Guilak F. A biomechanical role for perlecan in the pericellular matrix of articular cartilage. *Matrix Biol*. 2012;31(6):320-327.
doi: 10.1016/j.matbio.2012.05.002
 188. Chen L, Yang J, Cai Z, et al. Mitochondrial-Oriented Injectable Hydrogel Microspheres Maintain Homeostasis of Chondrocyte Metabolism to Promote Subcellular Therapy in Osteoarthritis. *Research*. 2024;7:0306.
doi: 10.34133/research.0306
 189. Quan H, He Y, Xiao P, et al. Scaffold-Mediated Microenvironmental Modulation Targeting Osteoclasts for ONFH Niche Reprogramming. *Research*. 2025;8:1027.
doi: 10.34133/research.1027
 190. Lampi MC, Reinhart-King CA. Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials. *Sci Transl Med*. 2018;10(422):eaao0475.
doi: 10.1126/scitranslmed.aao0475
 191. Liu E, Zhu D, Gonzalez Diaz E, Tong X, Yang F. Gradient Hydrogels for Optimizing Niche Cues to Enhance Cell-Based Cartilage Regeneration. *Tissue Eng Part A*. 2021;27(13-14):929-939.
doi: 10.1089/ten.tea.2020.0158
 192. Galarraga JH, Locke RC, Witherel CE, et al. Fabrication of MSC-laden composites of hyaluronic acid hydrogels reinforced with MEW scaffolds for cartilage repair. *Biofabrication*. 2022;14(1):014106.
doi: 10.1088/1758-5090/ac3acb
 193. Asadikorayem M, Brunel LG, Weber P, Heilshorn SC, Zenobi-Wong M. Porosity dominates over microgel stiffness for promoting chondrogenesis in zwitterionic granular hydrogels. *Biomater Sci*. 2024;12(21):5504-5520.
doi: 10.1039/D4BM00233D
 194. Walker M, Pringle EW, Ciccone G, et al. Mind the Viscous Modulus: The Mechanotransductive Response to the Viscous Nature of Isoelastic Matrices Regulates Stem Cell Chondrogenesis. *Adv Healthc Mater*. 2024;13(9):e2302571.
doi: 10.1002/adhm.202302571
 195. Ai C, Liu L, Wong K, Tan XH, Goh JCH. The effect of chondroitin sulfate concentration and matrix stiffness on chondrogenic differentiation of mesenchymal stem cells. *Biomater Sci*. 2023;11(13):4557-4573.
doi: 10.1039/D2BM01980A
 196. Roncada T, Bonithon R, Blunn G, Roldo M. Soft substrates direct stem cell differentiation into the chondrogenic lineage without the use of growth factors. *J Tissue Eng*. 2022;13:20417314221122121.
doi: 10.1177/20417314221122121
 197. Žigon-Branc S, Markovic M, Van Hoorick J, et al. Impact of Hydrogel Stiffness on Differentiation of Human Adipose-Derived Stem Cell Microspheroids. *Tissue Eng Part A*. 2019;25(19-20):1369-1380.
doi: 10.1089/ten.tea.2018.0237
 198. Bachmann B, Spitz S, Schädler B, et al. Stiffness Matters: Fine-Tuned Hydrogel Elasticity Alters Chondrogenic Redifferentiation. *Front Bioeng Biotechnol*. 2020;8:373.
doi: 10.3389/fbioe.2020.00373
 199. Lee J, Jeon O, Koh J, et al. Micromechanical property mismatch between pericellular and extracellular matrices regulates stem cell articular and hypertrophic chondrogenesis. *Matter*. 2023;6(2):475-492.

- doi: 10.1016/j.matt.2022.11.008
200. Chen Z, Lv Z, Zhuang Y, *et al.* Mechanical Signal-Tailored Hydrogel Microspheres Recruit and Train Stem Cells for Precise Differentiation. *Adv Mater.* 2023;35(40):2300180.
doi: 10.1002/adma.202300180
 201. Chaudhuri O, Gu L, Klumpers D, *et al.* Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater.* 2016;15(3):326-334.
doi: 10.1038/nmat4489
 202. Chaudhuri O, Gu L, Darnell M, *et al.* Substrate stress relaxation regulates cell spreading. *Nat Commun.* 2015;6(1):6364.
doi: 10.1038/ncomms7365
 203. Shi N, Wang J, Tang S, *et al.* Matrix Nonlinear Viscoelasticity Regulates Skeletal Myogenesis through MRTF Nuclear Localization and Nuclear Mechanotransduction. *Small.* 2024;20(9):e2305218.
doi: 10.1002/smll.202305218
 204. Davidson MD, Prendergast ME, Ban E, *et al.* Programmable and contractile materials through cell encapsulation in fibrous hydrogel assemblies. *Sci Adv.* 2021;7(46):eabi8157.
doi: 10.1126/sciadv.abi8157
 205. Bertsch P, Sacco P. The role of non-linear viscoelastic hydrogel mechanics in cell culture and transduction. *Mater Today Bio.* 2025;34:102188.
doi: 10.1016/j.mtbio.2025.102188
 206. Nam S, Stowers R, Lou J, Xia Y, Chaudhuri O. Varying PEG density to control stress relaxation in alginate-PEG hydrogels for 3D cell culture studies. *Biomaterials.* 2019;200:15-24.
doi: 10.1016/j.biomaterials.2019.02.004
 207. Bauer A, Gu L, Kwee B, *et al.* Hydrogel substrate stress-relaxation regulates the spreading and proliferation of mouse myoblasts. *Acta Biomater.* 2017;62:82-90.
doi: 10.1016/j.actbio.2017.08.041
 208. Liu Y, Li L, Li X, *et al.* Viscoelastic hydrogels regulate adipose-derived mesenchymal stem cells for nucleus pulposus regeneration. *Acta Biomater.* 2024;180:244-261.
doi: 10.1016/j.actbio.2024.04.017
 209. Zhao D, Li Y, Xiang L, *et al.* Cell Shock Absorption via Stress Relaxation Hydrogel Microspheres for Alleviating Endoplasmic Reticulum Stress in Chondrocytes. *Research.* 2025;8:0777.
doi: 10.34133/research.0777
 210. Roth JG, Huang MS, Navarro RS, Akram JT, LeSavage BL, Heilshorn SC. Tunable hydrogel viscoelasticity modulates human neural maturation. *Sci Adv.* 2023;9(42):eadh8313.
doi: 10.1126/sciadv.adh8313
 211. Li H, Zhao T, Yuan Z, *et al.* Cartilage lacuna-biomimetic hydrogel microspheres endowed with integrated biological signal boost endogenous articular cartilage regeneration. *Bioact Mater.* 2024;41:61-82.
doi: 10.1016/j.bioactmat.2024.06.037
 212. Baptista D, Teixeira L, van Blitterswijk C, Giselbrecht S, Truckenmüller R. Overlooked? Underestimated? Effects of Substrate Curvature on Cell Behavior. *Trends Biotechnol.* 2019;37(8):838-854.
doi: 10.1016/j.tibtech.2019.01.006
 213. Schamberger B, Ziege R, Anselme K, *et al.* Curvature in Biological Systems: Its Quantification, Emergence, and Implications across the Scales. *Adv Mater.* 2023;35(13):e2206110.
doi: 10.1002/adma.202206110
 214. Lee SJ, Yang S. Substrate Curvature Restricts Spreading and Induces Differentiation of Human Mesenchymal Stem Cells. *Biotechnol J.* 2017;12(9).
doi: 10.1002/biot.201700360
 215. Ding S-L, Zhao X-Y, Xiong W, *et al.* Cartilage Lacuna-Inspired Microcarriers Drive Hyaline Neocartilage Regeneration. *Adv Mater.* 2023;35(30):2212114.
doi: 10.1002/adma.202212114
 216. Anderson DE, Johnstone B. Dynamic Mechanical Compression of Chondrocytes for Tissue Engineering: A Critical Review. *Front Bioeng Biotechnol.* 2017;5:76.
doi: 10.3389/fbioe.2017.00076
 217. Vainieri ML, Wahl D, Alini M, van Osch GJVM, Grad S. Mechanically stimulated osteochondral organ culture for evaluation of biomaterials in cartilage repair studies. *Acta Biomater.* 2018;81:256-266.
doi: 10.1016/j.actbio.2018.09.058
 218. Farcasanu MV, Ruiz TdLH, Brito FMJdS, *et al.* Dynamic Compression Improves Chondrogenesis in the Tissue Engineered Model of Cartilage. *Biotechnol Bioeng.* 2025;122(9):2574-2591.
doi: 10.1002/bit.29026
 219. Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. *J Orthop Res.* 2002;20(4):842-848.
 220. Mesallati T, Buckley CT, Nagel T, Kelly DJ. Scaffold architecture determines chondrocyte response to externally applied dynamic compression. *Biomech Model Mechanobiol.* 2013;12(5):889-899.
doi: 10.1007/s10237-012-0451-2
 221. Zhao W, Cai G, Qian J, Geng J, Yao X, Zhang Y. Protein conformational transition microenvironment in silk fibroin hydrogels: proliferation and chondrogenesis of encapsulated stem cells. *Regen Biomater.* 2025;12:rbaf102.
doi: 10.1093/rb/rbaf102
 222. Schneider MC, Lalitha Sridhar S, Vernerey FJ, Bryant

- SJ. Spatiotemporal neocartilage growth in matrix-metalloproteinase-sensitive poly(ethylene glycol) hydrogels under dynamic compressive loading: an experimental and computational approach. *J Mater Chem B*. 2020;8(14):2775-2791.
doi: 10.1039/C9TB02963J
223. Xie W, Wei X, Kang H, *et al*. Static and Dynamic: Evolving Biomaterial Mechanical Properties to Control Cellular Mechanotransduction. *Adv Sci*. 2023;10(9):e2204594.
doi: 10.1002/advs.202204594
224. Alanko J, Mai A, Jacquemet G, *et al*. Integrin endosomal signalling suppresses anoikis. *Nat Cell Biol*. 2015;17(11):1412-1421.
doi: 10.1038/ncb3250
225. Alanko J, Ivaska J. Endosomes: Emerging Platforms for Integrin-Mediated FAK Signalling. *Trends Cell Biol*. 2016;26(6):391-398.
doi: 10.1016/j.tcb.2016.02.001
226. Rainero E, Norman JC. Endosomal integrin signals for survival. *Nat Cell Biol*. 2015;17(11):1373-1375.
doi: 10.1038/ncb3261
227. Zhao Y, Dong X, Li Y, *et al*. Integrated Cross-Scale Manipulation and Modulable Encapsulation of Cell-Laden Hydrogel for Constructing Tissue-Mimicking Microstructures. *Research*. 2024;7:0414.
doi: 10.34133/research.0414
228. Shi S, Zhang W, Yu Y, *et al*. Dynamic matrix engineering promotes nascent protein deposition to drive cell migration and expedite Re-epithelization in chronic wound. *Bioact Mater*. 2026;56:455-467.
doi: 10.1016/j.bioactmat.2025.10.020
229. Dai S, Huang B, Wang P, *et al*. Exosome-anchored scholizite conversion coating on titanium with long-term stability and immunomodulatory ability to enhance osteogenesis. *Chem Eng J*. 2025;503:158142.
doi: 10.1016/j.cej.2024.158142
230. Li Q, He X-L, Chen S-J, *et al*. Excessive collagen type VII mediates pleural fibrosis via increasing extracellular matrix stiffness. *J Clin Invest*. 2025;135(24)
doi: 10.1172/JCI188822
231. Koo JH, Guan K-L. Interplay between YAP/TAZ and Metabolism. *Cell Metab*. 2018;28(2):196-206.
doi: 10.1016/j.cmet.2018.07.010
232. Totaro A, Panciera T, Piccolo S. YAP/TAZ upstream signals and downstream responses. *Nat Cell Biol*. 2018;20(8):888-899.
doi: 10.1038/s41556-018-0142-z
233. Li X, Liu S, Han S, *et al*. Dynamic Stiffening Hydrogel with Instructive Stiffening Timing Modulates Stem Cell Fate In Vitro and Enhances Bone Remodeling In Vivo. *Adv Healthc Mater*. 2023;12(29):e2300326.
doi: 10.1002/adhm.202300326
234. İyisan N, Hausdörfer O, Wang C, *et al*. Mechanoactivation of Single Stem Cells in Microgels Using a 3D-Printed Stimulation Device. *Small Methods*. 2024;8(12):e2400272.
doi: 10.1002/smt.202400272
235. Jiang Y, Zhang H, Wang J, Liu Y, Luo T, Hua H. Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J Hematol Oncol*. 2022;15(1):34.
doi: 10.1186/s13045-022-01252-0
236. Qin L, He T, Chen S, *et al*. Roles of mechanosensitive channel Piezo1/2 proteins in skeleton and other tissues. *Bone Res*. 2021;9(1):44.
doi: 10.1038/s41413-021-00168-8
237. Xiao B. Mechanisms of mechanotransduction and physiological roles of PIEZO channels. *Nat Rev Mol Cell Biol*. 2024;25(11):886-903.
doi: 10.1038/s41580-024-00773-5
238. Wang L, You X, Lotinun S, Zhang L, Wu N, Zou W. Mechanical sensing protein PIEZO1 regulates bone homeostasis via osteoblast-osteoclast crosstalk. *Nat Commun*. 2020;11(1):282.
doi: 10.1038/s41467-019-14146-6
239. Wu Y, Xu X, Liu F, *et al*. Three-Dimensional Matrix Stiffness Activates the Piezo1-AMPK-Autophagy Axis to Regulate the Cellular Osteogenic Differentiation. *ACS Biomater Sci Eng*. 2023;9(8):4735-4746.
doi: 10.1021/acsbomaterials.3c00419
240. Chen W, Yi Z, Wang X, *et al*. Composite hydrogel-microsphere delivery system promotes early nerve-mediated bone regeneration and late-stage mechanotransduction-driven bone remodeling via sequential release of NGF and Yoda1. *Bioact Mater*. 2026;57:531-550.
doi: 10.1016/j.bioactmat.2025.10.040
241. Du J, Zu Y, Li J, *et al*. Extracellular matrix stiffness dictates Wnt expression through integrin pathway. *Scientific Reports*. 2016;6:20395.
doi: 10.1038/srep20395
242. Xue C, Chu Q, Shi Q, Zeng Y, Lu J, Li L. Wnt signaling pathways in biology and disease: mechanisms and therapeutic advances. *Signal Transduct Target Ther*. 2025;10(1):106.
doi: 10.1038/s41392-025-02142-w
243. Zhou Q, Ren X, Oberoi MK, *et al*. β -Catenin Limits Osteogenesis on Regenerative Materials in a Stiffness-Dependent Manner. *Adv Healthc Mater*. 2021;10(23):e2101467.
doi: 10.1002/adhm.202101467
244. Zhou Q, Lyu S, Bertrand AA, *et al*. Stiffness of Nanoparticulate Mineralized Collagen Scaffolds Triggers Osteogenesis

- via Mechanotransduction and Canonical Wnt Signaling. *Macromol Biosci.* 2021;21(3):e2000370.
doi: 10.1002/mabi.202000370
245. Deng A, Zhang H, Hu Y, *et al.* Microsphere Strategy to Generate Conformal Bone Organoid Units with Osteoimmunomodulation and Sustainable Oxygen Supply for Bone Regeneration. *Adv Sci.* 2025;12(32):e01437.
doi: 10.1002/advs.202501437
246. Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. *Nat Rev Mol Cell Biol.* 2014;15(12):802-812.
doi: 10.1038/nrm3896
247. Saraswathibhatla A, Indana D, Chaudhuri O. Cell-extracellular matrix mechanotransduction in 3D. *Nat Rev Mol Cell Biol.* 2023;24(7):495-516.
doi: 10.1038/s41580-023-00583-1
248. Ji C, McCulloch CA. TRPV4 integrates matrix mechanosensing with Ca²⁺ signaling to regulate extracellular matrix remodeling. *FEBS J.* 2021;288(20):5867-5887.
doi: 10.1111/febs.15665
249. Yang Y, Xu Z, He S, *et al.* Developmental dynamics mimicking inversely engineered pericellular matrix for articular cartilage regeneration. *Biomaterials.* 2025;317:123066.
doi: 10.1016/j.biomaterials.2024.123066
250. Zhou R, Fu W, Vasylyev D, Waxman SG, Liu C-J. Ion channels in osteoarthritis: emerging roles and potential targets. *Nat Rev Rheumatol.* 2024;20(9):545-564.
doi: 10.1038/s41584-024-01146-0
251. Lin DH, Hoelz A. The Structure of the Nuclear Pore Complex (An Update). *Annu Rev Biochem.* 2019;88(1):725-783.
doi: 10.1146/annurev-biochem-062917-011901
252. Bley CJ, Nie S, Mobbs GW, *et al.* Architecture of the cytoplasmic face of the nuclear pore. *Science.* 2022;376(6598):eabm9129.
doi: 10.1126/science.abm9129
253. Li Z, Tang J, Zhou L, *et al.* MicroSphere 3D Structures Delay Tissue Senescence through Mechanotransduction. *ACS Nano.* 2025;19(2):2695-2714.
doi: 10.1021/acsnano.4c14874
254. Yu Z-H, Ji Y-C, Li K, *et al.* Stiffness of the extracellular matrix affects apoptosis of nucleus pulposus cells by regulating the cytoskeleton and activating the TRPV2 channel protein. *Cell Signal.* 2021;84:110005.
doi: 10.1016/j.cellsig.2021.110005
255. Zhu M, Zhang H, Zhou Q, *et al.* Dynamic GelMA/DNA Dual-Network Hydrogels Promote Woven Bone Organoid Formation and Enhance Bone Regeneration. *Adv Mater.* 2025;37(24):e2501254.
doi: 10.1002/adma.202501254
256. Zheng R, Zhang N, Mao S, *et al.* Fast bone regeneration using injectable fully biomimetic organoids with biomineralized and organic microenvironments. *Biomater Sci.* 2025;13(2):486-495.
doi: 10.1039/D4BM01181C
257. Gao Y, Li Q, Du Z, *et al.* HAMA-SBMA hydrogel with anti-inflammatory properties delivers cartilage organoids, boosting cartilage regeneration. *J Nanobiotechnol.* 2025;23(1):401.
doi: 10.1186/s12951-025-03475-y
258. Chen Z, Bo Q, Wang C, Xu Y, Fei X, Chen R. Single BMSC-derived cartilage organoids for gradient heterogeneous osteochondral regeneration by leveraging native vascular microenvironment. *J Nanobiotechnol.* 2025;23(1):325.
doi: 10.1186/s12951-025-03403-0
259. Luo P, Cheng Y, Luo Y, *et al.* Hydrogel Enhanced Organoid Multidirectional Differentiation via Yap/Tead4 Mechanotransduction for Accelerated Tissue Regeneration. *ACS Appl Mater Interfaces.* 2025;17(26):37601-37616.
doi: 10.1021/acsaami.5c06161
260. Auletta B, Chiolerio P, Cecconi G, *et al.* Tissue-engineered neuromuscular organoids. *Commun Biol.* 2025;8(1):1074.
doi: 10.1038/s42003-025-08484-z
261. Kleuskens MWA, Crispim JF, van Doeselaar M, van Donkelaar CC, Janssen RPA, Ito K. Neo-cartilage formation using human nondegenerate versus osteoarthritic chondrocyte-derived cartilage organoids in a viscoelastic hydrogel. *J Orthop Res.* 2023;41(9):1902-1915.
doi: 10.1002/jor.25540
262. Menssen DMA, Feenstra JCA, Janssen RPA, Abinzano F, Ito K. Cartilage Organoids from Articular Chondroprogenitor Cells and Their Potential to Produce Neo-Hyaline Cartilage. *Cartilage.* 2025;19476035241313179.
doi: 10.1177/19476035241313179
263. Shen CY, Zhou QR, Wu X, *et al.* Accelerating cartilage regeneration with DNA-SF hydrogel sustained release system-based cartilage organoids. *Mil Med Res.* 2025;12(1):39.
doi: 10.1186/s40779-025-00625-z
264. Shen C, Wang J, Li G, *et al.* Boosting cartilage repair with silk fibroin-DNA hydrogel-based cartilage organoid precursor. *Bioact Mater.* 2024;35:429-444.
doi: 10.1016/j.bioactmat.2024.02.016
265. Sun Y, You Y, Wu Q, Hu R, Dai K. Senescence-targeted MicroRNA/Organoid composite hydrogel repair cartilage defect and prevention joint degeneration via improved chondrocyte homeostasis. *Bioact Mater.* 2024;39:427-442.
doi: 10.1016/j.bioactmat.2024.05.036
266. Wang J, Chen X, Li R, *et al.* Standardization and consensus in the development and application of bone organoids. *Theranostics.* 2025;15(2):682-706.

doi: 10.7150/thno.105840

267. Wang H, Li J, Feng H, *et al.* Engineering injectable composite scaffolds for enhanced bone healing: Integration of stem cells, hydrogels, and microspheres. *Chem Eng J.* 2025;507:160593.
doi: 10.1016/j.cej.2025.160593
268. Zhang L, Zhang Y, Wan Z, *et al.* Piezo1 mediated scaffold-free rapid generation of self-mineralized bone organoids via activating Wnt signaling. *Mater Today Bio.* 2026;36:102620.
doi: 10.1016/j.mtbio.2025.102620
269. Ding H, Chen D, Tan X, *et al.* Enhanced Bone Repair using Callus Organoids Derived from Urine-Derived Stem Cells with Silk Fibroin. *Adv Healthc Mater.* 2025;14(23):e2501852.
doi: 10.1002/adhm.202501852
270. Yang Z, Wang B, Liu W, *et al.* In situ self-assembled organoid for osteochondral tissue regeneration with dual functional units. *Bioact Mater.* 2023;27:200-215.
doi: 10.1016/j.bioactmat.2023.04.002
271. Shahriyari M, Rinn M, Hofemeier AD, Babych A, Zimmermann WH, Tiburcy M. Protocol to develop force-generating human skeletal muscle organoids. *STAR Protoc.* 2024;5(1):102794.
doi: 10.1016/j.xpro.2023.102794
272. Price FD, Matyas MN, Gehrke AR, *et al.* Organoid culture promotes dedifferentiation of mouse myoblasts into stem cells capable of complete muscle regeneration. *Nat Biotechnol.* 2025;43(6):889-903.
doi: 10.1038/s41587-024-02344-7
273. Chang TL, Borelli AN, Cutler AA, Olwin BB, Anseth KS. Myofibers cultured in viscoelastic hydrogels reveal the effects of integrin-binding and mechanosensing on muscle satellite cells. *Acta Biomater.* 2025;192:48-60.
doi: 10.1016/j.actbio.2024.11.044
274. Das S, Hilman MC, Yang F, Mourkioti F, Yang W, Cullen DK. Motor neurons and endothelial cells additively promote development and fusion of human iPSC-derived skeletal myocytes. *Skelet Muscle.* 2024;14(1):5.
doi: 10.1186/s13395-024-00336-4
275. Serna C, 3rd, Sandepudi K, Keate RL, *et al.* Incorporation of decellularized-ECM in graphene-based scaffolds enhances axonal outgrowth and branching in neuro-muscular co-cultures. *Sci Prog.* 2024;107(3):368504241281469.
doi: 10.1177/00368504241281469
276. Wang R, Wang F, Lu S, *et al.* Adipose-derived stem cell/FGF19-loaded microfluidic hydrogel microspheres for synergistic restoration of critical ischemic limb. *Bioact Mater.* 2023;27:394-408.
doi: 10.1016/j.bioactmat.2023.04.006
277. Fang T, Zhang H, Xie Y, *et al.* Centimeter-Scale Self-Assembling Tendon Organoids Drive Tissue Regeneration. *Adv Sci.* 2025;12(43):e09453.
doi: 10.1002/advs.202509453
278. Graça AL, Kroner-Weigl N, Reyes Alcaraz V, Müller-Deubert S, Rudert M, Docheva D. Demonstration of Self-Assembled Cell Sheet Culture and Manual Generation of a 3D Tendon/Ligament-Like Organoid by using Human Dermal Fibroblasts. *J Vis Exp.* 2024;208.
doi: 10.3791/66047
279. Ding Z, Cai Y, Sun H, *et al.* Janus hydrogel microrobots with bioactive ions for the regeneration of tendon-bone interface. *Nat Commun.* 2025;16(1):2189.
doi: 10.1038/s41467-025-57499-x
280. Ma L, Zhang Z, Mu Y, *et al.* Generation of engineered human spinal cord organoid in channel-patterned collagen and transplantation for spinal cord regeneration. *Biomaterials.* 2026;327:123753.
doi: 10.1016/j.biomaterials.2025.123753
281. Nishijima T, Okuyama K, Shibata S, *et al.* Novel artificial nerve transplantation of human iPSC-derived neurite bundles enhanced nerve regeneration after peripheral nerve injury. *Inflamm Regen.* 2024;44(1):6.
doi: 10.1186/s41232-024-00319-4
282. Wang Z, Liu R, Liu Y, *et al.* Human Placenta Decellularized Extracellular Matrix Hydrogel Promotes the Generation of Human Spinal Cord Organoids with Dorsoventral Organization from Human Induced Pluripotent Stem Cells. *ACS Biomater Sci Eng.* 2024;10(5):3218-3231.
doi: 10.1021/acsbomaterials.4c00067
283. Wang L, Gan J, Xu Z, *et al.* Laminin-Modified Porous GelMA Microspheres Sustain a Pro-Neurogenic Niche for Neural Stem Cells Transplantation in Spinal Cord Injury. *Adv Funct Mater.* 2025;35(50):e10129.
doi: 10.1002/adfm.202510129
284. Zhou R, Brislinger D, Fuchs J, *et al.* Vascularised organoids: Recent advances and applications in cancer research. *Clin Transl Med.* 2025;15(3):e70258.
doi: 10.1002/ctm2.70258
285. Sun D, Zhang K, Zheng F, *et al.* Matrix Viscoelasticity Controls Differentiation of Human Blood Vessel Organoids into Arterioles and Promotes Neovascularization in Myocardial Infarction. *Adv Mater.* 2025;37(5):e2410802.
doi: 10.1002/adma.202410802
286. Meijer EM, Koch SE, van Dijk CGM, *et al.* 3D Human iPSC Blood Vessel Organoids as a Source of Flow-Adaptive Vascular Cells for Creating a Human-Relevant 3D-Scaffold Based Macrovascular Model. *Adv Biol.* 2023;7(1):e2200137.
doi: 10.1002/adbi.202200137
287. Lou X, Wang F, Lv X, *et al.* Sequential angiogenic-osteogenic coupling via a spatiotemporally graded hydrogel enables vascularized bone organoids for critical-sized calvarial defect

- reconstruction. *Compos Part B Eng.* 2025;302:112553.
doi: 10.1016/j.compositesb.2025.112553
288. Jin X, Huang L, Wang X, *et al.* Synovial organoids: From fundamental construction to groundbreaking applications in arthritic disorders. *J Orthop Translat.* 2025;54:26-36.
doi: 10.1016/j.jot.2025.07.004
289. Wang X, He J, Zhang Q, He J, Wang Q. Constructing a 3D co-culture in vitro synovial tissue model for rheumatoid arthritis research. *Mater Today Bio.* 2025;31:101492.
doi: 10.1016/j.mtbio.2025.101492
290. Wang F, Liu J, Wen J, Sun Y, He M. Synovial Organoids and Cell Culture Models: An Ideal Platform for Exploring Traditional Chinese Medicine in Rheumatoid Arthritis. *ACS Omega.* 2025;10(33):36904-36916.
doi: 10.1021/acsomega.5c06035
291. Jiao Y, Lu S, Zhang J, Zhen J. Applications in osteochondral organoids for osteoarthritis research: from pathomimetic modeling to tissue engineering repair. *Front Bioeng Biotechnol.* 2025;13:1629608.
doi: 10.3389/fbioe.2025.1629608
292. Gao Q, Zhang X, Makarczyk MJ, *et al.* Macrophage phenotypes modulate neoangiogenesis and fibroblast profiles in synovial-like organoid cultures. *Osteoarthr Cartil.* 2025;33(5):590-600.
doi: 10.1016/j.joca.2025.02.777
293. Khan S, Cho H, Hasty KA, Brown T, Bhogoj S, Subramanian A. Synoviocyte-chondrocyte triculture model for early-stage PTOA: fibronectin fragment-induced catabolic effects *in vitro* and *in vivo*. *Front Bioeng Biotechnol.* 2025;13:1683333.
doi: 10.3389/fbioe.2025.1683333