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REVIEW ARTICLE

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

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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

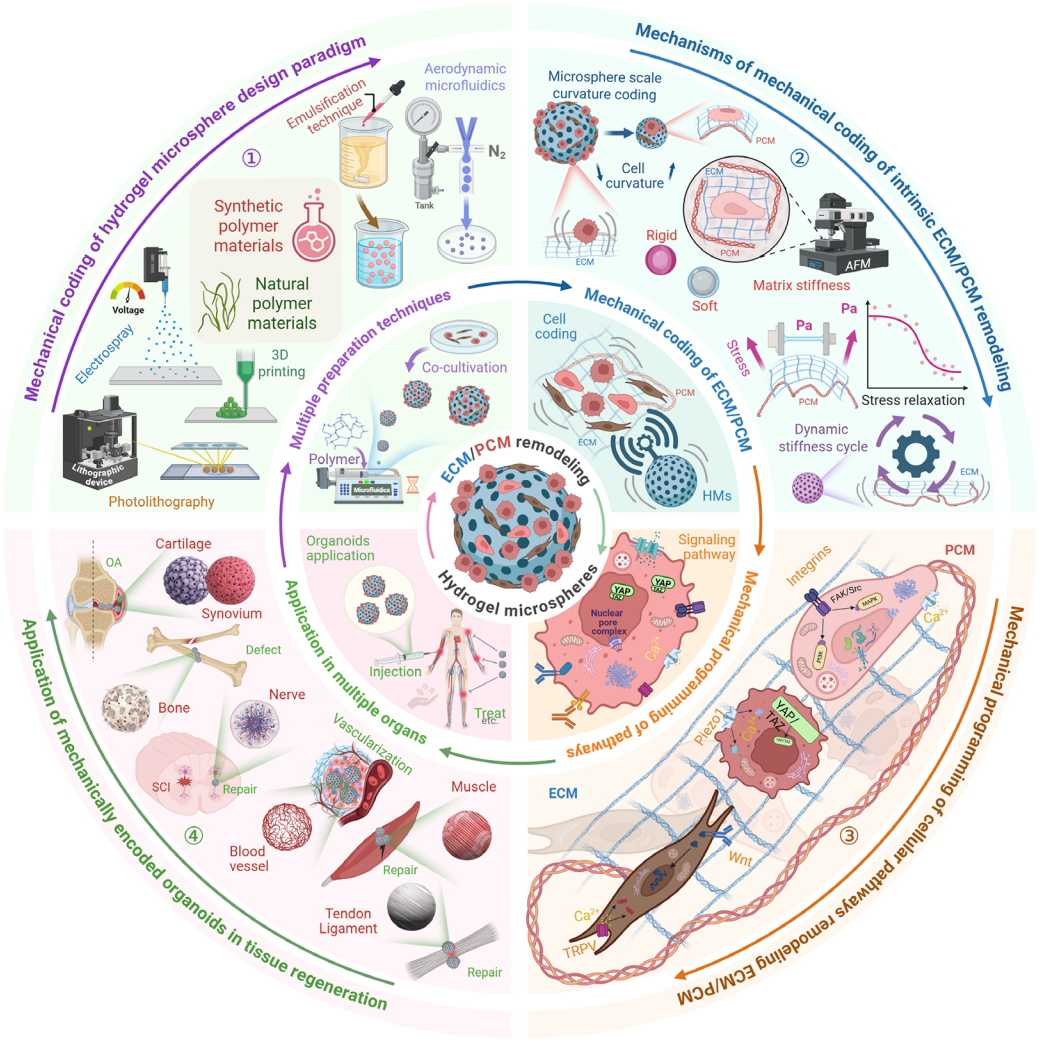

Figure 1. Schematic of mechanical encoding in hydrogel microsphere-mediated ECM/PCM remodeling and skeletal muscle organoid regeneration. (i) Design strategies for mechanically encoded hydrogel microspheres: Aerodynamic microfluidics, 3D bioprinting, lithography, and microfluidics. (ii) Mechanical encoding 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 applications[21] (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	*In situ* 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, *in situ* 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 *in situ* 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-1α (SDF-1α), 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-1α 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-β1; 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 HMs[66].

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 matrices[85] 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 release[92]; 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 autophagy[105] (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.

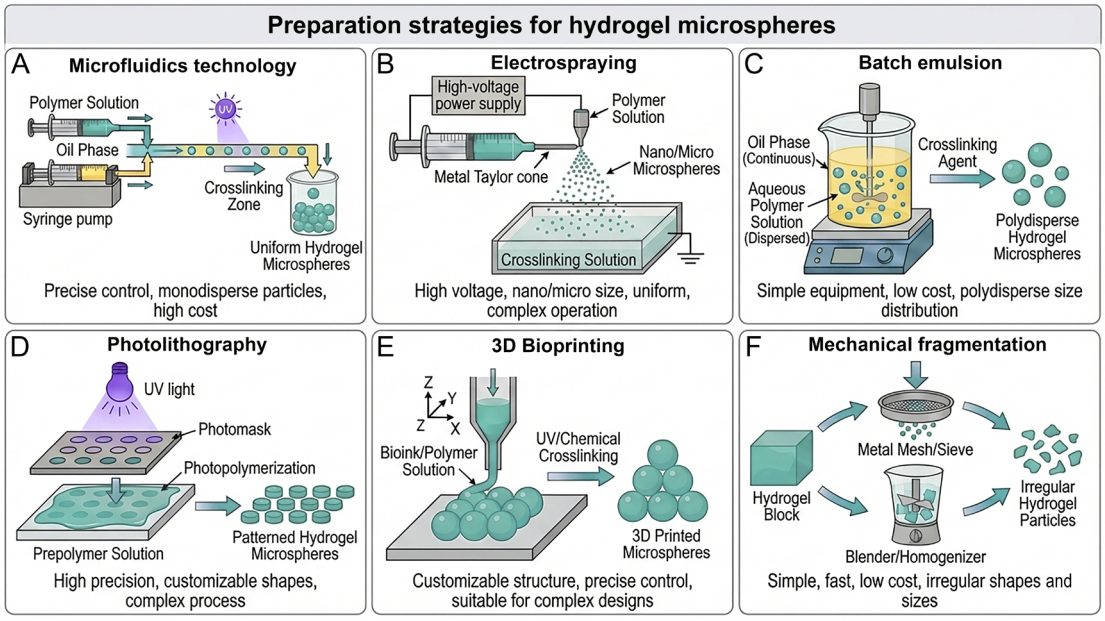

Figure 2. Fabrication strategies for hydrogel microspheres. (A) Microfluidics: Precision generation of monodisperse droplets via micro-scale channels. Coupled with photo-initiated or ionic cross-linking, this technique produces highly uniform microspheres with tailor-made dimensions and geometries.[105] (B) Electrospraying: 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 interfaces[115] (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 manufacturing[9] (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 interactions[138] (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, Puiggalí-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-β3/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 functions[142] (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 μg/mL). Their findings revealed that low-modulus matrices (2 kPa) combined with sparse ligand density (2 μg/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 hydrazone 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 → high curvature → attenuated cytoskeletal tension → 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

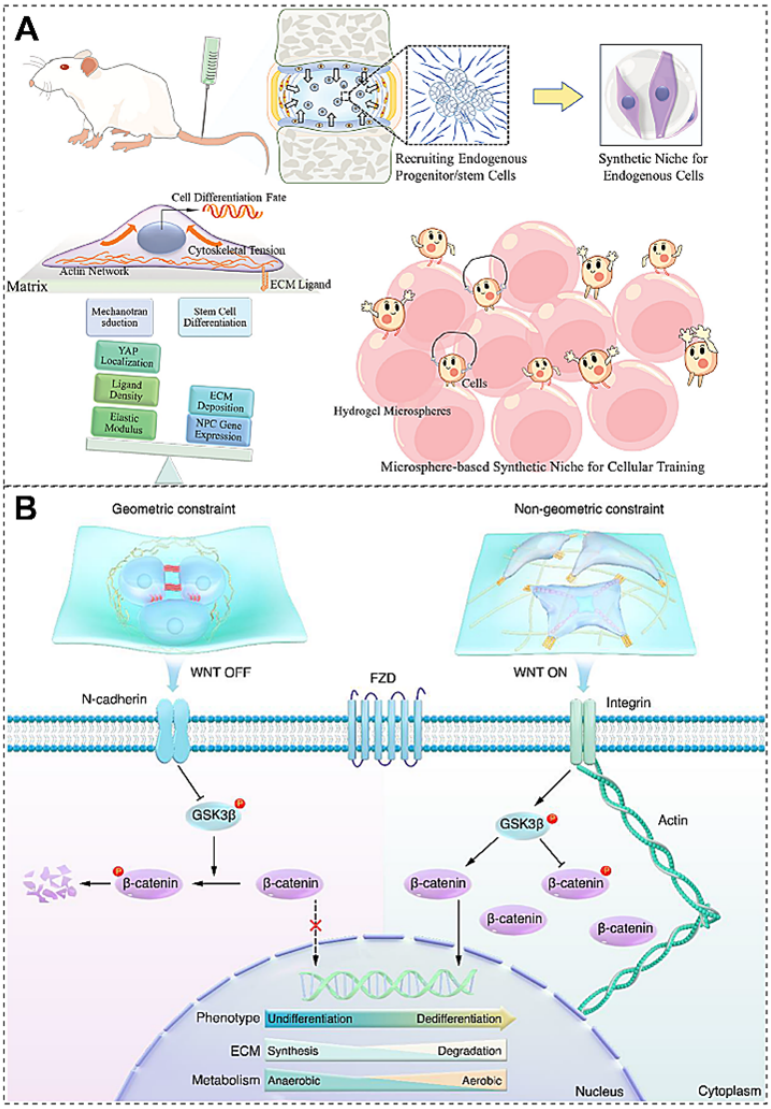

Figure 3. Regulation of extracellular matrix by microsphere mechanical stiffness and curvature. (A) Diagram of mechanical signal-tailored hydrogel microspheres capable of recruiting and training stem cells for directional differentiation to promote nucleus pulposus regeneration. Reprinted with permission 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; GSK3β: 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 αβ 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–Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} transients propagate through three therapeutic axes to orchestrate matrix remodeling: the Piezo1–Ca^{2+}–calcineurin/nuclear factor of activated T cells pathway regulating neurogenesis; the Piezo1–Ca^{2+}–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 osteogenesis[239]; and the Piezo1–Ca^{2+}–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 Ca^{2+} 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 tissues[260] (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 fusion[263] 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; *in situ* 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]); *in situ* 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 repair[264] (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 configurations[267](Figure 5)—leverage distinct mechanical

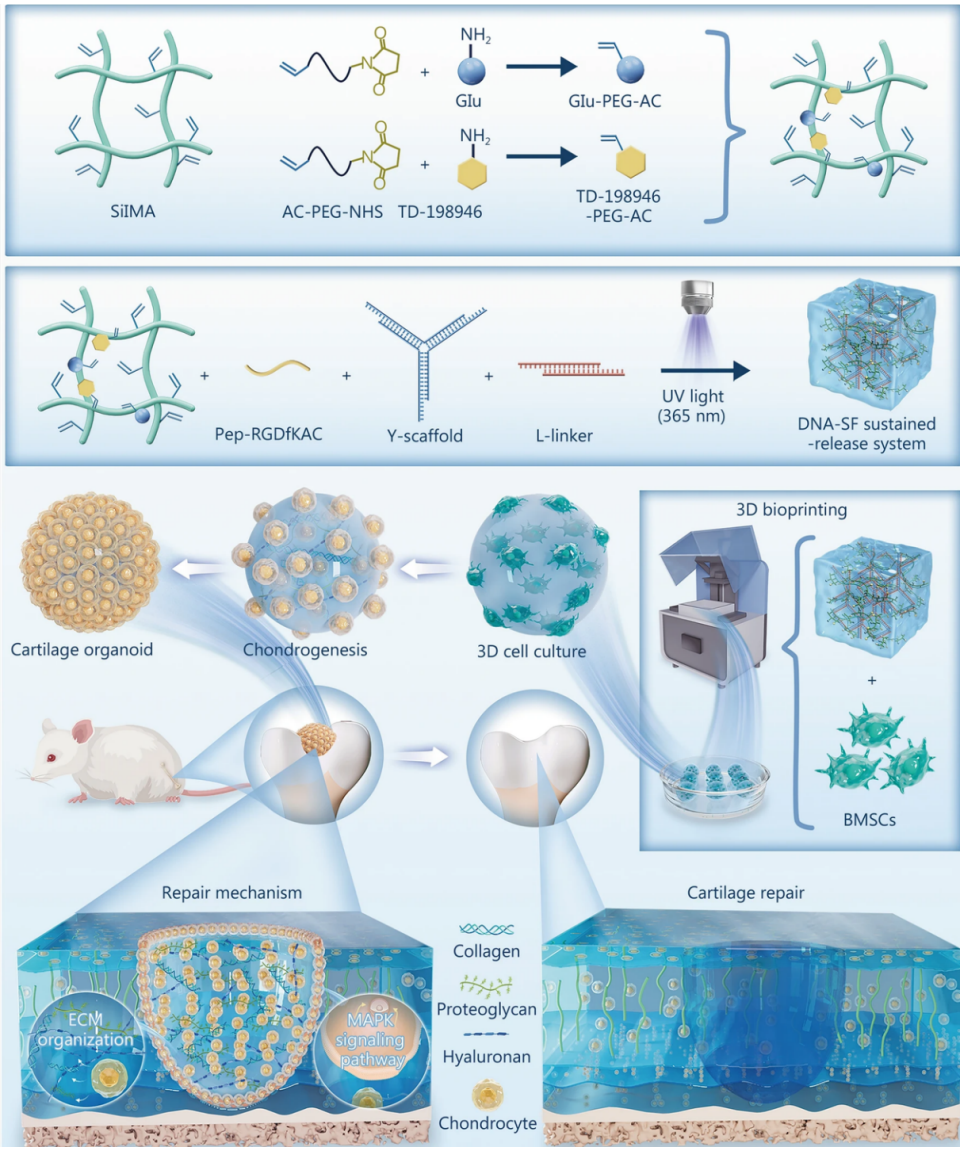

Figure 4. Preparation of cartilage organoids from hydrogel microspheres. DNA–SF sustained-release system synthesis and its mechanism for cartilage repair. Reprinted with modifications from Ref.[264]

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; NH_2: Amino group; Pep-RGDfKAC: RGD-containing peptide modified with D-Phe-Lys and an AC functional group; SilMA: 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]

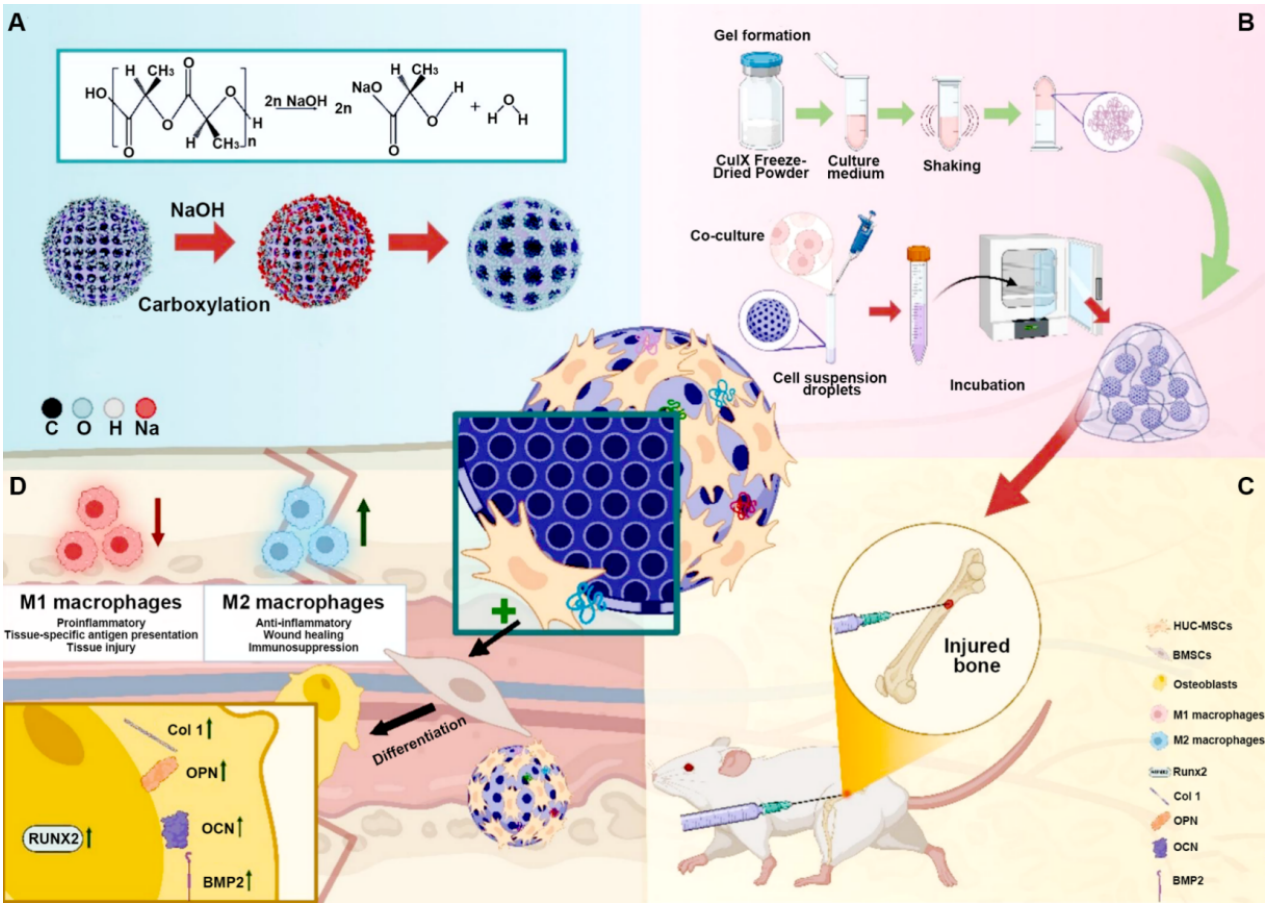

Figure 5. Preparation of bone organoids using hydrogel microspheres. (A) Schematic diagram of Cull X II@PDLLA@HUC-MSCs (CPH) synthesis pathway and bone defect repair after transplantation. Schematic representation of the carboxylation mechanism of PDLLA microspheres. (B) CPH synthetic pathway. (C) The 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; CH_3: 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 repair[276] (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

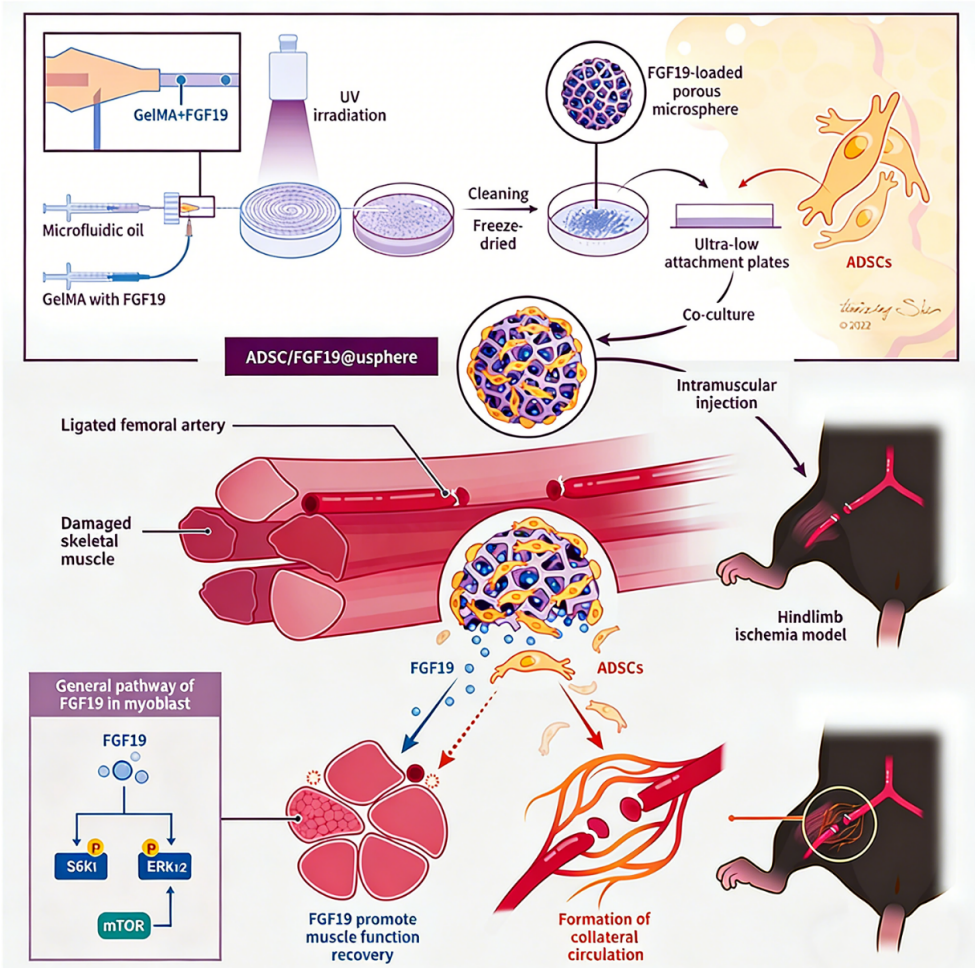

Figure 6. Preparation of skeletal muscle organoids using hydrogel microspheres. Schematic illustration of the synthetic process of ADSC/FGF19@μspheres and ischemic limb restoration. Uniform droplets of GelMA hydrogel with FGF19 were first prepared with the assistance of microfluidic technology, followed 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 dimethyloxalylglycine-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 organoids[287] (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

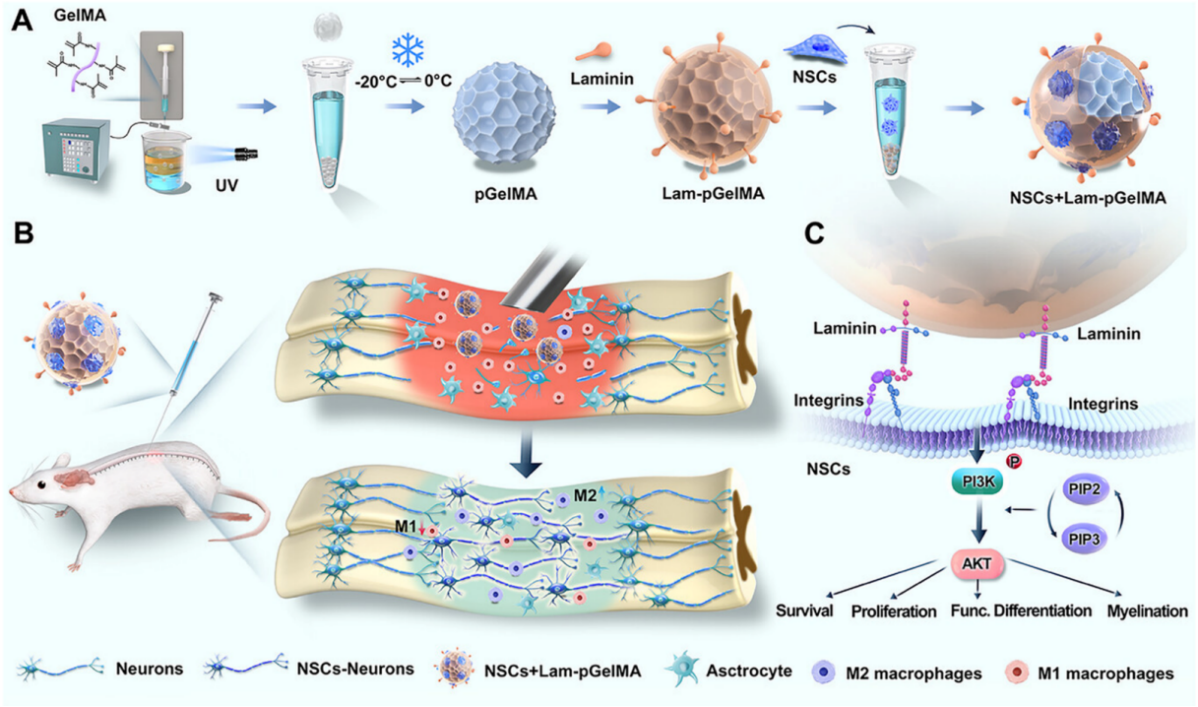

Figure 7. Preparation of nerve organoids using hydrogel microspheres. Schematic illustrating the fabrication of Lam-pGelMA microspheres and the therapeutic effect of NSCs loaded on the functionalized microspheres for spinal cord injury repair. (A) The workflow depicts the fabrication process of Lam-pGelMA 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; PIP_2: Phosphatidylinositol 4,5-bisphosphate; PIP_3: 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:

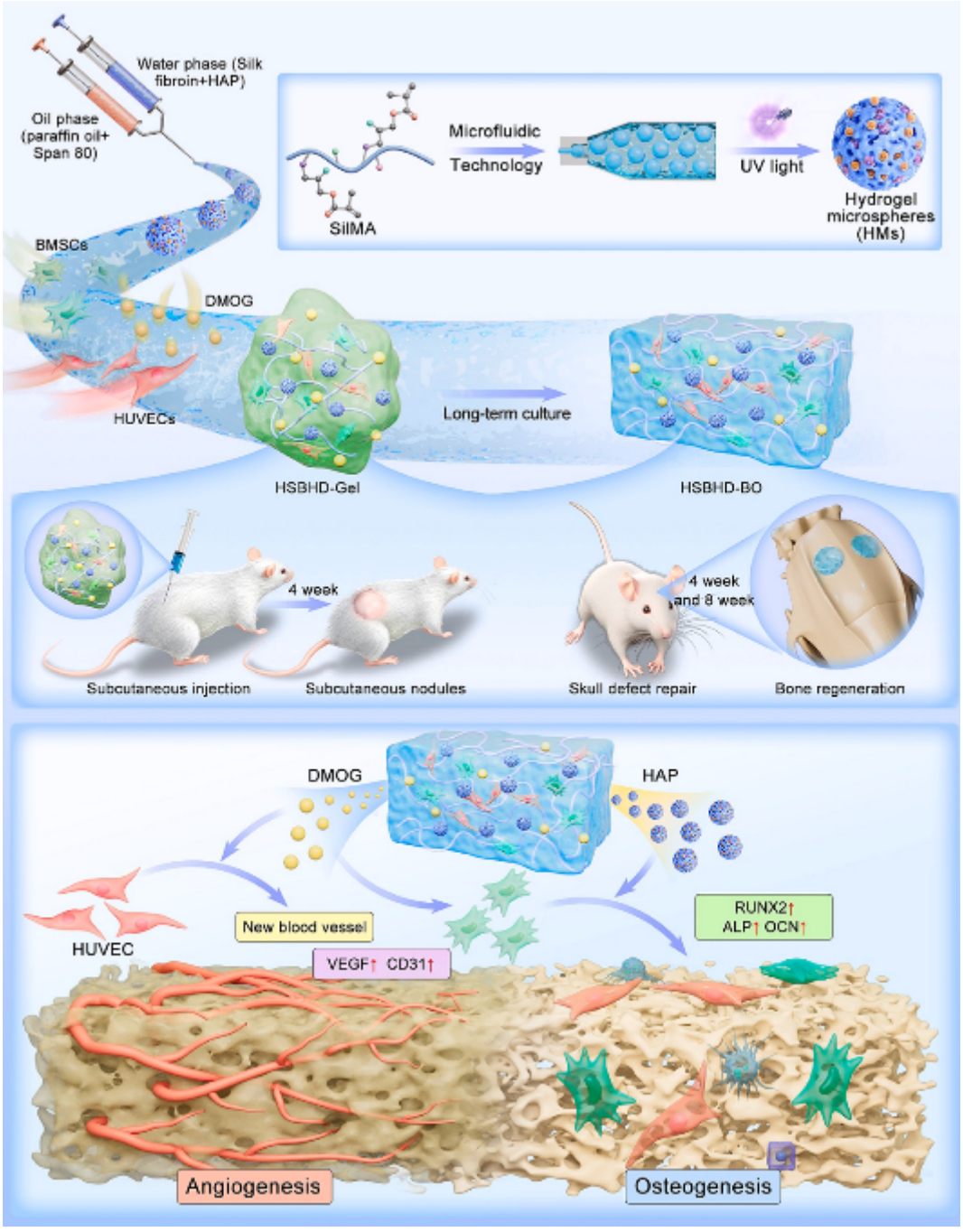

Figure 8. Preparation of vascular and bone organoids using hydrogel microspheres. Schematic diagram of the design and application of a hydrogel system integrating angiogenic and osteogenic components for vascularized bone organoid construction and bone defect repair.[287] Reprinted with permission from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

Abbreviations: ALP: Alkaline phosphatase; BMSCs: Bone marrow-derived mesenchymal stem cells; CD31: Cluster of differentiation 31; DMOG: Dimethyloxalylglycine; 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-1β 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.

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Conflict of interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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