Journal of Orthopedics and Orthopedic Surgery

Review Article Open Access

Biomimetic Cartilage Regeneration: From Zonal Design to Immune Challenges

Varsha A M[1], Stefanie A. Sydlik[2]*, Juhi Singh[1]*

[1]Department of Bioengineering, Indian Institute of Science, Bengaluru, Karnataka, India
[2]Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA

Article Info

Article Notes
Received: July 20, 2026
Accepted: September 28, 2026

***Correspondence:**
*Dr. Stefanie A. Sydlik, Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA.
Email: ssydlik@andrew.cmu.edu

*Dr. Juhi Singh, Department of Bioengineering, Indian Institute of Science, Bengaluru, Karnataka, India.
Email: juhisingh@iisc.ac.in

©2026 Sydlik SA, Singh J. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License.

Keywords
Osteoarthritis
Cartilage Regeneration
Inflammation
Localized Delivery
Injectable Hydrogels
3D-printed Scaffolds

Abstract

Cartilage regeneration remains constrained by the complex interaction of structure, mechanics, and inflammation as well as the tissue's weak intrinsic healing capacity. The emphasis of recent advancements has evolved to biomimetic approaches that mimic the dynamic homeostasis, compositional gradients, and zonal architecture of native cartilage. This review examines how scaffold design is affected by changes between healthy and diseased cartilage, notably when it addresses mechanotransduction, extracellular matrix turnover, and inflammatory signalling. This review highlights approaches, including injectable hydrogels and implantable structures, that integrate biochemical, mechanical, and cellular gradients to control spatial organization and functional development. How the immunological and inflammatory responses impair scaffold performance and how emerging material approaches, such as immunomodulation and controlled release, minimize these effects are the central focus. In the end, trade-offs between clinical translation and biomimetic complexity, considering scalability, fabrication, and regulatory constraints are weighed. Furthermore design methods which achieve a balance between translational feasibility and physiological fidelity are proposed.

Introduction

A strong, avascular material termed cartilage cushions the exteriors of articulating bones to make way for painless, smooth movement.[1] Persistent cytokine-driven degradation erodes this cushion in inflammatory joint diseases like osteoarthritis (OA), resulting in chronic discomfort, significant loss of mobility, and poor quality of life.[2] OA alone harmed an estimated 607 million individuals worldwide in 2021; this burden has increased by about 137% since 1990 and is still increasing due to obesity and population aging.[3] Analgesics, non-steroidal anti-inflammatory medications, and intra-articular corticosteroid or hyaluronic acid injections are the pillars of current clinical management; donor-site morbidity, high cost, fibrocartilage formation, and the risk of immune rejection remain obstacles to surgical options such as microfracture, autografts, and allografts.[4,5] These limitations have fuelled interest in scaffold- and hydrogel-based methods that can promote genuine cartilage regeneration. In addition to addressing how the inflammatory microenvironment threatens scaffold performance and emerging counter-strategies, this review compares healthy and OA-altered cartilage, examines tissue engineering techniques that mimic native gradients and matrix remodelling, and discusses the realistic trade-offs between construct complexity, manufacturability, and clinical deployability.

Specifically, this review addresses four questions: (1) which native features of cartilage must a cartilage mimetic scaffold reproduce; (2) how the inflammatory microenvironment impacts the constructs placed in the cartilage defect; (3) which material strategies counter the inflammation, and at what level of evidence; (4) and trade-offs that exist between biomimetic complexity, manufacturability, and clinical deployability, separating each strategy from clinical use.

Natural Cartilage

The mechanical function of articular cartilage, a dense, avascular connective tissue, depends on the precise alignment of zonal architecture, extracellular matrix (ECM) composition, and chondrocyte-driven homeostasis (Figure 1).[1] In terms of structure, the tissue is separated into four distinct zones. Flattened chondrocytes with collagen fibers oriented parallel to the articular surface are identified in the superficial (tangential) zone, which has the lowest proteoglycan (PG) content and the highest lubricin concentration. These properties offer crucial shear resistance and lubrication, keeping the coefficient of friction (COF) remarkably low, between 0.002 and 0.02.[6] The middle (transitional) zone, which functions as a mechanical bridge and has rounded chondrocytes embedded in a collagen network that is orientated randomly, lies beneath it. Columnar chondrocytes are distributed along thick collagen fibers oriented perpendicular to the joint surface in the deep (radial) zone, having the highest concentration of PG and maximum compressive stiffness. Lastly, the tidemark acts as a barrier against subchondral vascular invasion and defines the limit of the calcified cartilage zone, which creates a mineralized interface that anchors the uncalcified tissue to the underlying subchondral bone.

The extracellular matrix is primarily held together by a type II collagen fibrillar scaffold and consists of 65–80% water.[7] This network is interconnected by minor collagens IX and XI, which help in regulating the interfibrillar connection and fibril width. The dominant PG, aggrecan, forms huge aggregates by adhering to hyaluronic acid backbones.[7] The densely packed glycosaminoglycan (GAG) side chains carry fixed negative charges that draw water in, generating osmotic swelling pressure that resists compressive loads (modulus 0.5–2 MPa).[6]

Chondrocytes, the sole resident cells, exist at low density and actively maintain homeostasis: the equilibrium between ECM synthesis and degradation.[8] These cells successfully adapt to their inherently hypoxic environment by stabilizing hypoxia-inducible factor-1 (HIF-1α)[9], clear damaged organelles via autophagy[10], and regulate matrix remodeling by responding to transforming growth factor-β (TGF-β) signaling.[2] Furthermore, they sense and respond to mechanical loads through mechanosensitive ion channels (Piezo1/2).[11] Chondrocytes also engage the matrix through integrins, principally α5β1, which act as mechanotransducers alongside the Piezo channels (Sections 3 and 6, Figure 1).[12] This tightly regulated homeostatic balance preserves tissue integrity under

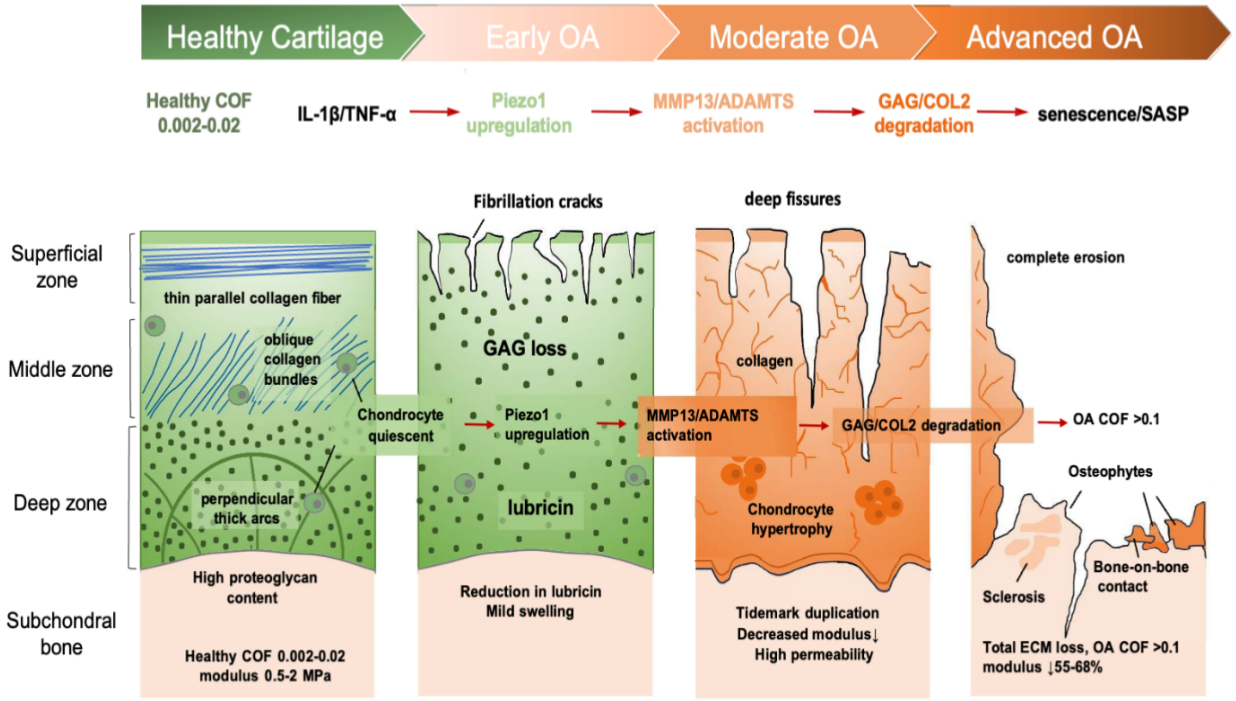

Figure 1: Schematic comparing the difference between healthy and diseased cartilage in terms of microenvironment, zonal organization, extracellular matrix and mechanical properties.

physiological loading but becomes critically disrupted under the inflammatory conditions detailed in Section 3.

Throughout this review, "biomimetic" refers to spatially resolved reproduction of at least one measurable native cartilage property: compositional, architectural, mechanical or immunological verified against a native-cartilage reference. Immunological mimicry is included wherein the host response is treated as a design target alongside stiffness and fiber orientation.

Physiological Differences: Healthy vs. Diseased Cartilage

OA causes an imbalance in the homeostasis wherein inflammatory IL-1β signals overactivate Toll-like receptors, NF-κB pathway and hypersensitive sensors (Piezo1)[11] triggering the production of destructive enzymes (matrix metalloproteinases (MMPs) and aggrecanase-2 (ADAMTS5)) that actively degrade the ECM.[2] Simultaneously, nitric oxide (NO) blocks the natural repair mechanisms leading to a cycle of unregulated degradation.[13] Early OA: PG/GAG loss results in increased leakiness (up 60-80%)[6], swelling, and softening (modulus drops 55-68%). Healthy cartilage has a smooth articular surface (Ra ≈ 80–110 nm by AFM)[14] and remarkably low boundary-mode friction (COF ≈ 0.001–0.2[15] tribometry). Late OA: wear, high friction (COF >0.1[16]), rough surface (40-200 nm; arithmetic mean roughness R^a by atomic force microscopy, profilometry values being higher and not interconvertible[14]), and disorganized collagen[17] (Figure 1). Zonal architecture is disrupted: cells age (senescence[8]), there is no lubricin, and cracks appear. Exercise promotes healthy remodeling[4] but exacerbates OA overload.[1] There are no cure medications (Disease-Modifying Osteoarthritis Drugs).[13] Regeneration could restore PG and mechanical strength[10] by using biomimetic scaffolds to target degraders and mimic architectural zones.

Two mechanisms warrant emphasis. Senescent chondrocytes acquire a senescence-associated secretory phenotype (SASP; Figure 1), ceasing division while secreting IL-6, IL-8 and MMP-3/13,[8] so an implanted scaffold meets a continuously regenerated rather than a decaying stimulus. MMP-cleaved fibronectin fragments also ligate the α5β1 integrin, driving a ROS and Rac1-dependent catabolic programme;[53] because α5β1 binds RGD, RGD-functionalized hydrogels engage the very receptor the degraded matrix exploits. The quantitative values above are pooled from small cadaveric series using non-interconvertible test modes and are best read as order-of-magnitude design targets.

Recent Approaches Mimicking Native Cartilage

Mimicking native cartilage's zonal architecture demands sophisticated strategies addressing compositional gradients, cellular optimization, mechanical stiffness gradients, and matrix remodelling (Figure 2). Most findings below derive from in vitro assays or small-animal models. Table 1 gives the evidence level, headline effect size and principal methodological caveat for each.

Compositional Gradients

Attempts to mimic the biochemical heterogeneity of native cartilage has driven the development of zone-specific scaffold compositions that recapitulate the tissue's layered organization.[18] Multilayer hydrogels combining chondroitin sulfate methacrylate (CSMA), gelatin methacrylate (GelMA), and nano-hydroxyapatite replicate the hydration, stiffness, and degradation profiles of each zone, inducing zone-specific extracellular matrix (ECM) deposition[19], while stereolithography apparatus (SLA) bioprinting of stratified GelMA/hyaluronic acid methacrylate (HAMA) blends achieves a 178% increase in type II collagen (COL2A1) expression versus homogeneous controls[20]. Key challenges remain diffusion instability and interlayer delamination. Clinical evidence for compositional gradient systems is currently limited to a single collagen/hydroxyapatite platform. A pilot study ($n = 30$) showed favorable 2-year clinical and MRI results, while a larger cohort ($n = 203$) demonstrated significant 24-month functional improvements. Conversely, the widely researched GelMA/HAMA-based gradients remain restricted to *in vitro* and small-animal models, leaving their long-term human efficacy unproven.

Progressive Cellular Optimization

Beyond scaffold composition, optimizing the cellular response through physical and biochemical stimuli has emerged as a critical strategy for guiding zone-specific differentiation. Electrical stimulation of mesenchymal stem cells (MSCs) markedly upregulates COL2, aggrecan (ACAN), and SRY-box 9 (SOX9).[21] Moreover, a joint-simulating bioreactor, coupling low oxygen tension with thermomechanical loading produced a synergistic effect leading to approximately 14-fold increase in chondrogenic gene expression.[22,23] Growth differentiation factor 5 (GDF-5)-preconditioned MSC-derived exosomes loaded in a bioprinted HAMA scaffold further offer a promising cell-free regenerative strategy with sustained in vivo release.[24] Preconditioning, stimulation, and GDF-5 therapies remain unproven in humans despite strong preclinical backing. However, a growth-factor-driven alternative has reached late-stage clinical trials: allogeneic chondrocytes engineered to overexpress TGF-β 1 significantly improved International Knee Documentation Committee (IKDC) score and pain scores compared to placebo at 24 months in a phase II study, and have now advanced to phase III evaluation.[25]

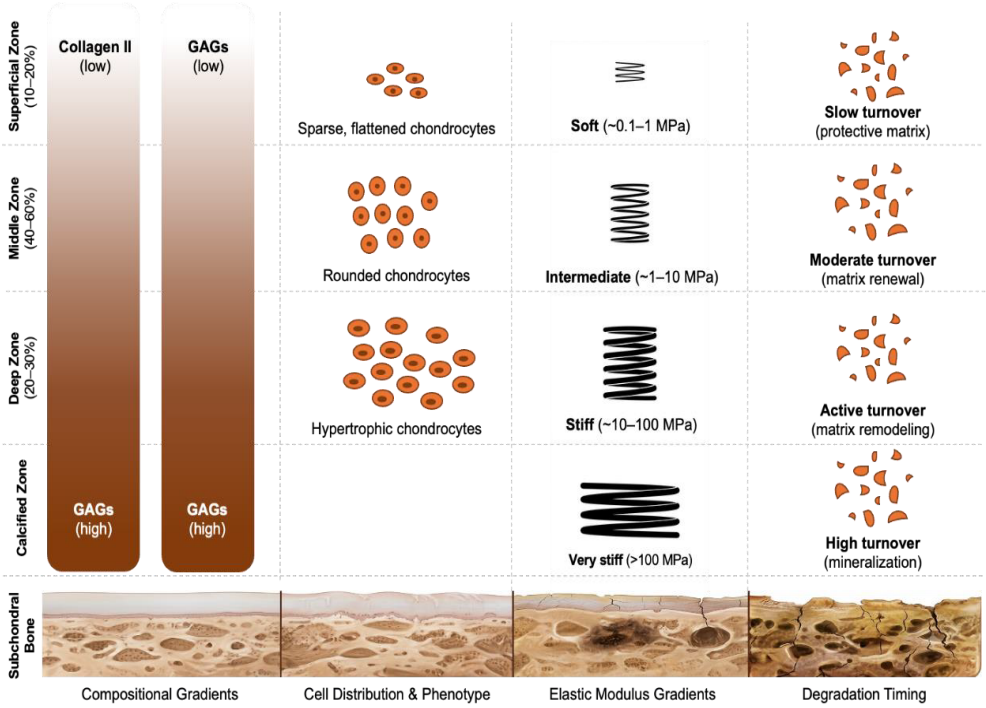

Figure 2: Schematic depiction of strategies employed to mimic native cartilage's zonal architecture addressing compositional gradients, cellular optimization, mechanical stiffness gradients, and matrix remodelling.

Mechanical Stiffness Gradients

The distinct mechanical environments across cartilage zones play an equally important role in directing cell fate. Bioinspired stiffness ramps direct zone-specific morphology, and melt electrowriting (MEW) of multiphasic polycaprolactone (PCL) microfibers embedded in GelMA/HAMA hydrogels generates depth-dependent compressive moduli that quantitatively match native cartilage loading mechanics.[26,27] Clinical precedent for depth-dependent stiffness transitions instead relies on compositional grading such as layered collagen-hydroxyapatite and aragonite-based scaffolds which have established multi-year human data. This gap highlights a major translational opportunity for architecturally engineered fiber gradients.

Matrix Remodelling

Achieving the appropriate balance between scaffold degradation and new tissue formation is fundamental to ensuring engineered constructs transition into functional cartilage (Figure 3, 4). MMP-sensitive polyethylene glycol (PEG) hydrogels enable dynamic scaffold turnover, and tuning collagen methacrylation degree to match degradation rate to chondrogenesis tempo significantly outperforms rapidly degrading counterparts in both in vitro and in vivo settings.[28,29] Another injectable ZnO-based hydrogel releases miR-17-5p in response to elevated MMP activity, simultaneously recruiting MSCs via zinc ion release and suppressing catabolic enzymes to restore ECM homeostasis in a rat OA model.[30] Scalability and delamination remain persistent challenges, and multi-axial bioprinting with real-time monitoring represents the most promising route toward human-scale repair. The chemical identity of the products released during turnover is itself a design variable (Section 8). Injectable cell-based hydrogels offer the strongest clinical proof for controlled remodeling. A first-in-human trial using allogeneic umbilical cord blood stem cells and hyaluronate showed safe, durable improvements over 7 years.[31] However, regulatory progress remains extremely slow: only one cell-based cartilage repair product has gained FDA approval in the past decade, highlighting a stark divide between extensive preclinical research and human translation.

Oxygen and Nutrient Transport in Thick Constructs

Zonal organization and mass transport compete directly in tissue engineering. Passive diffusion sustains cell viability only 100–200 μm from a free surface, beyond which a hypoxic and ultimately necrotic core develops.[32] Because full-thickness cartilage is 2–4 mm deep, any full-depth, cell-laden construct is inherently transport-limited. The distinction is critical: 1–6% oxygen supports healthy HIF-1α-driven SOX9 expression, whereas levels below 1% trigger ROS accumulation, inflammatory NF-κB activation, and apoptosis.[33] An over-thick or over-seeded construct thus generates the very inflammatory phenotype it was designed to resist. While increased porosity, printed channels, and oxygen-releasing materials extend viable depth, they sacrifice mechanical modulus, add

Table 1: Evidence level, headline quantitative claim, methodological caveat, translational limitation and approximate technology readiness level (TRL, 1–9) for the strategies reviewed in Sections 4 and 5. TRL values are approximate and intended for relative comparison.

Strategy	Evidence Level	Headline Quantitative Claim	Methodological Caveat	Principal Translational Limitation	Approx. TRL
Section 4.1 — Compositional Gradients					
Multilayer/gradient hydrogel (CSMA–GelMA–nHA; SLA GelMA/HAMA)[19,20]	In vitro (limited small animal)	Exhibits biomechanical and biochemical gradient like natural cartilage	In vitro study	Interlayer delamination; diffusion instability	2–3
Section 4.2 — Progressive Cellular Optimization					
Acoustic cell patterning[21]	In vitro	Mimics cellular and extracellular matrix organization similar to deep zone of native articular cartilage	Mimics only the deep zone; requires integration with other cartilage zones	Complex acoustic pressure fields required to achieve zonal gradient	2-3
Electrical stimulation of hMSCs[22]	In vitro	Enhances the chondrogenic differentiation of hMSCs	Induces stress in cells, does not mimic complex native cartilage architecture	Parameter settings for electrical stimulation need standardization	2-3
GDF-5-preconditioned MSC-derived exosomes in bioprinted HAMA scaffold[24]	Small animal	Sustained in vivo exosome release; cell-free regenerative strategy	Small-animal model only; no mechanical or long-term functional endpoint reported	Exosome standardization and scalability; bioprinting complexity	2–3
TGF-β1-overexpressing allogeneic chondrocytes (Invossa-like)[25]	Phase II RCT (human); phase III initiated	Significant improvement in IKDC and pain scores vs placebo at 24 months	Genetically modified cell product; regulatory suspension in some jurisdictions over safety concerns	ATMP/gene therapy classification; manufacturing complexity; prior regulatory setbacks	6–7
Section 4.3 — Mechanical Stiffness Gradients					
MEW fiber-reinforced hydrogel[26]	In vitro	Depth-dependent modulus matching native values	Directly measured property benchmarked to native tissue; but in vitro and non-inflammatory	Slow fabrication; untested under inflammation	3
Section 4.4 — Matrix Remodelling					
MMP-sensitive PEG hydrogels[29]	In vitro;	Tuned degradation rate matched to chondrogenesis tempo; outperformed rapidly degrading counterparts	Inter- and intra-donor variability by age affects gel-to-tissue transition; limited biological replicates	Scalability; delamination in multilayer formats	3
Injectable ZnO-based hydrogel (miR-17-5p release)[30]	Small animal (rat OA)	MSC recruitment via Zn^{2+} release; catabolic enzyme suppression; ECM homeostasis restored	Single rat OA model; short follow-up; zinc dose–toxicity window not fully characterized	Metal ion toxicity at higher doses; no large-animal or human data	3
Allogeneic umbilical cord blood MSCs + hyaluronate (cell-based injectable)[31]	First-in-human trial; 7-year follow-up	Safe, durable clinical improvements over 7 years	Small cohort; single-centre; no randomized comparator	Two-stage; ATMP regulatory pathway; only one FDA-approved cell-based cartilage product in a decade	7–8
Section 5.1 — Injectable, Shape-Conforming Scaffolds					
Injectable biopolymer hydrogel (alginate, chitosan, GelMA, HA)[34]	Small animal (rat, rabbit)	Sustained KGN release; immature ECM by 2–4 weeks	Resorption times from rodent models; no comparison performed in an inflamed joint	Mechanical weakness; accelerated enzymatic resorption; no zonal control	3–4
Cryogel sponge[35]	Small animal (rabbit)	Zonal GAG deposition; superior infiltration vs hydrogel	Single rabbit study, short follow-up; lot-to-lot variability unquantified	Fibrous encapsulation under an M1-dominant response	3–4

Double-network injectable hydrogel (CSMA / poly(L-lysine))[36]	In vitro	Improved compressive strength vs single network	In vitro only; no in vivo or OA data	Zonal control still absent	3
Section 5.2 — Architecturally Defined Scaffolds					
Electrospun nanofibrous membrane[37]	Small animal (immunocompetent)	Suppression of pro-inflammatory cytokines in vivo	Small group sizes; no load-bearing assessment reported	Thin constructs only; limited load-bearing capacity	3
dECM / functionalized dECM scaffold[38]	Small animal; some large-animal work	MSC recruitment, chondrogenesis, M2 polarization	Donor variability; bioactivity sensitive to sterilization; effect sizes not pooled across studies	Regulatory complexity; supply and standardization	3–4
3D-bioprinted zonal scaffold[39]	In vitro; small animal	Patient-specific anatomical geometry	Mechanical mismatch rarely quantified against native tissue values	Resolution limits preclude true zonal architecture; cost	2–3
Section 5.3 — Marketed Comparators (Clinical Benchmarks)					
Cell-free aragonite osteochondral implant (marketed comparator)[40,41]	Multicentre RCT, n = 247; 24–60-month follow-up	Superior KOOS and IKDC vs debridement/ microfracture; greater MRI defect fill	Open-label; comparator is debridement/ microfracture rather than an active scaffold	Not zonally biomimetic; not designed to modulate inflammation	9
Collagen-based MACT scaffold (marketed comparator)[58]	Case series, level 4 (n = 23, 24 months); phase III RCT registered (NCT01656902), not reported	Satisfactory 2-year clinical and MOCART outcomes for defects >2 cm²	Single-centre, uncontrolled, small n; no published randomized comparator	Two-stage, cell-based, high cost; ATMP regulatory burden	9
Section 7.1 — Controlled-Release Platforms					
Chitosan NPs co-loaded with fisetin and KGN in HA hydrogel[42]	In vitro	Sustained chondroprotective release outperformed free-drug controls	In vitro only; no OA model; no mechanical assessment	Translation to in vivo; nanoparticle clearance and retention unknown	2–3
Stimulus-responsive MMP-sensitive microspheres (hydroxychloroquine release)[43]	Small animal (rat/ mouse OA)	ROS scavenging; HIF-1α downregulation	Trigger (MMP activity) is spatially heterogeneous and load-dependent; burst release risk	Payload stability; no human data	3
Tetrahedral framework nucleic acid NPs (ginsenoside Rb1)[44]	Small animal	Deep cartilage penetration; prolonged joint residence	Novel delivery vehicle; limited replication; no mechanical or structural cartilage endpoints	Manufacturing complexity of nucleic acid nanostructures; cost; no large-animal data	2–3
Genetically engineered chondrocyte-mimetic nanoplatform[45]	Small animal (OA model)	Synergistic IL-1β blockade + mitochondrial repair; disease modification	Complex multi-functional platform; small-animal OA model only	Regulatory complexity of multi-target nanotherapeutics; scalability	2–3
Enzyme-sensitive linker platform (MMP-reporter/trigger)	Small animal	Delivered dose tracks local protease activity (theranostic)	MMP activity is spatially heterogeneous; mean-calibrated dosing may under-dose active foci	Payload stability; calibration of dose–response in human joints unknown	2–3
ADAMTS5-responsive silk fibroin hydrogel (miR-17-3p LNPs)[46]	Small animal (rat, microfracture model)	Self-regulating feedback loop; NF-κB suppression; inflammatory niche reprogramming	Single-model validation; long-term silencing durability of miRNA unknown	Silk fibroin immunogenicity; LNP stability in vivo	3
Section 7.2 — Surface and Composition Modulation					
RGD-modified HAMA hydrogel with KGN and Klotho[47]	Small animal (OA model)	Simultaneous ROS scavenging, macrophage reprogramming (M2), and chondrogenesis	Multi-component system; difficult to attribute effects to individual components	Klotho sourcing and stability; regulatory complexity of multi-active hydrogel	3
HA / chondroitin sulfate NF-κB suppression[48]	In vitro (inflammatory chondrocytes)	Direct suppression of NF-κB; reduced MMP-driven ECM degradation	In vitro inflammatory chondrocyte model only; no in vivo validation	Translation gap; no mechanical or structural cartilage data	2

Exosome-loaded bilayer hydrogel (MSC exosomes + ROS-responsive diclofenac) [49]	Small animal	Dual anti-inflammatory + pro-chondrogenic activity	Exosome batch variability; bilayer integration not mechanically characterized	Exosome standardization; ROS-responsive release kinetics in human joints unvalidated	2–3
dECM scaffold with Treg-conditioned medium[50]	Small animal	M2 macrophage polarisation via biological resolution programme (not small-molecule)	Ill-defined biologic payload; Treg-CM composition variable	Standardization of conditioned medium; regulatory classification	2–3
Sensor-integrated scaffolds (pH, pO_2, lactate, MMP, cytokine monitoring)[51]	Conceptual / early prototype	Real-time diagnostic feedback loop for inflammation status	Unproven in cartilage; power supply, biofouling, calibration drift constraints	Technical maturity; biocompatibility of embedded electronics; cost	1–2

Table 2: Proposed quantitative working ranges for zonal cartilage construct design. These values are defensible starting points drawn from native-tissue measurements, published preclinical data, and applicable regulatory standards; no consensus specification for zonal cartilage constructs yet exists.

Design Parameter	Proposed Working Range	Rationale / Native Reference	Governing Standard(s)
Number of cell-laden zones	≤2–3 distinct zones	Beyond 3 zones, interlayer delamination risk exceeds fidelity gained; current clinical constructs are monophasic or biphasic[40, 41,52]	ISO 10993-5 (cytotoxicity per zone); EU ATMP classification applies if cells are included (Regulation EC 1394/2007)
Inter-layer modulus ratio	≤10:1 (within one order of magnitude)	Native cartilage superficial-to-deep modulus ratio ≈5–10:1 [626]; mismatch beyond this causes stress concentration and delamination	ASTM F2150 (test method for unconfined compression); ISO 14243 (joint simulator loading)
Bulk compressive modulus (equilibrium)	0.3–2.0 MPa	Native articular cartilage: 0.5–2.0 MPa equilibrium aggregate modulus[6]; values below 0.3 MPa risk collapse under physiological loads	ASTM F2150; ISO 7206 (implant testing); ISO 14243 (knee-simulator cycles)
Pore size (hydrogel / sponge zone)	100–400 µm (interconnected)	Pores <100 µm restrict cell infiltration; pores >400 µm compromise chondrocyte phenotype retention and reduce surface area for cell attachment	ASTM F2450 (porosity measurement); ISO 10993-6 (local tissue response)
Construct thickness (cell-laden)	≤2.0 mm per cell-laden zone (≤4 mm total)	Passive O_2 diffusion limit ≈100–200 µm from free surface[32]; full-thickness cartilage is 2–4 mm; thicker cell-laden zones produce a necrotic/inflammatory core (Section 4.5)	No specific ISO standard; thickness justified by oxygen diffusion modelling and in vitro viability data
Degradation-product local pH	≥5.5 within degrading zone	PLGA/PLA depots can reach pH ≈2.8 locally[66]; pH <5.5 shifts macrophages to M1 and triggers TNF-α/IL-1β release (Section 8)	ISO 10993-13/14/15 (degradation assessment); ISO 10993-5 (cytotoxicity of degradation extracts)
Terminal sterilization dose / method	≤25 kGy gamma; EtO residuals per ISO 10993-7	Gamma >25 kGy causes chain scission in PCL/PLGA, accelerating hydrolysis and altering immunological profile[53,71]; autoclaving stiffens hydrogels	ISO 11137 (radiation sterilization, 25 kGy substantiation); ISO 11135 (EtO sterilization); ISO 10993-7 (EtO residual limits)
Fabrication time (per construct)	≤60 min (for single-stage surgical use)	Intraoperative scaffold preparation must fit within a single surgical session; prolonged fabrication times raise cost and limit clinical adoption[54,55]	ISO 13485 (Quality management system for medical devices); cGMP if classified as ATMP
Shelf life stability	≥12 months at 2–8 °C (cell-free); shorter for cell-laden	Cell-free off-the-shelf availability is a key advantage over autologous cell-based products; shelf life must survive supply-chain storage	ISO 11607 (packaging and sterile barrier); ICH Q1A (stability testing guidance, applicable to ATMP)

fabrication steps, or require complex controlled release.[32] Consequently, this transport constraint is why clinical constructs remain either thin or cell-free. Table 2 collects these and the other quantitative design criteria with their governing standards.

Broad Classification of Regeneration Strategies

Scaffolds that respond fluidly to defect geometries without imposing internal structure and those aimed to generate architecturally controlled constructs are the two main categories of current cartilage regeneration strategies.

Injectable, Shape Conforming Scaffolds

Although injectable biopolymer hydrogels based on alginate, chitosan, GelMA, and hyaluronic acid (HA) provide minimally invasive administration, they exhibit poor zonal control, rapid resorption that results in immature ECM within two to four weeks, and mechanical weakness. By enhancing adhesion and

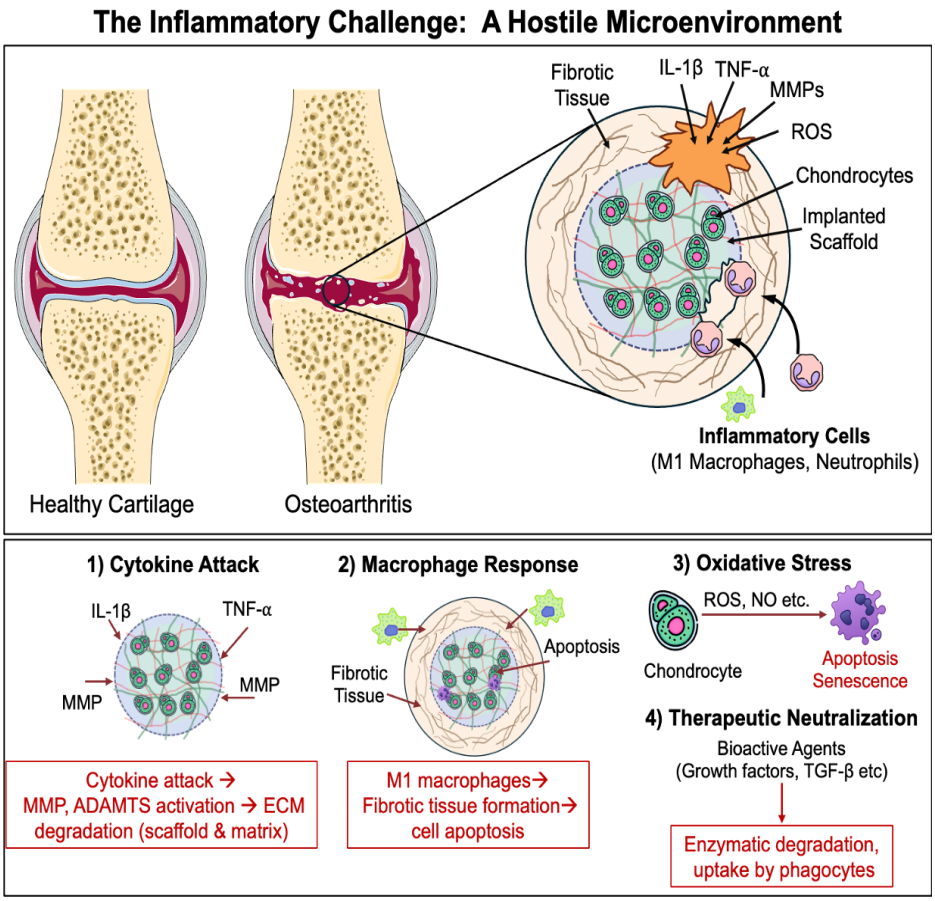

Figure 3: Overview of inflammatory and immune responses impacting the scaffold after implantation at the cartilage defect site.

prolonging chondrogenic release, catechol-chitosan systems in addition to kartogenin (KGN)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles partially work over these limitations.[34] Cryogel sponges, macroporous networks created by cryopolymerization, have demonstrated zonal GAG deposition in rabbit osteochondral defects and outperform hydrogels in cell infiltration.[35] Double-network injectable hydrogels of methacrylated CS with synthetic poly(L-Lysine) improve mechanical strength but poor zonal control persists.[36]

Architecturally Defined Scaffolds

Advanced biomimetic scaffolding strategies aim to replicate the intricate structural and biochemical complexities of native cartilage while actively modulating the local immune environment. Electrospun nanofibrous membranes replicate native collagen fibril architecture and have been shown to suppress pro-inflammatory cytokines, protecting engineered cartilage from immune rejection in vivo.[37] Decellularized ECM (dECM) scaffolds preserve biochemical cues impossible to replicate synthetically, and functionalized variants simultaneously recruit synovial MSCs, drive chondrogenesis, and modulate macrophage polarization.[38] Three-dimensional (3D) bioprinted scaffolds offer anatomical complexity but face persistent mechanical mismatch and resolution limits that currently preclude accurate zonal architecture.[39]

Clinical Status and Levels of Evidence

None of the biomimetic strategies reviewed here has been evaluated in humans yet (Table 1). However, the system which has reached patients is simpler: a cell-free aragonite biphasic implant outperformed debridement and microfracture on Knee injury and Osteoarthritis Outcome Score, IKDC score and MRI defect filling in a 26-centre randomized trial of 247 patients, with five-year follow-up and approval in 2022.[40,41] Matrix-associated autologous chondrocyte transplantation on a bilayered type I collagen sponge gave satisfactory two-year clinical and MOCART outcomes for defects larger than 2 cm^2, albeit as a single-center case series at level 4 evidence.[58] A phase III randomized comparison against microfracture is registered (NCT01656902) but has not, to our knowledge, been reported in full. Both these systems are non-gradient and do not modulate inflammation. The sophistication described in Sections 4 and 7 is precisely what remains untested. Preclinical endpoints also map poorly onto the outcomes deciding clinical success at 24–60 months;

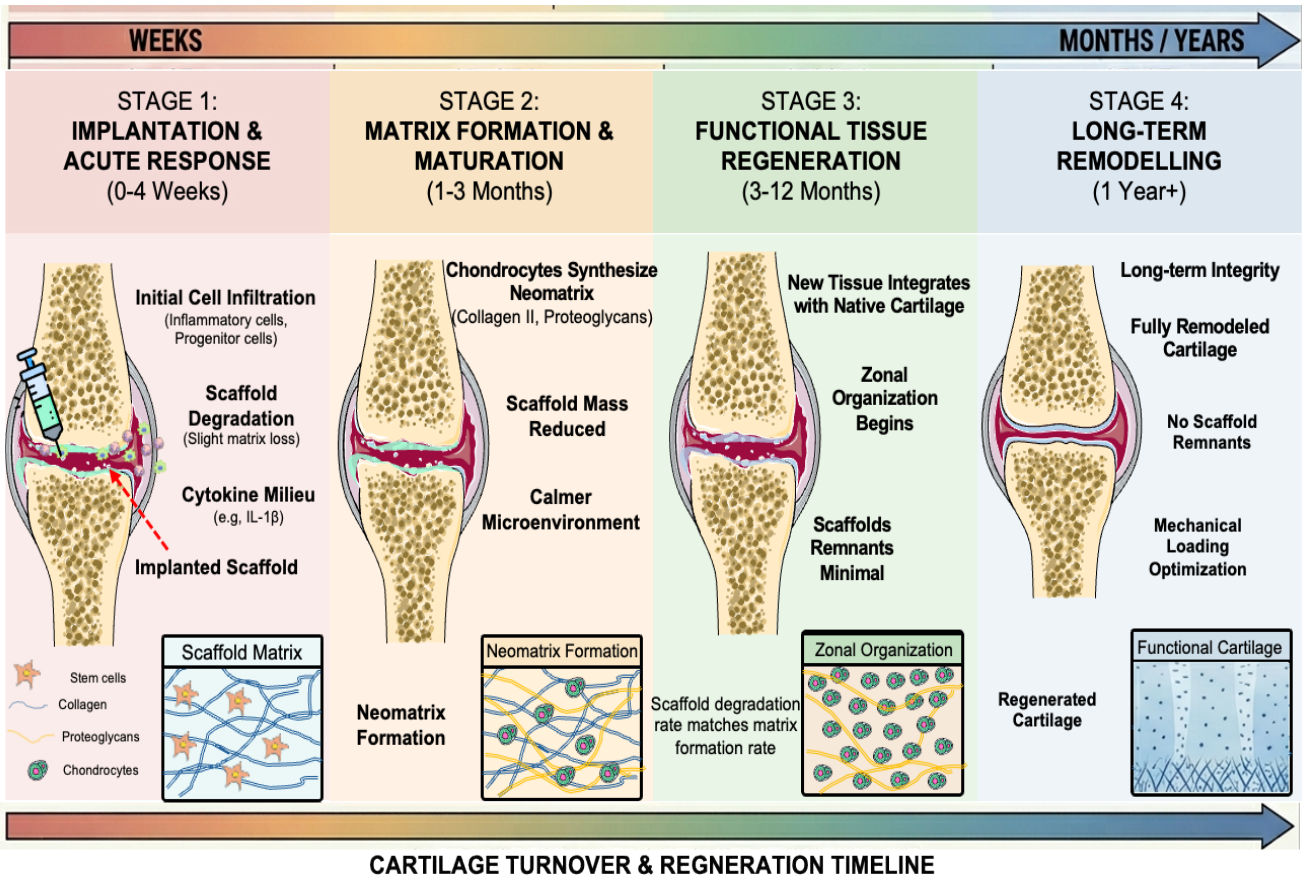

Figure 4: Schematic depiction of events that unfold after scaffold implantation (impact of inflammatory and immune responses) and sequential events and timeline leading to complete regeneration.

closing that gap may matter more than added complexity. Table 3 relates lesion size, location and patient age to construct class on the basis of this evidence.

How Inflammatory and Immune Responses Disrupt Scaffolds and the Cartilage Microenvironment

The hostile inflammatory environment of a cartilage defect threatens any regenerative construct (Figure 3, 4). Intra-articular pro-inflammatory mediators (IL-1β, IL-6, IL-17, IL-18, TNF-α) and elevated reactive oxygen species (ROS) disrupt the immune microenvironment within the joint, driving synovitis and progressive degradation.[56] IL-1β and TNF-α induce MMP activity that cleaves the collagen and proteoglycan networks scaffolds depend upon for structural integrity and chondrogenic signalling.[43] For conformable hydrogels that are already mechanically weak, this enzymatic attack accelerates scaffold breakdown beyond the two-to-four week window, collapsing the provisional matrix before any mature cartilage tissue can form. Even architecturally stronger constructs such as decellularized ECM or electrospun membranes are vulnerable, since early pro-inflammatory conditions attenuate protective type-2 immune programmes and produce lasting effects on how the scaffold remodels over time.[43,57]

Macrophage behaviour upon biomaterial implantation is equally critical: M1-dominated responses lead to chronic inflammation and fibrous encapsulation.[58] This is particularly harmful for injectable hydrogels and cryogel sponges, where fibrous encapsulation physically blocks the cell infiltration and glycosaminoglycan deposition these scaffolds rely upon for zonal matrix formation.[58]

Two important aspects that need consideration are macrophage phenotype and presence of other immune cells. First, macrophage phenotype is partly epigenetically encoded. This means that chromatin and methylation changes acquired during an initial pro-inflammatory encounter impacts the later immune responses (trained immunity).[59] This implies a window effect wherein an early M1-dominated environment leaves an imprint that later anti-inflammatory release cannot fully reverse.[60] This suggests for concentrating immunomodulation in the first days after implantation rather than as sustained background release.[61]

Second, macrophages are not the only relevant population. Regulatory T cells accumulate around implanted ECM and are required for constructive rather than fibrotic remodeling. These cells act through IL-10 and TGF-β to

suppress M1, restrain MMP expression and stabilize M2.[62] High-molecular-weight hyaluronic acid, already a common scaffold component, itself induces macrophage IL-10. This possibly explains immunological behavior usually attributed to mechanics alone in case of hyaluronic acid hydrogels/ scaffolds. Integrin signaling links both the arms: RGD functionalization engages the same α5β1 receptor that fibronectin fragments exploit (Section 3), an ambiguity unresolved experimentally in OA.[63]

Oxidative stress further adds to the damage: hypoxia-driven ROS accumulation activates NF-κB, upregulates MMPs, and triggers chondrocyte apoptosis.[64] This undermines the cell survival strategies that nanofibrous scaffolds depend upon. In thick constructs that hypoxia is partly self-inflicted (Section 4.5). The inflammatory microenvironment also blunts locally delivered bioactive agents, indicating that scaffold design must address immune conditions alongside mechanical and architectural requirements.[52] None of the mechanistic findings summarized in this section have yet been confirmed in a large-animal OA model or a human joint, a gap that limits how directly they can be extrapolated to clinical scaffold performance.

Recent Strategies to Tackle OA Inflammation

It has been established that OA is a combination

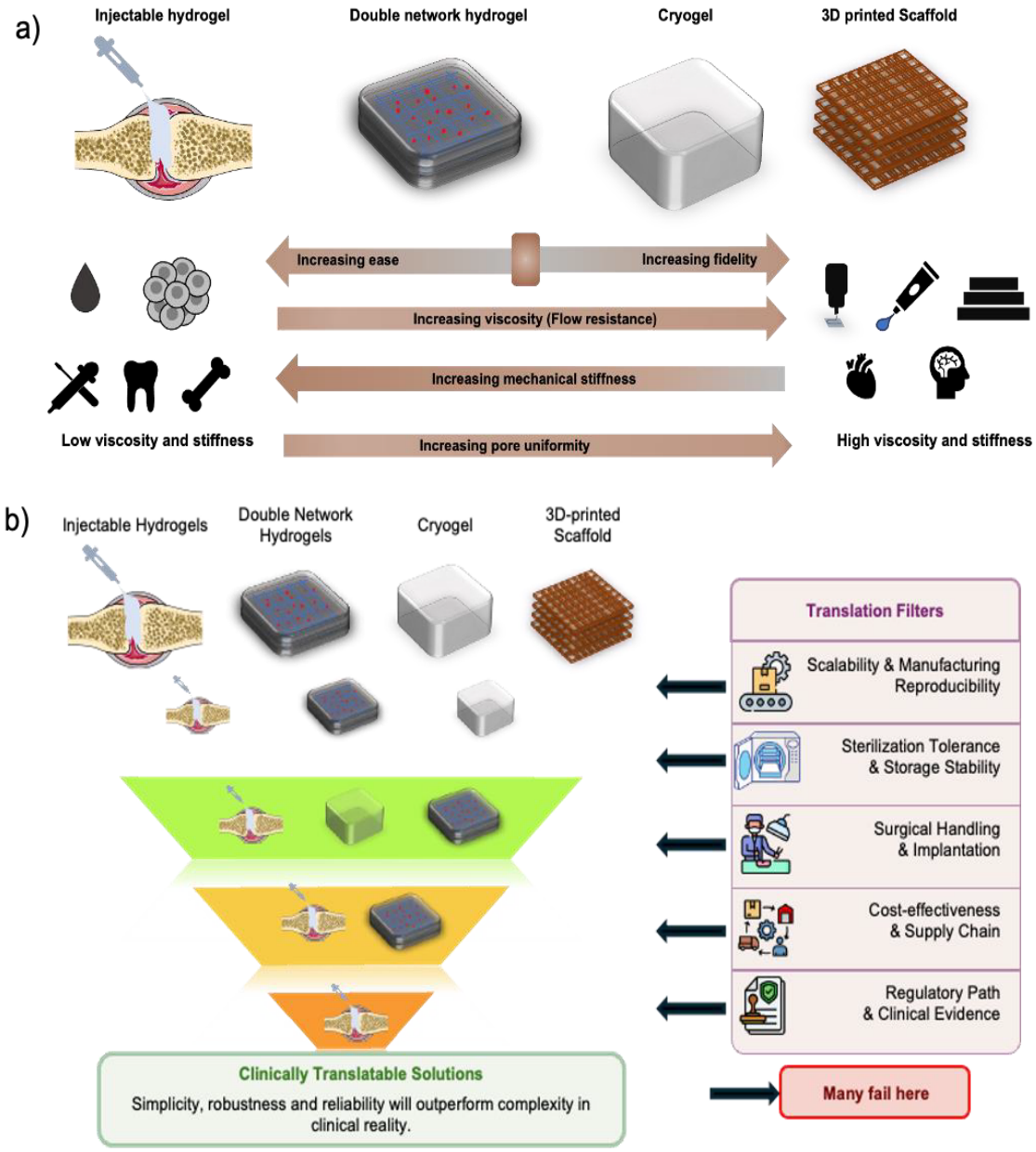

Figure 5: (a) Achieving the balance between ease and fidelity is essential for successful regeneration of cartilage defect and clinical deployment. (b) Translation filters that often hinder the clinical translation of cartilage-mimic technologies.

Table 3: Provisional decision matrix relating lesion size, anatomical location, and patient profile to suggested construct class. Scenarios 1–3 are based on randomized or high-level cohort evidence. Scenarios 4–5 are extrapolations from preclinical data and clinical reasoning. Reference numbers correspond to the manuscript reference list.

Clinical Scenario	Lesion Size	Location	Patient Profile	Suggested Construct Class	Rationale	Principal Caveat	Evidence Base
1	<2 cm²	Femoral condyle (contained)	Age <40; active	Microfracture ± acellular scaffold augmentation (e.g. collagen I/III membrane, chitosan-based scaffold)	Small contained defects respond well to marrow stimulation; scaffold augmentation stabilizes the clot and may improve repair durability vs microfracture alone[4,5]	Fibrocartilage rather than hyaline repair; limited long-term durability beyond 5 years[4]	RCT-level (microfracture); cohort-level for scaffold augmentation[4,5]
2	2–6 cm²	Femoral condyle or trochlea	Age <45; moderate–high demand	Cell-free biphasic or osteochondral scaffold (e.g. aragonite-based implant)	Multicentre RCT (n = 247) demonstrated superior KOOS, IKDC, and MRI defect filling vs microfracture/debridement at 24–60 months[40,41]; avoids second surgery and ATMP classification	Not zonally biomimetic; does not address inflammation; comparator was microfracture, not an active scaffold[40]	RCT-level[40,41]
3	2–6 cm²	Femoral condyle or trochlea	Age <50; failed prior microfracture	Matrix-associated autologous chondrocyte implantation (MACI) or collagen-based MACT	MACI demonstrated significantly better outcomes than microfracture for defects >3 cm² [452]; suitable for revision after failed marrow stimulation	Two-stage procedure; ATMP regulatory pathway; high cost; donor-site morbidity[52]	RCT-level (SUMMIT)[4]; level 4 for MACT[52]
4	>6 cm²	Femoral condyle (with subchondral involvement)	Age <50; high demand	Osteochondral allograft transplantation or next-generation biphasic immunomodulatory construct (investigational)	Large defects with bone loss exceed microfracture and single-phase scaffold capacity; osteochondral allografts restore both cartilage and subchondral architecture[4,5]; immunomodulatory biphasic constructs are the proposed next-generation approach but remain investigational	Allograft: limited supply, disease transmission risk, variable incorporation; immunomodulatory constructs: no human data (TRL 3–4)[5]	Cohort/case-series level (allograft)[4,5]; preclinical only for immunomodulatory constructs
5	2–4 cm²	Patellofemoral joint	Age >45; low demand; early–moderate OA (KL grade II–III)	Injectable immunomodulatory hydrogel (cell-free, single-stage)	Patellofemoral biomechanics and thin cartilage make rigid implants prone to failure; injectable hydrogels conform to irregular geometry and can be delivered arthroscopically[34,47]; older, lower-demand patients benefit most from minimally invasive, single-stage approaches	Mechanical weakness; no zonal control; accelerated enzymatic resorption; all evidence is preclinical (TRL 3–4)[34,36]	Preclinical only (in vitro and small-animal models)[34,36,47]

of mechanical wear and persistent inflammatory microenvironment in the cartilage. Thus, scaffolds for cartilage regeneration must provide structural support while addressing inflammation and is achieved through two broad strategies: (1) spatiotemporally controlled therapeutic delivery; and (2) scaffold surface or composition designs that respond to the immune microenvironment directly.[65]

Controlled-Release Platforms

By incorporating therapeutics within micro/nanoparticles or hydrogel networks, controlled-release platforms sustain local drug release over days to weeks, unlike single injections cleared within hours. Biocompatible carriers composed of natural and synthetic polymers have been designed for sustained delivery of drugs, recombinant proteins, and gene sequences within the OA joint while reducing systemic side effects.[66] For example, chitosan nanoparticles co-loaded with fisetin and kartogenin (KGN) in a hyaluronic acid hydrogel sustained chondroprotective release that outperformed free-drug controls.[42] Stimulus-responsive systems release payload only when triggered by the OA microenvironment; for instance, MMP-sensitive microspheres release hydroxychloroquine in response to elevated MMP-13, scavenging ROS and downregulating HIF-1α.[43] Tetrahedral framework nucleic

acid nanoparticles loaded with ginsenoside Rb1 similarly achieved deep cartilage penetration and prolonged joint residence.[44] At the cellular scale, genetically engineered chondrocyte-mimetic nanoplatforms have combined IL-1β blockade with mitochondrial repair, demonstrating synergistic disease modification in OA models.[45] Another strategy is employing enzyme-sensitive linker as a reporter as well as a trigger, so delivered dose tracks local protease activity.[67,68] Its unexamined weakness is that intra-articular MMP activity is spatially heterogeneous and load-dependent, so mean-calibrated dosing may under-dose an active focus. Some recent designs leverage enzymatic degradation as a therapeutic mechanism. An ADAMTS5-responsive silk fibroin hydrogel breaks down in proportion to local aggrecanase activity, releasing miR-17-3p lipid nanoparticles. This suppresses NF-κB signaling upstream of ADAMTS5, creating a self-regulating feedback loop that reprogrammes the inflammatory niche during microfracture repair.[46]

Surface and Composition Modulation

Surface and composition modulation makes the scaffold itself immunologically instructive, primarily by steering macrophage polarization from pro-inflammatory M1 toward tissue-healing M2 phenotypes.[59] Crucially, biomaterial properties including surface chemistry, hydrophilicity, and stiffness can be deliberately tuned to favour the M2 phenotype and suppress chronic inflammation.[69,70] One well-demonstrated strategy is the RGD-modified hyaluronic acid methacrylate hydrogel co-loaded with KGN and the anti-aging protein Klotho, which simultaneously scavenged ROS, reprogrammed macrophages toward an anti-inflammatory state, and promoted chondrogenesis in OA models.[47] In order to directly suppress NF-κB signaling, reduce MMP-driven ECM degradation, and restore matrix homeostasis in inflammatory chondrocytes, a complementary strategy incorporated hyaluronic acid and chondroitin sulfate within scaffold compositions.[48] Exosome-loaded bilayer hydrogels combine a lower layer that delivers MSC-derived exosomes with a ROS-responsive upper layer that releases diclofenac, thereby reducing inflammation and promoting chondrogenesis.[49]

Two promising strategies are advancing regenerative tissue engineering. The first employs decellularized extracellular matrix (dECM) scaffolds loaded with regulatory T cell-conditioned medium.[50] Instead of merely suppressing inflammation, these biological payloads actively recruit a resolution programme by inducing M2 macrophage polarization (an anti-inflammatory, pro-healing cellular phenotype) without relying on small-molecule drugs, though at the expense of using an ill-defined biologic. The second strategy incorporates embedded microsensors into load-bearing scaffolds to monitor parameters such as pH, partial pressure of oxygen, lactate concentration, matrix metalloproteinase (MMP) activity, and specific cytokine profiles.[51] This establishes a diagnostic feedback loop to differentiate resolving from persistent inflammation. Although unproven in cartilage and constrained by technical issues like power supply, biofouling, and calibration drift, these sensors may only require a two-week functional lifespan during the key diagnostic window rather than multi-year longevity.

Taken as a whole, these methods support a fundamental idea: the immunological microenvironment must be regarded as a primary engineering target rather than an afterthought when designing scaffolds for OA.

Degradation Products and Their Immunological Consequences

In context of cartilage regeneration, scaffold/implant degradation is usually treated as a mechanical problem. However, the chemical identity of what is released matters independently. Aliphatic polyesters hydrolyze autocatalytically to acidic products: lactic and glycolic acid from PLGA and PLA, acidic oligomers from PCL. The local pH within a degrading depot can approach 2.8 further inciting immune response. Macrophages exposed to PLGA degradation extracts shift toward M1 with elevated TNF-α and IL-1β.[71] PLA products also reprogramme immune cell metabolism, which is why buffering pH alone does not abolish the response. Consequently, KGN-loaded PLGA nanoparticles (Section 5.1) release a chondrogenic payload and a pro-inflammatory by-product on overlapping timescales.[34] Moreover, gamma sterilization of PCL accelerates subsequent hydrolysis, so a regulatory decision alters immunological profile.[53] The effect is geometry-dependent, worse in dense lattices than thin membranes. Buffering agents, chitosan blending and reduced glycolide fraction mitigate it, but degradation is typically assessed in excess buffer, masking the confined acidification that actually occurs in a defect (Table 2).

Balancing Simplicity, Scale and Clinical Success

Cartilage tissue engineering has a persistent issue: the structures that most accurately mimic natural zonal complexity are frequently the most difficult to develop repeatedly and on a clinical scale (Figure 5). Although multi-layered bioprinted scaffolds with gradient compositions and embedded bioactive factors can closely resemble the architecture of healthy cartilage, their production requires advanced equipment, precise process control, and prolonged production times that raise production costs while lowering batch consistency.[54,55] Single-component injectable hydrogels, on the other hand, provide simple preparation, low-cost raw materials, and minimally invasive delivery; nonetheless, they usually compromise mechanical strength and zonal organization, resulting in

fibrocartilaginous healing tissue with limited durability.[72] While delamination between phases and lot-to-lot variability are still issues, cryogels and double-network hydrogels provide better cell penetration and stiffness without the need for additive manufacturing.[35,36]

Another factor that is frequently overlooked is introduced by terminal sterilization. While gamma irradiation induces chain scission in polyesters like PCL and PLGA, altering resorption profiles and possibly compromising integrated medicinal particles, autoclaving can stiffen polymer matrices and accelerate degradation kinetics.[73] Although ethylene oxide more consistently maintains bulk mechanical qualities, there is a chance that cytotoxic residue will become trapped in porous structures.[73] Therefore, a design limitation that has to be addressed from the beginning is finding a sterilizing method that maintains the structural and bioactive integrity of a particular construct. Clinical translation will ultimately favor designs that achieve an equilibrium between biological sophistication and a manufacturing and sterilizing procedure that is simple enough to guarantee reproducibility at high production levels.

These constraints are standardized: ISO 11137 governs radiation sterilization including substantiation of the 25 kGy dose, ISO 11135 ethylene oxide, and ISO 10993-7 residual limits, recently tightened for small implantable devices and thus relevant to porous constructs. Decisively, they constrain material selection and must be settled before formulation lock, not validated afterwards. Incorporating cells further moves the product onto an advanced therapy medicinal product (ATMP) pathway. Table 2 proposes quantitative working ranges, notably a cap of two or three cell-laden zones, beyond which delamination risk outgrows the fidelity gained, and an inter-layer modulus ratio within an order of magnitude.

Future Perspectives & Conclusions

Effective cartilage regeneration requires structural mimicry and regulation of the inflammatory microenvironment to proceed concurrently. Current zonal scaffolds direct spatially coordinated chondrogenesis, but are studied almost exclusively under idealized conditions bearing little resemblance to an inflamed OA joint. Closing this gap requires built-in immunomodulatory architecture: stimulus-responsive release, macrophage-polarizing surface chemistry, and real-time sensing of pH, ROS, or MMP activity to provide feedback after implantation.

Set against clinical evidence, the field is less advanced than preclinical evidence suggests. None of the zonal, gradient, or immunomodulatory constructs reviewed here has been evaluated in humans. The systems reaching patients are architecturally simpler and immunologically inert. One leading system is a cell-free aragonite osteochondral implant, which proved superior to debridement and microfracture on KOOS, IKDC, and MRI defect fill in a 247-patient, 5-year multicentre randomized trial. Another is a collagen-based MACT scaffold, which remains supported only by level 4 case series with its phase III comparison unreported. Cell-based products carrying stronger biological rationales, TGF-β1-overexpressing allogeneic chondrocytes now in phase III, and umbilical cord blood MSCs with seven-year follow-up reach patients only through the costly ATMP route. Consequently, cell-free biphasic constructs carrying a single defined immunomodulatory function are closest to translation.

Two barriers are most limiting. The first is the absence of a validated terminal sterilization route for hydrogel composites, a precondition for marketability often addressed too late after formulation is fixed. The second is controlling the M1/M2 macrophage balance during the critical fortnight post-implantation, a window in which the immune response is set and not fully recoverable afterwards. Manufacturing scale-up remains serious but tractable, as modular assembly of quality-controlled layers offers a known route to scale without losing zonal control.

The immediate next step is a twelve-month ovine or caprine study in a surgically induced OA joint, comparing a bilayer construct with and without an early immunomodulatory phase, powered on defect filling and repair-tissue mechanics. If positive, the first-in-human trial should compare against an active scaffold rather than microfracture powered on 24-month KOOS and IKDC with MOCART defect fill as a secondary endpoint, followed to five years for commensurability with the aragonite dataset. Joint inflammatory state at implantation must be a prespecified stratification variable.

Regulatory classification belongs among early design inputs, as the cost gap between device and ATMP pathways currently exceeds the demonstrated performance gap, favouring cell-free constructs that recruit host cells. A realistic five-year translation sequence spans sterilization and degradation-product standardization in years 0–2, large-animal demonstration of early-window immunomodulation in years 2–4, and a first-in-human study of cell-free biphasic constructs in 2–6 cm^2 lesions by years 4–5 (Table 3), while closed-loop sensing constructs remain near a decade away. Cartilage tissue engineering will become routine practice only when immunological, engineering, and regulatory challenges are solved together, and currently, the least sophisticated constructs are closest to achieving this.

Acknowledgment

The authors would like to acknowledge the use of image(s) provided by Servier Medical Art (https://smart.servier.com), licensed under CC BY 4.0 (https://

creativecommons.org/licenses/by/4.0/) to design the schematics. Icons from Flaticon.com have been also used to design the schematics. All the schematics have been designed using Microsoft PowerPoint.

References

1. Rehman S, Iqbal S, Shahid MU, et al. Cartilage: Structure, Function, and the Pathogenesis of Osteoarthritis. In Advancements in Synovial Joint Science - Structure, Function, and Beyond. IntechOpen. 2024. https://doi.org/10.5772/intechopen.1003264
2. Houard X, Goldring MB, Berenbaum F. Homeostatic Mechanisms in Articular Cartilage and Role of Inflammation in Osteoarthritis. Curr Rheumatol Rep 2013; 15(11): 375. https://doi.org/10.1007/s11926-013-0375-6
3. Qiao L, Li M, Deng F, et al. Epidemiological Trends of Osteoarthritis at the Global, Regional, and National Levels from 1990 to 2021 and Projections to 2050. Arthritis Res Ther. 2025; 27(1): 199. https://doi.org/10.1186/s13075-025-03658-w
4. Kutaish H, Klopfenstein A, Adorisio SNO, et al. Current Trends in the Treatment of Focal Cartilage Lesions: A Comprehensive Review. 2025. https://doi.org/10.1530/EOR-2024-0083
5. Dell'Isola A, Recenti F, Giardulli B, et al. Osteoarthritis Year in Review 2025: Epidemiology and Therapy. Osteoarthritis and Cartilage. 2025; 33(11): 1300-1306. https://doi.org/10.1016/j.joca.2025.08.015
6. Krakowski P, Rejniak A, Sobczyk J, et al. Cartilage Integrity: A Review of Mechanical and Frictional Properties and Repair Approaches in Osteoarthritis. Healthcare. 2024; 12(16): 1648. https://doi.org/10.3390/healthcare12161648
7. Belluzzi E, Todros S, Pozzuoli A, et al. Human Cartilage Biomechanics: Experimental and Theoretical Approaches towards the Identification of Mechanical Properties in Healthy and Osteoarthritic Conditions. Processes. 2023; 11(4): 1014. https://doi.org/10.3390/pr11041014
8. Goldring MB, Marc KB. Cartilage Homeostasis in Health and Rheumatic Diseases. Arthritis Research & Therapy. 2009.
9. Michelacci YM, Baccarin RYA, Rodrigues NNP. Chondrocyte Homeostasis and Differentiation: Transcriptional Control and Signaling in Healthy and Osteoarthritic Conditions. Life. 2023; 13(7): 1460. https://doi.org/10.3390/life13071460
10. Lotz MK, Caramés B. Autophagy and Cartilage Homeostasis Mechanisms in Joint Health, Aging and OA. Nat Rev Rheumatol. 2011; 7(10): 579-587. https://doi.org/10.1038/nrrheum.2011.109
11. Wang N, Lu Y, Rothrauff BB, et al. Mechanotransduction Pathways in Articular Chondrocytes and the Emerging Role of Estrogen Receptor-α. Bone Res. 2023; 11(1): 13. https://doi.org/10.1038/s41413-023-00248-x
12. Loeser RF. Integrins and Chondrocyte–Matrix Interactions in Articular Cartilage. Matrix Biology. 2014; 39: 11-16. https://doi.org/10.1016/j.matbio.2014.08.007
13. Yunus MHM, Nordin A, Kamal H. Pathophysiological Perspective of Osteoarthritis. Medicina. 2020; 56(11): 614. https://doi.org/10.3390/medicina56110614
14. Ihnatouski M, Pauk J, Karev D, et al. AFM-Based Method for Measurement of Normal and Osteoarthritic Human Articular Cartilage Surface Roughness. Materials. 2020; 13(10): 2302. https://doi.org/10.3390/ma13102302
15. Link JM, Salinas EY, Hu JC, et al. The Tribology of Cartilage: Mechanisms, Experimental Techniques, and Relevance to Translational Tissue Engineering. Clinical Biomechanics. 2020; 79: 104880. https://doi.org/10.1016/j.clinbiomech.2019.10.016
16. Wan H, Ren K, Kaper HJ, et al. A Bioinspired Mucoadhesive Restores Lubrication of Degraded Cartilage through Reestablishment of Lamina Splendens. Colloids and Surfaces B: Biointerfaces. 2020; 193: 110977. https://doi.org/10.1016/j.colsurfb.2020.110977
17. Troeberg L, Nagase H. Proteases Involved in Cartilage Matrix Degradation in Osteoarthritis. Biochim Biophys Acta. 2012; 1824(1): 133-145. https://doi.org/10.1016/j.bbapap.2011.06.020
18. Yu L, Cavelier S, Hannon B, et al. Recent Development in Multizonal Scaffolds for Osteochondral Regeneration. Bioactive Materials. 2023; 25: 122-159. https://doi.org/10.1016/j.bioactmat.2023.01.012
19. Çelik E, Bayram C, Denkbaş EB. Zone-Inspired Hydrogel Constructs Promote Spatially Controlled Chondrogenesis for Osteochondral Regeneration. Sci Rep. 2025; 16(1): 3370. https://doi.org/10.1038/s41598-025-33222-0
20. Shopperly LK, Spinnen J, Krüger JP, et al. Blends of Gelatin and Hyaluronic Acid Stratified by Stereolithographic Bioprinting Approximate Cartilaginous Matrix Gradients. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2022; 110(10): 2310-2322. https://doi.org/10.1002/jbm.b.35079
21. Armstrong JPK, Pchelintseva E, Treumuth S, et al. Tissue Engineering Cartilage with Deep Zone Cytoarchitecture by High-Resolution Acoustic Cell Patterning. Advanced Healthcare Materials. 2022; 11(24): 2200481. https://doi.org/10.1002/adhm.202200481
22. Lehmenkötter N, Greven J, Hildebrand F, et al. Electrical Stimulation of Mesenchymal Stem Cells as a Tool for Proliferation and Differentiation in Cartilage Tissue Engineering: A Scaffold-Based Approach. Bioengineering. 2024; 11(6): 527. https://doi.org/10.3390/bioengineering11060527
23. Stampoultzis T, Guo Y, Nasrollahzadeh N, et al. Low-Oxygen Tension Augments Chondrocyte Sensitivity to Biomimetic Thermomechanical Cues in Cartilage-Engineered Constructs. iScience. 2023; 26(8). https://doi.org/10.1016/j.isci.2023.107491
24. Zheng Y, Fu L, Zhang Z, et al. Three-Dimensional Bioprinting of Growth Differentiation Factor 5-Preconditioned Mesenchymal Stem Cell-Derived Exosomes Facilitates Articular Cartilage Endogenous Regeneration. ACS Nano. 2025; 19(16): 15281-15301. https://doi.org/10.1021/acsnano.4c13492
25. Lee B, Parvizi J, Bramlet D, et al. Results of a Phase II Study to Determine the Efficacy and Safety of Genetically Engineered Allogeneic Human Chondrocytes Expressing TGF-B1. J Knee Surg. 2020; 33(02): 167-172. https://doi.org/10.1055/s-0038-1676803
26. Pahoff S, Weekes A, Mertano H, et al. Engineering Biomimetic Zonal Properties in Fibre-Reinforced Hydrogels for Functional Cartilage Tissue Engineering in Vitro. Materials Today Bio. 2025; 35: 102550. https://doi.org/10.1016/j.mtbio.2025.102550
27. Peng Y, Zhuang Y, Liu Y, et al. Bioinspired Gradient Scaffolds for Osteochondral Tissue Engineering. Exploration. 2023; 3(4): 20210043. https://doi.org/10.1002/EXP.20210043
28. Liu Q, Dai W, Gao Y, et al. The Degradation Rate of Collagen-Based Hydrogels Regulates Chondrogenic Differentiation of Bone Marrow Mesenchymal Stem Cells. Collagen & Leather. 2025; 7(1): 39. https://doi.org/10.1186/s42825-025-00221-w
29. Maples MM, Schneider MC, Bryant SJ. Impact of Inter- and Intra-Donor Variability by Age on the Gel-to-Tissue Transition in MMP-Sensitive PEG Hydrogels for Cartilage Regeneration. ACS Appl Bio Mater. 2023; 6(7): 2677-2689. https://doi.org/10.1021/acsabm.3c00082
30. Li W, Shi Z, Jing H, et al. Streamlined Metal-Based Hydrogel Facilitates Stem Cell Differentiation, Extracellular Matrix Homeostasis and Cartilage Repair in Male Rats. Nat Commun. 2025; 16(1): 4344. https://doi.org/10.1038/s41467-025-59725-y

31. Lim HC, Park YB, Ha CW, et al. Allogeneic Umbilical Cord Blood–Derived Mesenchymal Stem Cell Implantation Versus Microfracture for Large, Full-Thickness Cartilage Defects in Older Patients: A Multicenter Randomized Clinical Trial and Extended 5-Year Clinical Follow-Up. Orthop J Sports Med. 2021; 9(1): 2325967120973052. https://doi.org/10.1177/2325967120973052

32. Wang Y, Chen Z, Huang W, et al. Oxygen-Releasing Biomaterials for Osteoarthritis: Advances in Managing the Hypoxic Joint Microenvironment. Front Cell Dev Biol. 2025. https://doi.org/10.3389/fcell.2025.1704327

33. Fan F, Yang C, Piao E, et al. Mechanisms of Chondrocyte Regulated Cell Death in Osteoarthritis: Focus on ROS-Triggered Ferroptosis, Parthanatos, and Oxeiptosis. Biochemical and Biophysical Research Communications. 2024; 705: 149733. https://doi.org/10.1016/j.bbrc.2024.149733

34. Li M, Li F, Xu J, et al. Bioadhesive Chitosan Hydrogel with Dynamic Covalent Bonds and Sustained Kartogenin Release for Endogenous Cartilage Regeneration. Front Bioeng Biotechnol. 2025. https://doi.org/10.3389/fbioe.2025.1606726

35. Yang D, Yang J, Chang SJ, et al. Exosome-Seeded Cryogel Scaffolds for Extracellular Matrix Regeneration in the Repair of Articular Cartilage Defects: An In Vitro and In Vivo Rabbit Model Study. Polymers. 2025; 17(7): 975. https://doi.org/10.3390/polym17070975

36. Singh J, Kadir JC, Orlando JD, et al. Biomimetic Double Network Hydrogels of Chondroitin Sulfate and Synthetic Polypeptides for Cartilage Tissue Engineering. Biomaterials Science. 2025; 13(15): 4211-4231. https://doi.org/10.1039/D5BM00296F

37. Zhang Y, Cai R, Li J, et al. The Immunosuppressive Niche Established with a Curcumin-Loaded Electrospun Nanofibrous Membrane Promotes Cartilage Regeneration in Immunocompetent Animals. Membranes. 2023; 13(3): 335. https://doi.org/10.3390/membranes13030335

38. Liao Z, Fu L, Li P, et al. Incorporation of Magnesium Ions into an Aptamer-Functionalized ECM Bioactive Scaffold for Articular Cartilage Regeneration. ACS Appl Mater Interfaces. 2023; 15(19): 22944-22958. https://doi.org/10.1021/acsami.3c02317

39. Zhao X, Li N, Zhang Z, et al. Beyond Hype: Unveiling the Real Challenges in Clinical Translation of 3D Printed Bone Scaffolds and the Fresh Prospects of Bioprinted Organoids. J Nanobiotechnol. 2024; 22(1): 500. https://doi.org/10.1186/s12951-024-02759-z

40. Altschuler N, Zaslav KR, Di Matteo B, et al. Aragonite-Based Scaffold Versus Microfracture and Debridement for the Treatment of Knee Chondral and Osteochondral Lesions: Results of a Multicenter Randomized Controlled Trial. Am J Sports Med. 2023; 51(4): 957-967. https://doi.org/10.1177/03635465231151252

41. Conte P, Anzillotti G, Crawford DC, et al. Differential Analysis of the Impact of Lesions' Location on Clinical and Radiological Outcomes after the Implantation of a Novel Aragonite-Based Scaffold to Treat Knee Cartilage Defects. International Orthopaedics (SICOT). 2024; 48(12): 3117-3126. https://doi.org/10.1007/s00264-024-06314-1

42. Nabizadeh Z, Nasrollahzadeh M, Heidari F, et al. A Drug-Loaded Nano Chitosan/Hyaluronic Acid Hydrogel System as a Cartilage Tissue Engineering Scaffold for Drug Delivery. International Journal of Biological Macromolecules. 2024; 283: 137314. https://doi.org/10.1016/j.ijbiomac.2024.137314

43. Xu Z, Ma J, Hu H, et al. Metal Ion-Crosslinking Multifunctional Hydrogel Microspheres with Inflammatory Immune Regulation for Cartilage Regeneration. Front Bioeng Biotechnol. 2025. https://doi.org/10.3389/fbioe.2025.1540592

44. Huang K, Li Q, Lin H, et al. Cartilage-Penetrating Framework Nucleic Acid Nanoparticles Ameliorate Osteoarthritis by Promoting Drug Delivery and Chondrocyte Uptake. Advanced Science. 2025; 12(26): 2502661. https://doi.org/10.1002/advs.202502661

45. Genetically engineered chondrocyte-mimetic nanoplatform attenuates osteoarthritis by blocking IL-1β and restoring sirtuin-3 | Science Advances. https://www.science.org/doi/10.1126/sciadv.adv4238 (accessed 2026-06-15)

46. Gao T, Li H, Yang Y, et al. An Aggrecanase-Responsive Delivery Hydrogel System of miRNA-17 Facilitates Microfracture-Mediated Articular Cartilage Regeneration by Reprogramming Hostile Inflammatory Niche. ACS Nano. 2025; 19(36): 32063-32081. https://doi.org/10.1021/acsnano.4c16049

47. Po Z, Xiao M, Han Z, et al. Bioactive Multifunctional Hydrogel to Ameliorate Adverse Joint Microenvironment and Enhance Cartilage Regeneration. Chemical Engineering Journal. 2025; 515: 163554. https://doi.org/10.1016/j.cej.2025.163554

48. Ma Y, Yang X, Jiang M, et al. Alone or in Combination, Hyaluronic Acid and Chondroitin Sulfate Alleviate ECM Degradation in Osteoarthritis by Inhibiting the NF-κB Pathway. J Orthop Surg Res. 2025; 20(1): 11. https://doi.org/10.1186/s13018-024-05411-6

49. Yang Q, Liu G, Chen G, et al. Novel Injectable Adhesive Hydrogel Loaded with Exosomes for Holistic Repair of Hemophilic Articular Cartilage Defect. Bioactive Materials. 2024; 42: 85-111. https://doi.org/10.1016/j.bioactmat.2024.08.018

50. Xiong Y, Mi BB, Lin Z, et al. The Role of the Immune Microenvironment in Bone, Cartilage, and Soft Tissue Regeneration: From Mechanism to Therapeutic Opportunity. Military Med Res. 2022; 9(1): 65. https://doi.org/10.1186/s40779-022-00426-8

51. Kumar R, Sporn K, Prabhakar P, et al. Next-Generation Biomaterials for Load-Bearing Tissue Interfaces: Sensor-Integrated Scaffolds and Mechanoadaptive Constructs for Skeletal Regeneration. Journal of Functional Biomaterials. 2025; 16(7): 232. https://doi.org/10.3390/jfb16070232

52. He S, Fu X, Wang L, et al. Self-Assemble Silk Fibroin Microcapsules for Cartilage Regeneration through Gene Delivery and Immune Regulation. Small. 2023; 19(40): 2302799. https://doi.org/10.1002/smll.202302799

53. del-Mazo-Barbara L, Gómez-Cuyàs J, Martínez-Orozco L, et al. In Vitro Degradation of 3D-Printed Polycaprolactone\biomimetic Hydroxyapatite Scaffolds: Impact of the Sterilization Method. Polymer Testing. 2024; 139: 108566. https://doi.org/10.1016/j.polymertesting.2024.108566

54. Olate-Moya F, Arens L, Wilhelm M, et al. Chondroinductive Alginate-Based Hydrogels Having Graphene Oxide for 3D Printed Scaffold Fabrication. ACS Appl Mater Interfaces. 2020; 12(4): 4343-4357. https://doi.org/10.1021/acsami.9b22062

55. Chen Q, Zou B, Wang X, et al. SLA-3d Printed Building and Characteristics of GelMA/HAP Biomaterials with Gradient Porous Structure. Journal of the Mechanical Behavior of Biomedical Materials. 2024; 155: 106553. https://doi.org/10.1016/j.jmbbm.2024.106553

56. Liu K, Zhang B, Zhang X. Promoting Articular Cartilage Regeneration through Microenvironmental Regulation. Jrnl of Immunology Research. 2024; 2024(1): 2314-8861. https://doi.org/10.1155/2024/4751168

57. Li W, Baurceanu I, Pal S, et al. Inflammatory Agonists Modulate the Host Response to Type 2 ECM Scaffold Immune Environment and Long-Term Remodeling After Severe Traumatic Injury. bioRxiv [Preprint, not peer-reviewed] November 11, 2025, p 2025.11.09.687444. https://doi.org/10.1101/2025.11.09.687444

58. Zhang R, Tan SF, Wang Y, et al. From Macrophage Polarization to Clinical Translation: Immunomodulatory Hydrogels for Infection-Associated Bone Regeneration. Front Cell Dev Biol. 2025. https://doi.org/10.3389/fcell.2025.1684357

59. Zou X, Xu H, Qian W. Macrophage Polarization in the Osteoarthritis Pathogenesis and Treatment. Orthopaedic Surgery. 2025; 17(1): 22-35. https://doi.org/10.1111/os.14302

60. Wang C, De Francesco R, Lamers LA, et al. Transcriptomic Profiling of Osteoarthritis Synovial Macrophages Reveals a Tolerized Phenotype Compounded by a Weak Corticosteroid Response. Rheumatology (Oxford). 2024; 64(2): 860-869. https://doi.org/10.1093/rheumatology/keae161

61. Brown BN, Valentin JE, Stewart-Akers AM, et al. Macrophage Phenotype and Remodeling Outcomes in Response to Biologic Scaffolds with and without a Cellular Component. Biomaterials. 2009; 30(8): 1482-1491. https://doi.org/10.1016/j.biomaterials.2008.11.040

62. Xiong Y, Mi BB, Lin Z, et al. The Role of the Immune Microenvironment in Bone, Cartilage, and Soft Tissue Regeneration: From Mechanism to Therapeutic Opportunity. Military Med Res. 2022; 9(1): 65. https://doi.org/10.1186/s40779-022-00426-8

63. Loeser RF. Integrins and Chondrocyte–Matrix Interactions in Articular Cartilage. Matrix Biology. 2014; 39: 11-16. https://doi.org/10.1016/j.matbio.2014.08.007

64. Fan F, Yang C, Piao E, et al. Mechanisms of Chondrocyte Regulated Cell Death in Osteoarthritis: Focus on ROS-Triggered Ferroptosis, Parthanatos, and Oxeiptosis. Biochemical and Biophysical Research Communications. 2024; 705: 149733. https://doi.org/10.1016/j.bbrc.2024.149733

65. Zhao R, Liang B, Shi Y, et al. Immunomodulatory Biomaterials for Osteoarthritis: Targeting Inflammation and Enhancing Cartilage Regeneration. Materials Today Bio. 2025; 34: 102100. https://doi.org/10.1016/j.mtbio.2025.102100

66. Wang D, Liu W, Venkatesan JK, et al. Therapeutic Controlled Release Strategies for Human Osteoarthritis. Advanced Healthcare Materials. 2025; 14(2): 2402737. https://doi.org/10.1002/adhm.202402737

67. Liu Y, Zhang Y, Yu C, et al. Organic Di-Selenide Hydrogel Microspheres for Multimodal Treatment of Osteoarthritis. Nat Commun. 2026; 17(1); 2300. https://doi.org/10.1038/s41467-026-68817-2

68. Li M, Deng T, Chen Q, et al. A Versatile Platform Based on Matrix Metalloproteinase-Sensitive Peptides for Novel Diagnostic and Therapeutic Strategies in Arthritis. Bioactive Materials. 2025; 47: 100-120. https://doi.org/10.1016/j.bioactmat.2025.01.011

69. Zhang J, Huang X, Wu X, et al. Injectable Glycosaminoglycan Hydrogel with Immunosuppressive Exosomes for Macrophage Polarization and Cartilage Repair in a Rheumatoid Arthritis Treatment. ACS Appl Mater Interface. 2025; 17(45): 61784-61798. https://doi.org/10.1021/acsami.5c16825

70. Jiang X, Sun Y, Lyu Y, et al. Hydrogels for Ameliorating Osteoarthritis: Mechanical Modulation, Anti-Inflammation, and Regeneration. BMEMat. 2024; 2(2): e12078. https://doi.org/10.1002/bmm2.12078

71. Ma S, Feng X, Liu F, et al. The Pro-Inflammatory Response of Macrophages Regulated by Acid Degradation Products of Poly(Lactide-Co-Glycolide) Nanoparticles. Engineering in Life Sciences. 2021; 21(10): 709-720. https://doi.org/10.1002/elsc.202100040

72. Parvin N, Joo SW, Mandal TK. Injectable Biopolymer-Based Hydrogels: A Next-Generation Platform for Minimally Invasive Therapeutics. Gels. 2025; 11(6): 383. https://doi.org/10.3390/gels11060383

73. Rizwan M, Chan SW, Comeau PA, et al. Effect of Sterilization Treatment on Mechanical Properties, Biodegradation, Bioactivity and Printability of GelMA Hydrogels. Biomed Mater. 2020; 15(6): 065017. https://doi.org/10.1088/1748-605X/aba40c