

REVIEW ARTICLE

Decellularized extracellular matrix bioinks for 3D bioprinting of the tendon–bone interface: From biomimetic gradients to regenerative healing

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Abstract

The tendon–bone interface anchors compliant tendon to rigid bone through continuous gradients in collagen orientation, proteoglycan content, mineralization, and local modulus. Following tendon–bone injury in adults, this spatiotemporal organization is difficult to restore, and repair typically produces a fibrous connection rather than a functional enthesis. The main barrier is not the absence of a single reparative factor but a spatiotemporal mismatch among immune resolution, regional cell identity, matrix maturation, controlled mineralization, and load transfer. Tissue-derived decellularized extracellular matrix (dECM) can retain source-associated matrix cues, while 3D bioprinting enables their controlled spatial placement. However, none of printability, increased lineage-marker expression, or a higher ultimate failure load alone is sufficient to establish functional enthesis regeneration. This review examines how dECM identity is retained during decellularization, solubilization, and printing; how regionalized constructs are designed; and how these strategies may influence cell identity, immune regulation, matrix remodeling, perfusion, and mechanical maturation. Current evidence supports the feasibility of dECM bioprinting for regionalized tissue formation and interface integration, but remains insufficient to establish tissue-specific potency, long-term gradient stability, or neotissue-mediated load transfer after scaffold degradation. We therefore propose a progressive evidence framework comprising material identity, manufacturing fidelity, regional tissue formation, host integration, and load takeover to distinguish proof of concept, enthesis-like tissue formation, and functional regeneration. Future progress will require reproducible, attributable, and translatable evidence generated using physicochemically matched controls, spatiotemporal quantification, and long-term loading models.

Keywords: Tendon–bone interface; Decellularized extracellular matrix; Bioink; 3D bioprinting; Functional gradient; Regeneration assessment

1. Introduction

The tendon–bone interface (TBI) is a specialized functional unit that anchors compliant tendon or ligament to rigid bone and enables efficient load transfer across tissues.^{1–6} Rotator cuff tears, ligament enthesis injuries, and related disorders can cause persistent pain and impaired function. Although surgical repair can restore anatomical continuity, healing commonly results in a fibrous attachment rather than regeneration of the native fibrocartilaginous enthesis.^{1,3,6} The native interface is characterized by coordinated gradients in collagen organization, proteoglycan content, mineralization, and local mechanical properties that reduce strain concentration between tendon and bone.^{4–6} The native spatial organization emerges during development through coordinated programs of cell-lineage specification, extracellular matrix (ECM) deposition, and mineralization under the influence of mechanical loading, but is only incompletely re-established following tendon–bone injury in adults.^{7–9} Accordingly, the regenerative objective extends beyond tissue reconnection to reconstruction of an integrated interface capable of durable load transmission.

Current approaches—including cell delivery, growth factors, mineralized materials, gradient scaffolds, and multiphasic constructs—can improve individual aspects of tendon–bone repair,^{10–16} but do not, by themselves, address the need to coordinate distinct tendon-side, transition-zone, and bone-side requirements across healing. Persistent inflammatory dysregulation and incomplete structural recovery can further disrupt this coordination.^{17,18} This challenge motivates regenerative platforms that combine region-relevant biological cues with controllable spatial manufacturing.

Decellularized extracellular matrix (dECM) offers a tissue-derived material context for such regionalized regeneration. By retaining subsets of native ECM proteins, glycosaminoglycans, adhesion ligands, and matrix-associated signals after decellularization, tissue-derived dECM can provide source-associated microenvironmental cues that single-component hydrogels cannot readily reproduce.^{19–29} Tendon-derived dECM (TdECM), cartilage-derived dECM (CdECM), and bone-derived dECM (BdECM) are therefore of particular interest for reconstructing regionally distinct microenvironments across the TBI. However, processing steps including decellularization, digestion, sterilization, crosslinking, and storage can alter matrix composition and biological activity. The relevance of dECM consequently depends on whether source-associated matrix information is reproducibly retained and remains biologically meaningful after processing. Three-dimensional (3D) bioprinting complements dECM by enabling controlled spatial

placement of materials, cells, and bioactive components.^{30–36} Multimaterial deposition, multi-nozzle printing, and related biofabrication approaches can organize distinct formulations into predefined regions or continuous gradients, thereby converting tissue-source information into spatially patterned constructs.^{37–44} Together, dECM and bioprinting provide a route for translating tissue-source information into spatially organized tendon–bone constructs.^{45–53}

Recent reviews have provided comprehensive perspectives on biomimetic strategies for tendon–bone repair, tissue-specific dECM bioinks, bioink formulation and printability, gradient scaffolds, and musculoskeletal bioprinting.^{10,14,40–44} However, as summarized in [Table 1](#), these reviews generally address different segments of the regenerative process rather than a cumulative evidential sequence linking retention of tissue-source information, spatial manufacturing, regional tissue formation, and eventual functional load transfer. Relevant literature was identified through targeted searches of the Web of Science Core Collection, with the final search conducted on July 15, 2026, using combinations of terms related to dECM, bioinks/3D bioprinting, tendon–bone/enthesis/rotator cuff, and gradient/interface regeneration; reference lists of key primary studies and recent reviews were also screened. Studies were included in the comparative evidence matrix ([Table 2](#)) when sufficient information was available on construct design, experimental model, comparators, follow-up, outcomes, and major limitations; studies lacking sufficient information for claim-level evaluation were not tabulated. The search was intended to identify representative studies for claim-level comparison rather than to perform a formal systematic review or meta-analysis. Evidence was classified as direct when dECM-integrated printed constructs were evaluated in tendon–bone or rotator cuff enthesis models, supportive when studies addressed a major component of the focal system, and adjacent when broader enthesis, ECM, immunological, vascular, or mechanobiological studies were used to assess biological plausibility.

To address this gap, we propose an author-defined five-level interpretive framework comprising material identity, manufacturing fidelity, regional tissue formation, host integration, and load takeover ([Figure 1](#)). The framework is intended for claim evaluation rather than as a field-wide consensus benchmark. Accordingly, the current review progresses from the native enthesis and dECM processing to spatial construct design, biological maturation, evidence grading, and translational readiness.

Table 1. Positioning of the present review relative to recent reviews

Review	Primary scope	dECM identity/processing	Spatial bioprinting/gradient design	Tendon–bone interface	Explicit evidence hierarchy used	Post-degradation load takeover considered
Lei <i>et al.</i> ¹⁰	Biomimetic strategies for TBI	Partial	Yes	Yes	No	No
Park <i>et al.</i> ⁴⁰	Tissue-specific dECM bioinks for musculoskeletal applications	Yes	Yes	Partial	No	No
Kim <i>et al.</i> ⁴¹	dECM-based bioinks and tissue-specific microenvironments	Yes	Yes	Not TBI-specific	No	No
Abaci & Guvendiren ⁴²	Design/rheology of dECM bioinks	Yes	Yes	No	No	No
De Santis <i>et al.</i> ⁴³	ECM-enriched bioinks	Yes	Yes	No	No	No
Zhang <i>et al.</i> ⁴⁴	Strategies to improve dECM printability	Yes	Yes	No	No	No
Chen <i>et al.</i> ¹⁴	Biomimetic gradient scaffolds for rotator cuff enthesis	Limited/not central	Yes	Yes	No	No
Present review	Evidence-based evaluation of dECM-bioprinted TBI regeneration	Yes	Yes	Yes	Five-level cumulative framework	Yes

Note: The comparison summarizes the principal scope of the cited reviews and is intended to position the present review without implying that individual prior reviews exclude all related topics. Abbreviations: dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; TBI: Tendon–bone interface.

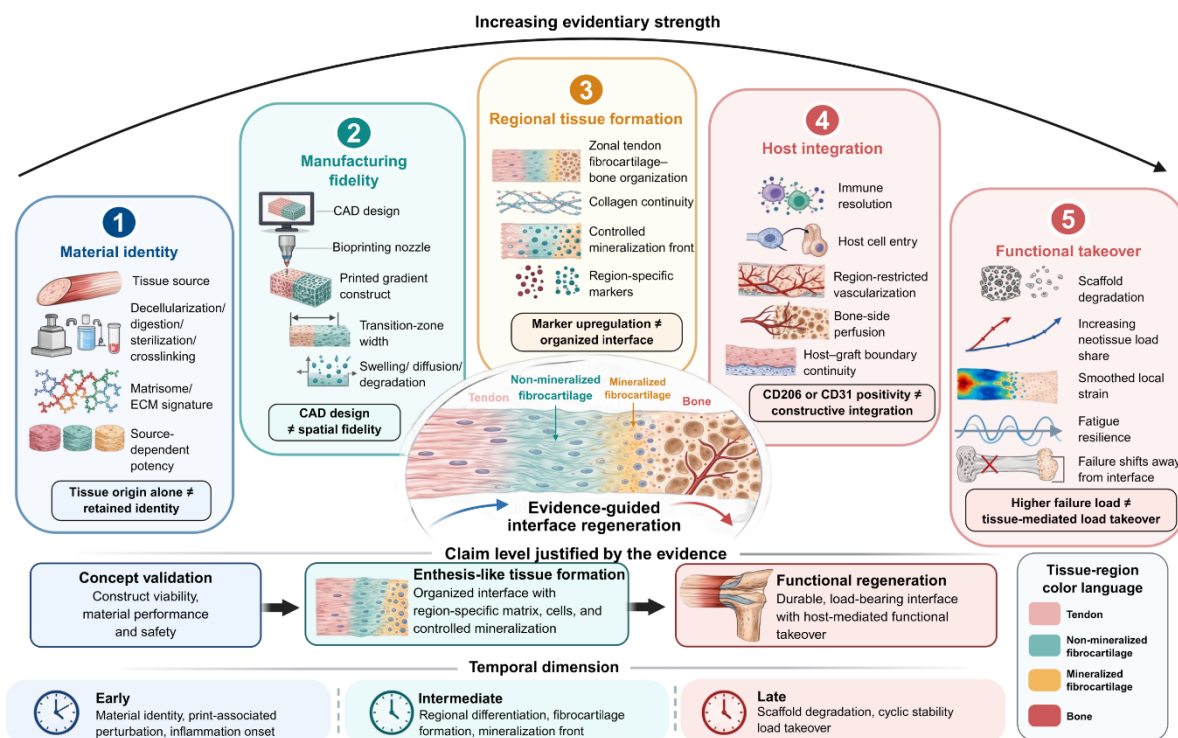


Figure 1. The proposed cumulative five-level evidence framework for evaluating decellularized extracellular matrix-bioprinting-mediated tendon–bone interface regeneration. The framework comprises five sequential levels of evidence—material identity, manufacturing fidelity, regional tissue formation, host integration, and load takeover—with increasing evidentiary strength from left to right. The central schematic depicts the continuous tissue transition from tendon through unmineralized and mineralized fibrocilage to bone. The lower section distinguishes proof of concept, enthesis-like tissue formation and functional regeneration, and summarizes the principal early-, intermediate-, and late-stage assessment priorities. A tissue-source label, a computer-aided design (CAD), increased marker expression, or improved maximum failure load alone is insufficient to demonstrate functional regeneration. Such a conclusion is supported only when neotissue maintains continuous load transmission and cyclic stability after scaffold degradation. Created in BioRender. Qing, H. (2026) <https://BioRender.com/ev9bfjg>.

Abbreviations: CAD: Computer-aided design; ECM: Extracellular matrix.

2. Native structure of the tendon–bone interface and challenges in injury healing

2.1. The four-zone gradient structure of the native tendon–bone interface

The native TBI generally comprises a tendon region, an unmineralized fibrocilage region, a mineralized fibrocilage region, and a bone region (Figure 2A).^{1–3} The tendon region consists of highly parallel collagen-fiber bundles and tenocytes and transmits longitudinal tensile forces generated by muscle contraction to bone. The unmineralized fibrocilage region lies between tendon and bone and is rich in type II collagen, aggrecan, and other proteoglycans. Its cartilage-like matrix accommodates local compression and shear deformation, preventing abrupt bending or delamination of collagen bundles near the bone surface.^{2,3} Toward the bone, the mineralized fibrocilage

region shows type X collagen expression, increased alkaline phosphatase activity, and progressively greater mineral deposition, enabling the cartilage-like matrix to transition gradually into a mineralized matrix. The bone region is dominated by mineralized type I collagen, hydroxyapatite, osteoblasts, and osteocytes, providing rigid support and long-term structural stability for tendon attachment.

Importantly, the function of the native TBI does not arise from the simple juxtaposition of four zones, but from the continuous spatial transitions among them. Using multiscale imaging and micromechanical analysis, Rossetti *et al.*⁴ showed that the tendon–bone insertion is not a homogeneous connecting band but a hierarchically heterogeneous structure jointly defined by collagen-fiber orientation, mineral distribution, matrix composition, and local mechanical response (Figure 2B and 2C). Genin *et al.*⁵ further showed that functional gradients in mineral

and collagen help reduce mechanical mismatch between compliant and rigid tissues. The native TBI can therefore be regarded as a functionally graded biological composite: collagen orientation, proteoglycan content, mineral deposition, and local stiffness do not vary independently, but are co-organized along the same spatial axis to enable smooth load transfer.^{4–6}

Developmentally, the enthesis forms as a continuous attachment unit rather than by late-stage joining of mature tendon and bone. Scleraxis (SCX)/SRY-box transcription factor 9 (SOX9)-associated progenitor populations and coordinated ECM and mineralization programs provide an important developmental reference for adult repair, although this program is only partially reactivated after injury.^{8,54–60}

This structural understanding imposes fundamental constraints on regenerative materials for TBI. Repair strategies should not merely reproduce a four-zone morphology, but should reconstruct continuously varying matrix identity, mineralization, and mechanical environments across the tendon-side region, fibrocartilaginous transition zone, and bone-side region. For dECM bioprinting, the key is therefore not simply to combine collagen, mineral, or hydrogel components, but to use spatially programmable, source-associated ECM cues to recreate regionalized microenvironments and a continuous mechanical transition among tendon, fibrocartilage, and bone.^{10,11}

2.2. The healing dilemma after injury

Repair after TBI injury is commonly described in terms of inflammatory, proliferative, and remodeling phases. This linear staging, however, primarily describes the temporal sequence of the injury response and does not explain why the adult organism rarely regenerates a native enthesis. More fundamentally, the adult response to TBI injury is not a reactivation of the embryonic enthesis-development program, but a reparative response that prioritizes rapid defect closure, restoration of initial continuity, and limitation of local tissue exposure.^{1,2,61} Consequently, a repaired tendon may become morphologically reattached to the bone surface without recovering the tissue composition, fibrocartilage zonation, mineralization transition, or mechanical transmission of the native TBI.

Inflammation itself does not signify failed repair: an early innate immune response is required for tissue clearance and initiation of subsequent repair. The problem is persistence, not initiation, of inflammation. In human tendon disease, impaired inflammatory resolution is associated with chronic pathology,¹⁷ creating a local environment that favors fibrotic rather than organized

matrix remodeling. At the TBI, the relevant regenerative requirement is therefore timely inflammatory resolution rather than complete suppression of early inflammation.

A central obstacle during the proliferative phase is not simply insufficient cell numbers or collagen deposition, but the lack of regional identity in the newly recruited cells and matrix. Native tendon–bone function depends on a continuous tissue transition among the tendon-side region, unmineralized fibrocartilage, mineralized fibrocartilage, and the bone-side region. In contrast, repair tissue after injury is commonly dominated by rapid deposition of types I and III collagen. Such deposition may strengthen attachment of the tendon to the bone surface in the short term, but it neither spontaneously recreates parallel collagen alignment on the tendon side nor reliably induces a stable SOX9-positive fibrocartilage-like phenotype in the intermediate region.^{1,3} Structure–function studies by Moffat *et al.*³ showed that the mechanical function of the native ligament–bone interface depends on the continuous transition formed jointly by unmineralized and mineralized fibrocartilage, precisely the region that is most difficult to reconstruct after injury. Suboptimal proliferative repair may therefore reflect not simply an inadequate repair response but also a lack of spatial organization: cells and ECM enter the defect without being allocated according to the regional identities of the tendon-side region, transition zone, and bone-side region.

Abnormal mineralization further amplifies spatial mismatch after tendon–bone injury. Insufficient bone-side mineralization can leave the tendon attached through mechanically weak fibrous tissue, whereas premature or ectopic mineralization toward the tendon can create a local stiffness discontinuity. The regenerative target is therefore not maximal mineral formation but a spatially controlled transition that remains coupled to collagen organization and the fibrocartilaginous buffer.^{4,5}

During remodeling, collagen crosslinking and fiber reorganization can improve bulk mechanical properties without restoring enthesis function. At the TBI, recovery depends on progressive anchorage of tendon collagen through fibrocartilage and mineralized fibrocartilage, because collagen orientation strongly influences local stress concentration.⁶ If remodeling merely densifies scar collagen without rebuilding this transition, the interface remains mechanically vulnerable; clinically, structural insufficiency and re-tearing can persist despite anatomical repair (Figure 2D).¹⁸ Failed adult enthesis healing can therefore be viewed as spatiotemporal decoupling among immune resolution, regional identity, mineralization, and mechanical maturation.

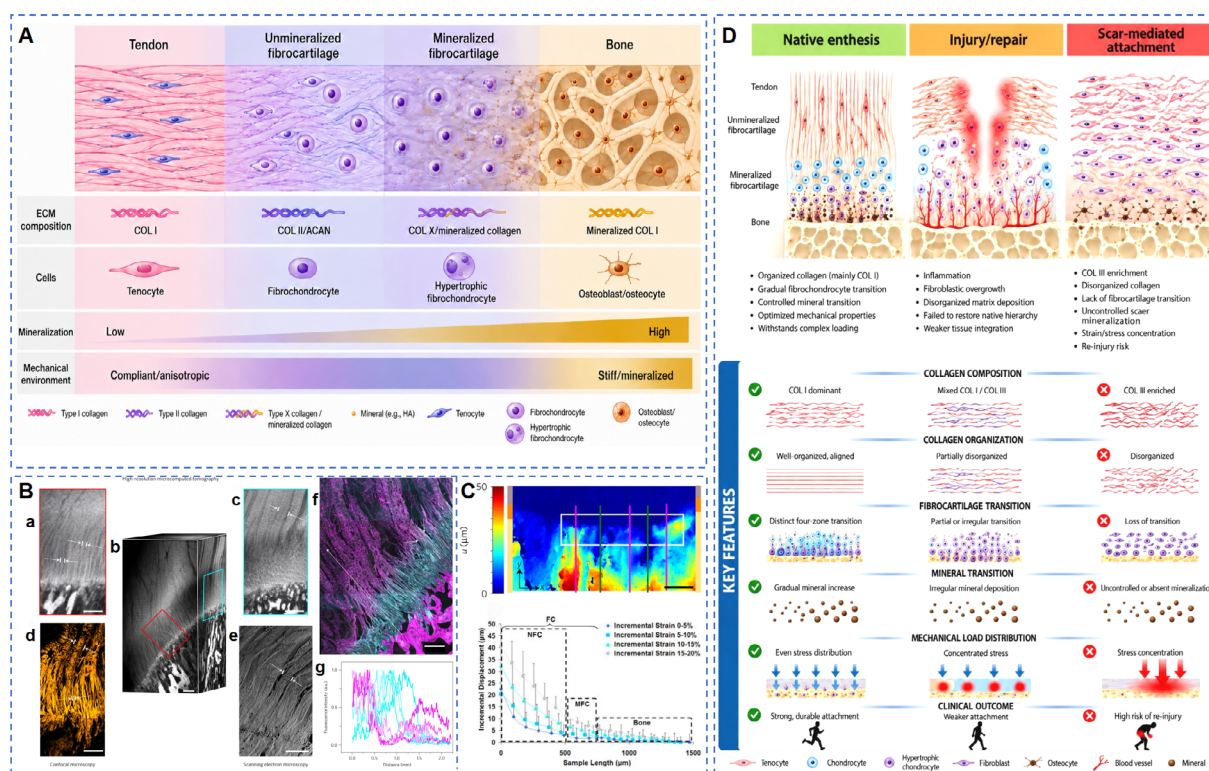


Figure 2. Multiscale structure, micromechanics and scar-mediated healing of the tendon–bone enthesis. (A) Idealized tissue organization of a fibrocartilaginous tendon–bone enthesis along the tendon–bone axis, comprising tendon, unmineralized FC, mineralized FC, and bone regions. Created in BioRender. Qing, H. (2026) <https://BioRender.com/8p0b9ej> (B) Multiscale imaging of the Achilles tendon–calcaneus enthesis shows that tendon collagen fibers reorganize, splay, and become finer near the bony insertion, accompanied by a transition from COL I-rich tendon matrix to COL II-rich interface matrix. Reprinted with permission from Rossetti *et al.*⁴ Copyright © 2017, Macmillan Publishers Limited, part of Springer Nature. (C) Representative displacement maps and incremental displacement curves during stepwise tensile loading show spatially heterogeneous deformation and progressive load transfer across unmineralized FC, mineralized FC, and bone. Reprinted with permission from Rossetti *et al.*⁴ Copyright © 2017, Macmillan Publishers Limited, part of Springer Nature. (D) Comparison of the native enthesis, adult injury and early repair, and the common outcome of scar-mediated healing. Native enthesis function depends on organized collagen architecture, appropriate fibrocartilaginous differentiation, controlled mineralization, and spatially distributed mechanical loading. Adult repair commonly features persistent inflammation, expansion of fibroblasts and myofibroblasts, disorganized deposition of types I and III collagen, incomplete restoration of the fibrocartilaginous transition, abnormal or absent mineralization, and persistent stress concentration, ultimately producing mechanically inferior tissue integration and increasing the risk of reinjury. Created in BioRender. Qing, H. (2026) <https://BioRender.com/kp80myr>.

Abbreviations: ACAN: Aggrecan; COL I: Type I collagen; COL II: Type II collagen; COL X: Type X collagen; ECM: Extracellular matrix; FC: Fibrocartilage; HA: Hydroxyapatite; MFC: Mineralized fibrocartilage; NFC: Nonmineralized fibrocartilage.

3. From decellularized extracellular matrix material information to spatially programmed tendon–bone constructs

3.1. Preservation of tissue-source information during decellularized extracellular matrix bioink processing

3.1.1. Source-dependent matrix identity after decellularization

For dECM to contribute meaningfully to tendon–bone regeneration, processing must preserve a distinguishable,

reproducible, and functionally meaningful matrix identity. Source-associated matrix identity should be supported by characterization of residual ECM composition and molecular profiles, including matrisome and proteomic analyses,^{62,63} whereas tissue-relevant potency should be supported by appropriate cellular assays rather than inferred from tissue source alone. Different tissue sources of dECM have plausible source-associated relevance to distinct regions of the TBI. TdECM is generally rich in type I collagen, decorin, biglycan, and other matrix components associated with collagen-fiber organization and maintenance of the tenogenic phenotype, making it

potentially relevant to recapitulating aspects of the tendon-side microenvironment. BdECM contains mineralization-related matrix proteins and osteogenic cues and may provide a bone-like matrix environment conducive to bone-side integration. CdECM and other fibrocartilage-relevant matrices contain type II collagen, aggrecan, and proteoglycans that may support cartilage-like matrix deposition in the transition zone; however, dedicated fibrocartilage-derived dECM has not been validated to the same extent in tendon–bone bioprinting. The value of source-specific dECM therefore depends on whether different tissue sources retain biologically meaningful matrix features relevant to the intended interface region.

Tissue specificity of dECM does not imply complete retention of biological function after processing. Decellularization reagents, digestion time, acidic solvents, sterilization methods, crosslinking strategies, and composite formulations may all alter protein composition, rheological behavior, and cell-regulatory capacity. Saldin *et al.*²⁹ emphasized that the biological function of dECM hydrogels is closely related to tissue source, preparation method, and gel architecture. In tendon–bone bioprinting, dECM is best viewed as a processed material whose value depends on retaining tissue-specific matrix cues. Whether these cues translate into effective regenerative signals depends on coordinated control throughout decellularization, solubilization, crosslinking, printing, and *in vivo* degradation. Because tissue source commonly covaries with formulation and construct properties, interpret source-associated responses at the integrated-construct level unless dECM-specific potency is isolated.

3.1.2. Preservation of matrix information during bioink formulation and printing

Three-dimensional bioprinting changes the role of dECM from a broadly distributed matrix material to a source-associated cue that can be positioned within predefined regions or gradients. Pati *et al.*³⁰ demonstrated that tissue-derived dECM can retain measurable source-associated biological activity after solubilization, gelation, and printing (Figure 3B, 3C, and 3F). The key implication is therefore not printability alone, but the possibility of translating retained tissue-source information into reproducible spatial patterns.

For cell-laden systems, suitability as a dECM bioink should be defined jointly by retained tissue-source potency and reproducible manufacturing performance, including rheology and gelation, deposition fidelity, and cell compatibility. The distinction between a cell-containing bioink and a cell-free biomaterial ink should follow established biofabrication terminology.^{31,64} TdECM

studies further show that digestion and solvent conditions alter printability, gelation, and cellular responses,^{37–39} demonstrating that dECM bioinks are processing-dependent material–cell systems rather than simply “dECM plus cells.” Processing variables should therefore be treated as determinants of both manufacturing fidelity and biological potency.

For TBI regeneration, dECM bioinks can be combined with region-specific cells, mineral phases, or soluble cues to create tendon-, transition-, and bone-directed microenvironments (Figure 3A, 3D, and 3E). These additions aim not to maximize construct complexity, but to convert tissue-derived matrix cues into localized tissue-derived matrix cues within spatially defined, verifiable regions while maintaining adequate printability and mechanical support. Because general dECM rheology, crosslinking, reinforcement, and printability have already been reviewed extensively,^{40–44} the present review considers these manufacturing variables only insofar as they affect retention and spatial translation of tissue-source information. Accordingly, the relevance of dECM bioinks to tendon–bone regeneration depends on preserving such information during processing and positioning it reproducibly across the interface.

3.2. Spatial translation of decellularized extracellular matrix information into tendon–bone interface architecture

3.2.1. Single-source decellularized extracellular matrix bioinks: Local tissue-biased microenvironments

Single-source dECM bioinks are most useful for testing whether source-associated ECM cues remain biologically informative after processing and printing, rather than for reconstructing the complete multiphasic interface. TdECM has been shown to support printable, tendon-biased cellular environments,³⁷ and tissue-specific dECM bioinks can support tendon- or ligament-like tissue formation in aligned constructs.⁴⁶ These studies support source-associated biological activity and local tendon-side applications, but do not establish fibrocartilage or bone-side regeneration. Single-source systems should therefore be interpreted primarily as potency and local-microenvironment validation platforms.

The limitations of a single-tissue-source dECM bioink are equally clear. TdECM may partially recapitulate source-associated cues of the tendon-side microenvironment, but cannot simultaneously provide the cartilage-like matrix cues required in the fibrocartilaginous transition zone and the mineralization cues required on the bone side. The true regenerative target at the TBI is not a single local tissue, but

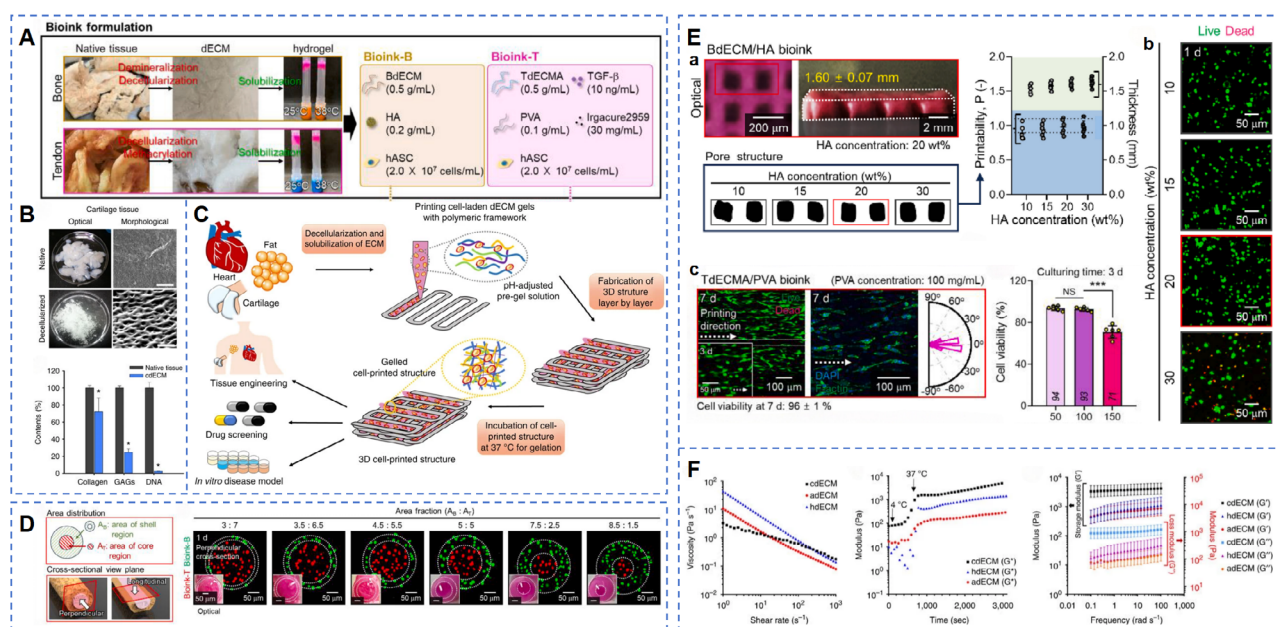


Figure 3. Manufacturing workflow, structure–process relationships and critical quality attributes of tissue-derived dECM bioinks for tendon–bone interface biofabrication. (A) Representative preparation and formulation of bone- and tendon-directed dECM bioinks. BdECM was combined with HA and hASCs, whereas TdECMA was combined with PVA, TGF- β , a photoinitiator, and hASCs. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (B) Representative morphological and biochemical quality-control assessment of cdECM, showing native and decellularized tissue morphology and changes in collagen, glycosaminoglycan, and DNA contents after decellularization. Reprinted with permission from Pati *et al.*³⁰ Copyright © 2014, Nature Publishing Group, a division of Macmillan Publishers Limited. (C) Typical fabrication workflow for a cell-laden, pepsin-solubilized dECM bioink, including tissue harvesting, decellularization, acidic solubilization, adjustment to physiological pH, cell encapsulation, layer-by-layer extrusion printing and thermally induced gelation. Reprinted with permission from Pati *et al.*³⁰ Copyright © 2014, Nature Publishing Group, a division of Macmillan Publishers Limited. (D) Core–shell construct printed using predefined cross-sectional ratios of bone-directed bioink-B and tendon-directed bioink-T. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (E) Representative manufacturing and cellular assessments of bone- and tendon-directed formulations. Bone-side assessments include pore fidelity, printed-filament thickness, and cell survival at different HA concentrations; tendon-side assessments include cell survival at different poly(vinyl alcohol) concentrations and alignment of cellular F-actin along the printing direction. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (F) Representative rheological curves of cartilage-, adipose- and cardiac-derived dECM precursor solutions, showing shear-thinning behavior, temperature-dependent gelation and predominantly solid-like viscoelastic behavior after gelation. Reprinted with permission from Pati *et al.*³⁰ Copyright © 2014, Nature Publishing Group, a division of Macmillan Publishers Limited. Abbreviations: adECM: Adipose-derived decellularized extracellular matrix; BdECM: Bone-derived decellularized extracellular matrix; cdECM: Cartilage-derived decellularized extracellular matrix; dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; GAGs: Glycosaminoglycans; HA: Hydroxyapatite; hASCs: Human adipose-derived stem cells; hdECM: Heart-derived decellularized extracellular matrix; PVA: Poly(vinyl alcohol); TdECMA: Methacrylated tendon-derived decellularized extracellular matrix; TGF- β : Transforming growth factor beta.

the continuously changing matrix identity, mineralization, and mechanical environment across the tendon-side region, fibrocartilage, and bone-side region. A single dECM bioink therefore cannot reproduce the complete gradient architecture of the TBI. Its principal value is to validate retention of source-associated biological activity and local microenvironmental effects after formulation and printing, thereby providing the basis for subsequent multisource patterning.

3.2.2. Multisource decellularized extracellular matrix patterning: Regionalization across the tendon–fibrocartilage–bone axis

Compared with a single-dECM system, regionalized printing with multiple dECM sources is more consistent with the structural logic of TBI regeneration. A useful working design principle is source-to-zone matching. TdECM is assigned to the tendon-side region to provide

tenogenic matrix cues, whereas CdECM or fibrocartilage-derived dECM may provide more directly relevant matrix information for the transition zone. BdECM is assigned to the bone-side region, optionally together with mineralized components, to support osseous integration. The key feature of this strategy is not simply stacking different materials, but using source-dependent matrix differences to confer an appropriate tissue identity on each region within the same construct. Chae *et al.*⁴⁵ used tissue-derived ECM bioinks to 3D-print a TBI construct for chronic rotator cuff repair, demonstrating that dECM from different tissues can create regionalized interface microenvironments. In a later study of a graded multitissue interface, Chae *et al.*⁴⁷ generated a gradient interface for rotator cuff regeneration by 3D cell printing (Figure 4A), further showing that multisource dECM or composite bioinks can spatially emulate the TBI's transitional architecture. Importantly, the intermediate region of the triphasic construct consisted of a mixed TdECM/BdECM bioink rather than an independently validated fibrocartilage-derived dECM. The study provides direct evidence for regionalized material patterning and enthesis-like tissue formation, but not for fibrocartilage-specific matrix identity or genuine fibrocartilage regeneration. Tissue source should consequently be interpreted as a regional design variable whose tissue-specific potency still needs to be disentangled from concurrent differences in material composition, cellular context, geometry, and other construct features.

Regionalized printing with multiple dECM sources should not be described as a static assembly in which one material is placed on the tendon side, another on the bone side, and a third between them. This strategy must address three questions: whether the different dECM sources genuinely retain distinguishable tissue-specific cues; whether these cues remain biologically active after digestion, crosslinking, and printing; and whether adjacent regions are separated by an abrupt interface or connected by a continuous transition generated through varying mixing ratios, mineralization, and cell density. Addressing these questions is necessary for multisource dECM printing to progress from material combination to spatial programming of interface regeneration.

3.2.3. Composite reinforcement: Coupling spatial fidelity with mechanical support

Composite reinforcement is often necessary because dECM hydrogels alone may not provide sufficient printability, shape retention, sutureability, or early mechanical support for tendon–bone implantation. Natural or synthetic polymers, mineral phases, and additional crosslinking strategies can therefore improve fabrication stability and mechanical support.^{42–44} However, these additions

simultaneously alter rheology, stiffness, degradation, pore architecture, and cellular signaling, making it difficult to attribute improved regenerative outcomes specifically to the tissue source of dECM. Unless source-specific dECM potency is isolated with appropriately matched controls, composite systems are more appropriately interpreted as integrated therapeutic constructs. In the tendon–bone setting, the design objective is consequently not to maximize the number of incorporated materials, but to retain regionally relevant dECM information while providing only the manufacturing and mechanical support required for a spatially stable interface construct.

A representative interface-specific example is the graded scaffold reported by Zhang *et al.*⁴⁸, in which TdECM, CdECM, and BdECM coatings were integrated with structural, compositional, and mechanical gradients (Figure 4C). This design illustrates the rationale for combining ECM-derived biological information with a mechanically supportive framework. Importantly, however, scaffold architecture, mechanical properties, regional composition, and dECM source were varied simultaneously, limiting attribution of the observed regenerative effects specifically to dECM. Thus, this study supports the value of an integrated gradient construct, but does not independently establish tissue-specific dECM potency.

3.2.4. Coordinated patterning of cells, bioactive cues, and printing modalities

Coordinated patterning of dECM bioinks, cells, bioactive factors, and printing paths can transform a material combination into a spatially controlled interface construct. Cells are not simply passive components within the bioink; they help establish regional identity and remodel the local matrix. Mesenchymal stem cells, tendon stem/progenitor cells, tenocytes, and lineage-directed progenitors can contribute to regional matrix formation, whereas spatially confined biochemical cues can further bias tenogenic, fibrocartilage-like, or osteogenic responses. In the current review, these cellular and biochemical components are considered primarily in terms of how they interact with dECM and are spatially organized within the printed construct, rather than as separate cell- or drug-delivery strategies. Supportive non-dECM studies have demonstrated that compartmentalized cell-laden constructs can generate regionally differentiated interface tissues. Cao *et al.*⁵¹ used stratified cell-laden hydrogels to reproduce tendon-, fibrocartilage-, and bone-directed compartments, whereas Bai *et al.*⁵² incorporated layer-specific growth-factor-loaded microspheres into a bioprinted living construct (Figure 4B), providing a clear example of how biochemical cues can be spatially confined

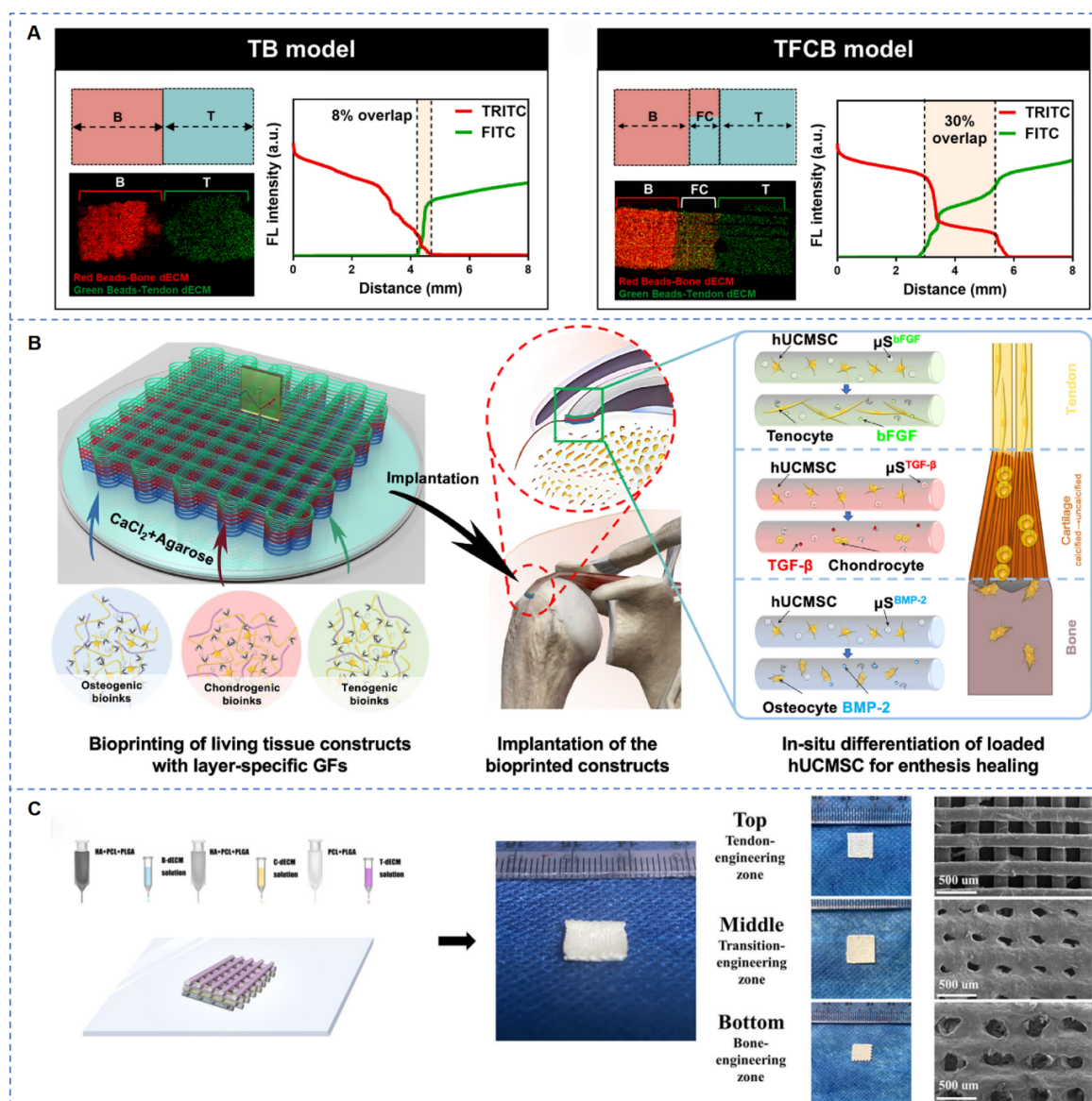


Figure 4. Spatial and spatiotemporal programming strategies for tendon–bone interface engineering constructs. (A) Representative comparison of a biphasic TB model and a triphasic TFCB model fabricated by 3D cell printing. Red fluorescent microspheres label BdECM, and green fluorescent microspheres label TdECM. In the TB model, the bone- and tendon-side regions are placed in direct apposition. The TFCB model contains an intermediate region formed from a mixed TdECM/BdECM bioink. Because the intermediate compartment consisted of mixed tendon- and bone-derived dECM, it should be interpreted as a fibrocartilage-directed design rather than an independently validated fibrocartilage-specific matrix. The wider overlap of the two fluorescence-intensity profiles in the TFCB model indicates greater interfacial mixing and spatial overlap of the initially deposited materials. Reprinted with permission from Chae *et al.*⁴⁷ Copyright © 2022, The Authors. (B) Schematic of a construct containing hUCMSCs and layer-specific GF-loaded microspheres. Microspheres in different regions contain bFGF, TGF- β , or BMP-2, with the aim of sustaining localized presentation of biochemical cues and inducing tendon-, cartilage-, and bone-directed differentiation after implantation at the rotator cuff enthesis. Reprinted with permission from Bai *et al.*⁵² Copyright © 2022, Acta Materialia Inc. (C) Fabrication process and regional morphology of a structure–composition–mechanics gradient scaffold modified with tissue-derived dECM coatings. Tendon-, cartilage-, and bone-derived dECM were assigned to the tendon-engineering, transition-engineering, and bone-engineering regions, respectively. Reprinted with permission from Zhang *et al.*⁴⁸ Copyright © 2023, American Chemical Society. Abbreviations: B: Bone; BdECM: Bone-derived decellularized extracellular matrix; bFGF: Basic fibroblast growth factor; BMP-2: Bone morphogenetic protein 2; CdECM: Cartilage-derived decellularized extracellular matrix; dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; FC: Fibrocartilage; FITC: Fluorescein isothiocyanate; FL: Fluorescence; GFs: Growth factors; HA: Hydroxyapatite; hUCMSCs: Human umbilical cord-derived mesenchymal stem cells; PCL: Polycaprolactone; PLGA: Poly(lactic-co-glycolic acid); T: Tendon; TdECM: Tendon-derived decellularized extracellular matrix; TB: Tendon–bone; TFCB: Tendon–fibrocartilage–bone; TGF- β : Transforming growth factor beta.

Table 2. Comparative evidence matrix for dECM bioprinting and related tendon–bone interface strategies: Claim level, confounders, missing decisive evidence, and translational limitations

Study	Construct/model & follow-up	Key comparator(s)/confounder(s)	Key evidence supporting claim	Claim level/highest claim supported	Missing decisive evidence/translational limitation
Direct evidence—dECM-integrated tendon–bone interface studies					
Chae <i>et al.</i> ⁴⁵	Spatially graded hBMMSC-laden TdECM/mixed dECM/BdECM zones integrated with a PU/PCL support patch; <i>in vitro</i> , 7–14 d; chronic rat rotator cuff repair, 9 wk.	Collagen or non-tissue-specific bioink, acellular printed patch, and surgical-repair controls. dECM source, cells, and construct architecture changed concurrently; residual synthetic support could contribute mechanically.	Zonal tenogenic, chondrogenic, and osteogenic responses; increased fibrocartilage-like tissue; improved histology and bulk mechanical outcomes.	Level 3—Regional tissue formation / enthesis-like tissue formation.	Missing evidence: source-specific dECM attribution, local-strain mapping, cyclic durability, and degradation-linked neotissue load takeover. Translational limitation: small-animal model, relatively short follow-up, and persistent synthetic structural support.
	Biphasic tendon–bone construct versus a triphasic tendon–fibrocartilage–bone construct containing a mixed TdECM/BdECM intermediate zone and hBMMSCs; <i>in vitro</i> , 14 d; chronic rat rotator cuff repair, 4, 8, and 12 wk.	Biphasic versus triphasic design and cell-free printed control. The intermediate zone simultaneously altered material composition and geometry and consisted of mixed TdECM and BdECM rather than fibrocartilage-specific dECM.	Wider interfacial overlap; regional marker and ECM distributions; improved longitudinal imaging, histology; gait, maximum load, and stiffness.	Level 3—Regional tissue formation / enthesis-like tissue formation; partial Level 4-relevant integration support.	Missing decisive evidence: the intermediate zone consisted of mixed TdECM/BdECM rather than an independently validated fibrocartilage-specific matrix. Fibrocartilage-specific matrix identity, transitional cell states, spatially controlled mineralization, degradation-linked local-strain mapping, cyclic durability, and neotissue-mediated load takeover were not directly established. Translational limitation: rat model, relatively short follow-up, and persistent contribution of the printed support to mechanics.
Zhang <i>et al.</i> ⁴⁸	3D-printed scaffold with graded structure, composition, and mechanical properties with zone-specific tendon-, cartilage-, and bone-related dECM coatings; <i>in vitro</i> lineage assays; rabbit rotator cuff repair, 8 and 16 wk.	Uncoated graded scaffold and non-graded/control groups. dECM coating effects were confounded by concurrent structural, compositional, and mechanical gradients; tissue-source potency was not isolated with source-swapped or physicochemically matched controls.	Zone-dependent tenogenic, chondrogenic, and osteogenic responses; graded tissue formation; improved histology and bulk biomechanics at 16 wk.	Level 3—Regional tissue formation / enthesis-like tissue formation.	Missing evidence: isolated dECM-specific potency, local-strain continuity, cyclic durability, and degradation-linked load takeover. Translational limitation: multicomponent scaffold complexity, dECM batch variability, and manufacturing reproducibility.
	Gradient core–shell construct combining BdECM/HAp and TdECMA/PVA/TGF- β bioinks with hASCs to generate bone-, interface-, and tendon-directed regions; <i>in vitro</i> , 28 d; rabbit rotator cuff repair, 8 wk.	Non-gradient bioprinted construct, repair-only, tear, and intact controls. Bioink composition, geometry, biochemical cues, and cellular context varied concurrently.	Regional OPN, ACAN, and TNMD signals; improved ECM organization and angiogenesis-related markers; increased maximum load and stiffness versus repair controls.	Level 3—Regional tissue formation, with partial Level 4-relevant integration support.	Missing evidence: immune/perfusion-based host-integration analysis, later time points, local-strain mapping, cyclic durability, and scaffold-degradation-linked load takeover. Translational limitation: complex, cell-laden, multicomponent manufacture and short <i>in vivo</i> follow-up.

(Cont'd...)

Table 2. (Continued)

Study	Construct/model & follow-up	Key comparator(s)/confounder(s)	Key evidence supporting claim	Claim level/highest claim supported	Missing decisive evidence/translational limitation
Supportive evidence—dECM bioinks and related interface models					
Pati <i>et al.</i> ³⁰	Adipose-, cartilage-, and cardiac-derived dECM bioinks containing tissue-relevant cells, deposited by extrusion and stabilized by thermal gelation; <i>in vitro</i> tissue analogs, 14 d.	Collagen controls and tissue-matched comparisons. The study was not designed for the tendon–bone interface and did not test an orthotopic load-bearing construct.	Decellularization quality, shear-thinning and temperature-dependent gelation, high cell viability, tissue-related gene expression, and ECM deposition.	Levels 1–2—Material identity and manufacturing fidelity/concept validation.	Missing evidence: tendon–bone spatial organization, regional tissue formation, host integration, and functional load transfer. Translational limitation: <i>in vitro</i> , non-TBI model without orthotopic or mechanically relevant validation.
Zhao <i>et al.</i> ³⁹	Tendon dECM bioinks prepared using three acidic solubilization systems to compare formulation-dependent gelation, rheology, and biological performance; <i>in vitro</i> BMSC printing and culture, 7 and 14 d.	Acid formulations at matched dECM concentration. Acid-dependent chemical/structural changes were not fully separable from rheological effects.	Acid-dependent differences in gelation, printability, cell viability, proliferation, and tenogenic responses.	Levels 1–2—Material identity/manufacturing fidelity / formulation validation.	Missing evidence: spatial interface manufacture, orthotopic host integration, and functional endpoints. Translational limitation: formulation-specific <i>in vitro</i> study with no long-term manufacturing or <i>in vivo</i> validation.
Chae <i>et al.</i> ⁴⁶	Tendon- and ligament-specific dECM bioinks used for support-bath printing of aligned, longitudinally channeled cell-laden constructs; <i>in vitro</i> , 7, 28, and 42 d; ectopic nude-mouse validation.	Collagen, tendon-derived dECM, and ligament-derived dECM constructs. The ectopic model did not reproduce tendon–bone integration or physiological loading.	Sustained cell viability; anisotropic organization; increased SCX/TNMD expression; improved construct mechanics and ectopic tendon-like tissue formation.	Level 2—Manufacturing fidelity, with Level 3—relevant tendon-like tissue support.	Missing evidence: fibrocartilage transition, bone integration, orthotopic host response, and interface-level load transfer. Translational limitation: ectopic validation and absence of physiological tendon–bone loading.
Kim and Kim ⁶⁵	Bioprinted myotendinous-junction model integrating muscle- and tendon-derived dECM with biochemical and biophysical cues; 2D culture, 14 d; 3D culture, 28 d.	Source-matched and mismatched ECM contexts. The model addressed a muscle–tendon rather than a TBI.	Source-related myogenic and tenogenic responses, regional organization, and interface-associated phenotypes.	Levels 1–2—Tissue-source matching and spatial concept validation.	Missing evidence: fibrocartilage/mineralized transition, bone-side integration, host response, and load takeover. Translational limitation: <i>in vitro</i> non-TBI interface model.

(Cont'd...)

Table 2. (Continued)

Study	Construct/model & follow-up	Key comparator(s)/confounder(s)	Key evidence supporting claim	Claim level/highest claim supported	Missing decisive evidence/translational limitation
Cao <i>et al.</i> ⁵¹	Supportive evidence—tendon–bone design or mechanism studies without dECM				
	Multiphasic PCL/PCL–TCP scaffold with stratified GelMA-encapsulated fibroblasts, BMSCs, and osteoblasts to emulate tendon, fibrocartilage, and bone regions; <i>in vitro</i> , 7 and 21 d; mouse subcutaneous implantation, 1–8 wk.	Phase-specific and acellular controls. The ectopic model lacked native tendon–bone geometry and physiological loading; no dECM component was present.	Intermediate-zone collagen II/chondrogenic features and spatially graded matrix/gene expression maintained <i>in vivo</i> .	Level 3—relevant design support—regional tissue formation; not direct evidence for dECM-bioprinted TBI regeneration.	Missing evidence: orthotopic integration, dECM attribution, local load transfer, and cyclic function. Translational limitation: ectopic implantation without native tendon–bone geometry or physiological loading.
Bai <i>et al.</i> ⁵²	Bioprinted living construct containing layer-specific bFGF-, TGF- β -, and BMP-2-loaded microspheres for tendon-, fibrocartilage-, and bone-directed signaling; <i>in vitro</i> regional differentiation; rabbit rotator cuff repair, 12 wk.	Scaffold and growth-factor control groups. Growth-factor identity, spatial pattern, and construct architecture changed together; no dECM component was present.	Aligned collagen, gradient fibrocartilage formation, improved histology and biomechanics, and ultimate failure load approaching native tissue.	Level 3—relevant design support—regional tissue formation; not direct evidence for dECM-bioprinted TBI regeneration.	Missing evidence: dECM-specific attribution, degradation-linked local-strain analysis, cyclic durability, and neotissue load takeover. Translational limitation: complex growth-factor delivery and unresolved long-term reproducibility/safety.
Jiang <i>et al.</i> ⁵³	Multilayer scaffold containing spatially pre-differentiated autologous ADMSCs and a melt-electrowritten mechanical support structure; <i>in vitro</i> differentiation; rabbit rotator cuff repair, 12 wk.	Acellular, undifferentiated-cell, and spatially differentiated ADMSC constructs. Architecture and cell state were assessed separately; no dECM component was present.	Improved histological score, collagen organization, and MRI T2 values; the scaffold improved mechanics, whereas added ADMSCs provided limited additional benefit.	Level 3—relevant design support—architecture-supported regional tissue formation; not direct evidence for dECM-bioprinted TBI regeneration.	Missing evidence: dECM attribution, degradation-linked local-strain mapping, cyclic durability, and load takeover. Translational limitation: complex cell-preparation/manufacturing workflow and persistent mechanical-support contribution.
Liu <i>et al.</i> ⁶⁶	Engineered tendon–fibrocartilage–bone composite with or without a BMSC sheet for zonal augmentation of rotator cuff repair; non-weight-bearing canine model, 6 wk.	Repair alone, composite alone, and composite plus BMSC sheet. The short, non-weight-bearing model reduced physiological loading demands; the construct was non-bioprinted and did not use dECM.	Improved histological score, fibrocartilage formation, collagen organization, and bulk biomechanics.	Level 3—relevant support—entheses-like tissue formation in a non-bioprinted model; not direct evidence for dECM-bioprinted TBI regeneration.	Missing evidence: long-term degradation, local-strain continuity, cyclic durability, and load takeover. Translational limitation: short-term non-weight-bearing model with limited physiological mechanical challenge.

Note: Evidence provenance (direct, supportive, and adjacent), as defined in the Introduction, is indicated by section grouping rather than as a separate column. Framework level indicates the strongest claim supported by the reported data and does not imply fulfillment of all criteria for that level. Abbreviations: ACAN: Aggrecan; ADMSCs: Adipose-derived mesenchymal stem cells; BDECm: Bone-derived decellularized extracellular matrix; bFGF: Basic fibroblast growth factor; BMP-2: Bone morphogenetic protein 2; BMSC: Bone marrow–derived mesenchymal stem cell; dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; GelMA: Gelatin methacryloyl; hAEC: Human adipose-derived stem cell; hBMSC: Human bone marrow–derived mesenchymal stem cell; MRI: Magnetic resonance imaging; OPN: Osteopontin; PCL: Polycaprolactone; PU: Polyurethane; PVA: Poly(vinyl alcohol); SCX: Scleraxis; T2: Transverse relaxation time; TBI: Tendon–bone interface; TCP: Tricalcium phosphate; TdECM: Tendon-derived decellularized extracellular matrix; TdECM: Tendon-derived decellularized extracellular matrix; TGF- β : Transforming growth factor beta; TNMD: Tenomodulin.

along the tendon–fibrocartilage–bone axis. Jiang *et al.*⁵³ similarly showed that spatial organization of differently conditioned cell populations can influence regional interface formation, and Du *et al.*⁵⁰ demonstrated that multicellular biomimetic printing can coordinate tendon- and bone-directed cellular environments within a single interface construct.

The printing modality determines how reproducibly these biological components can be positioned while maintaining construct integrity. Multinozzle and coaxial printing enable spatial separation or core–shell organization of different formulations, whereas photocrosslinking and hybrid hydrogel–polymer frameworks can improve geometric fidelity or mechanical support. These modalities should therefore be interpreted as manufacturing tools for realizing a biological design rather than as independent evidence of regeneration. Among dECM-integrated studies, the gradient core–shell construct reported by Kim *et al.*⁴⁹ provides a particularly relevant example: bone- and tendon-directed dECM formulations, cells, biochemical cues, and geometric patterning were integrated within a single regionalized construct, resulting in region-specific matrix responses. Thus, the relevant endpoint is not construct complexity itself, but whether the intended spatial pattern is manufactured reproducibly, persists during remodeling, and is translated into regionally appropriate neotissue.

Collectively, current design strategies progress from validating local tissue-source effects to spatially organizing multiple matrix, cellular, and mechanical cues across the tendon–bone axis. Their importance lies not in increasing construct complexity, but in determining whether an intended material pattern can be translated into regionalized biological responses. Representative strategies, outcomes, and evidential limitations are compared in Table 2.

3.3. Biological maturation of regionalized decellularized extracellular matrix constructs: Evidence and mechanistic interpretation

3.3.1. Regional cell-state specification and matrix identity

Interface regeneration requires spatially coordinated cell states across tendon, fibrocartilage, and bone rather than maximal induction of any single lineage. Developmental evidence indicates that the fibrocartilaginous attachment zone contains overlapping lineage programs, whereas adult repair often lacks the spatial matrix context needed to reproduce this sequence.^{1,3,8} The mechanistic question for dECM bioprinting is therefore whether spatially patterned tissue-derived cues can bias where tenogenic, cartilage-like, and osteogenic states emerge.

Current evidence is more consistent with dECM modulating lineage responsiveness than with dECM source directly determining cell fate. TdECM retains matrix components associated with tenogenic microenvironments and can support SCX- and tenomodulin (TNMD)-associated responses and type I collagen matrix formation.^{37,40,41} Adjacent lineage studies further support SCX–TNMD-associated tenogenic regulation, although these responses remain dependent on cellular and microenvironmental context.^{67,68} CdECM or other fibrocartilage-relevant matrices provide plausible sources of cartilage-associated ECM cues; however, their source-specific potency in dECM-bioprinted tendon–bone constructs remains incompletely established.⁶⁹ BdECM, mineralized components, and osteogenic cues can support runt-related transcription factor 2/Sp7 transcription factor-associated osteogenic responses.^{70,71} These source-associated effects do not map one-to-one onto terminal lineages; instead, they modify cellular responsiveness to differentiation signals. Regional identity therefore depends on where these programs emerge and how they overlap; a homogeneous increase in one lineage-associated program would not by itself recreate the native interface. The intended outcome is a controlled progression from predominantly tenogenic states through an SCX/SOX9-associated transition state toward osteogenic/mineralized states, with continuity and spatial overlap being more important than a sharply defined three- or four-layer morphology.^{4,5,8}

Among the biological responses considered here, restoration of regional cell and matrix identity currently has the strongest direct support from dECM-integrated tendon–bone bioprinting studies. In two related studies, Chae *et al.*⁴⁵ and Chae *et al.*⁴⁷ demonstrated regionally distinct microenvironments and tissue responses in dECM-integrated constructs, with representative region-specific cellfirst paragraphmatrix responses from Chae *et al.*⁴⁷ shown in Figure 5A; a gradient core–shell study⁴⁹ (Figure 5D) and a multicellular printing study⁵⁰ further showed that coordinated cellular, material, and geometric patterning can promote multiregional organization. These observations are consistent with the hypothesis that tissue-derived matrix cues, printed architecture, and intercellular interactions jointly influence where lineage-associated states emerge. A plausible working model is that initial matrix and geometric cues bias regional cell states, after which newly deposited ECM and the evolving mechanical environment help stabilize or redirect those states. This interpretation remains inferential, but it explains why short-term increases in lineage markers should not be equated with stable regional identity. Because dECM source, cellular composition, printing geometry, and bioactive factors

are often altered simultaneously, the observed regional phenotypes should generally be attributed to the integrated construct unless the contribution of dECM itself has been isolated experimentally. The mechanistic endpoint is therefore not transient lineage-marker elevation, but stable coupling among regional cell identity, neotissue ECM, and local mechanics.

3.3.2. Immune resolution and construct remodeling

Direct evidence that dECM bioprinting causally regulates the immune trajectory of tendon–bone regeneration remains limited. Supportive and adjacent evidence indicates that regenerative immune regulation depends on the magnitude, timing of resolution, and regional distribution of inflammation rather than on a simple M1-to-M2 transition. An early innate response is required for tissue clearance and recruitment of reparative cells, and experimental macrophage depletion can impair subsequent tendon repair.^{72–75} Conversely, persistent inflammatory signaling or failed resolution can sustain fibroblast and myofibroblast activation, disorganized collagen deposition, and fibrosis.^{72,76,77} Macrophage states are continuous and highly microenvironment-dependent rather than two mutually exclusive subsets.⁷⁸ The appropriate objective is therefore timely transition from inflammatory clearance toward resolution, matrix reorganization, and tissue maturation rather than simple suppression of M1-associated signals or maximization of an M2-like phenotype.

Supportive evidence from studies of ECM-scaffold immunology indicates that dECM is not immunologically inert. Host responses reflect several coupled material attributes, including residual cellular or antigenic burden, retained matrix composition, crosslinking and pore architecture, and degradation kinetics and products.^{27,29,79–83} Cellular remnants or excessive crosslinking can favor persistent inflammation or impede matrix renewal, whereas selected ECM degradation products can support constructive macrophage and progenitor-cell responses.^{19,20,79,80} Adaptive immune components also contribute to ECM-scaffold remodeling, indicating that dECM immunomodulation cannot be reduced to macrophage polarization alone.⁸⁴ For xenogeneic dECM, residual antigens such as α -Gal add source-dependent risk.⁸⁵ These broader scaffold studies support processing quality and degradation as determinants of host response, but do not by themselves establish a dECM-specific immune mechanism in bioprinted tendon–bone constructs.

The immune effects of dECM also vary with tissue source and processing because matrix composition, residual antigens, crosslinking, and degradation kinetics

differ among formulations.^{19,22,79,80,84,85} At the TBI, the preferred trajectory may also differ regionally: tendon-side repair requires inflammatory resolution without persistent fibrosis, the unmineralized transition should avoid excessive vascular and inflammatory infiltration and premature mineralization, and bone-side maturation requires compatible inflammatory resolution and angiogenic–osteogenic coupling. A uniformly M2-skewed macrophage state throughout the construct should therefore not be assumed to be optimal. Regional control of material composition, pore architecture, crosslinking, and degradation may provide a route toward spatially distinct immune environments, although this remains to be tested directly. Direct dECM–TBI studies have mainly demonstrated regional tissue formation, histological integration, angiogenesis-associated responses, and improved bulk mechanics, with relatively limited temporal or spatial characterization of immune-cell states.^{45,47,49} Supportive evidence from the non-dECM tendon–bone model shown in Figure 5B indicates that macrophage–stromal paracrine interactions can influence tenogenic and osteogenic cell behavior,²¹ but this should not be interpreted as direct evidence of a dECM-specific immune mechanism. Direct mechanistic attribution will require region-resolved immune profiling and perturbation or rescue experiments.

3.3.3. Matrix continuity and controlled mineralization

Regional expression of tenogenic, cartilage-like, and osteogenic markers is only the starting point of interface regeneration. Direct dECM–TBI studies support regional ECM deposition, fibrocartilage-like tissue formation, and spatially patterned mineralization, but they do not yet establish the specific mechanosensory pathways that mediate these outcomes.^{45,47–49} Whether the enthesis can ultimately withstand repeated tensile, compressive, and shear loading depends on whether these cellular states are translated into neotissue ECM with continuous spatial organization: collagen bundles aligned with the principal loading direction on the tendon side; a fibrocartilaginous matrix in the intermediate region capable of dissipating transverse compressive and shear strains; and a spatially confined, progressively maturing mineralized transition on the bone side. The low stress concentration in the native enthesis does not arise from an isolated increase in collagen, proteoglycans, or mineral, but from coordinated variation in collagen orientation, mineral content, and local mechanical properties along the same spatial axis.^{3–6,86,87}

On the tendon side, dECM provides a tissue-derived environment favorable for cell adhesion, tenogenic matrix synthesis, and collagen assembly rather than pre-

existing load-bearing collagen bundles. Preparation of tendon tissue as a printable bioink requires comminution, enzymatic digestion, and re-gelation and therefore cannot fully preserve the long-range continuity or hierarchical alignment of native collagen. The degree of digestion also substantially affects ink viscosity, gelation, and printing fidelity.³⁸ Collagen organization should consequently be regarded as a reassembly process driven jointly by matrix cues, geometric constraints, and cellular traction. Printed geometry and cellular traction can provide early directional organization for cell alignment and collagen deposition, but this local organization must subsequently mature across filament and material boundaries. Ker *et al.*⁸⁸ showed that combining directional architecture with spatial biochemical cues can simultaneously influence differentiation and alignment, providing adjacent support for this design principle. Studies of tissue-specific dECM printing similarly suggest that coupling bioink source with printing architecture is more informative than increasing dECM content alone.^{45–47} Nevertheless, load-bearing collagen architecture requires fibril maturation, crosslinking, and continuous integration across printed regions. TdECM is therefore better understood as supporting collagen reassembly than as directly reproducing mature tendon architecture.^{3–6}

Formation of the transition zone depends on gradual overlap between tendon-like collagen architecture and a hydrated cartilage-like matrix within a confined space. Enthesis fibrocartilage is not ordinary cartilage placed between tendon and bone, but an interface tissue formed where tenogenic and chondrogenic programs partially overlap and tensile and compressive loading converge. Developmental studies show that SCX/SOX9-associated progenitors contribute to the formation of cartilage-to-tendon or cartilage-to-ligament attachment regions, indicating continuous lineage and spatial origins for this zone.⁸ Its regenerative target is therefore not to maximize the total amount of cartilage-related matrix, but to create within a limited region a buffer that remains continuously connected to both tendon-side collagen and bone-side mineralized tissue. Collagen bundles should continue to enter the tendon-proximal side; type II collagen, aggrecan, and hydrated proteoglycans should progressively accumulate centrally; and restricted hypertrophic and mineralized phenotypes should emerge only toward bone. The transition-zone matrix must remain confined between tendon and bone, because excessive expansion of a cartilage-like region may interrupt collagen continuity and bone-side anchorage. Multisource dECM and gradient-printing studies support the view that spatial coupling favors regionalized cartilage-like matrix formation,^{45,47,49,51,52,66} although the specific contribution of

dECM to fibrocartilage identity and maturation remains incompletely resolved.

Adjacent mechanobiology studies indicate that mechanical loading can influence cell recruitment and ECM maturation during tendon–bone healing, but these pathways have not been established as mediators of dECM-bioprinted constructs. Appropriate mechanical stimulation can influence fibrochondrocyte responses through transient receptor potential vanilloid 4 (TRPV4)-related mechanosensing,⁸⁹ while transforming growth factor beta 1 activation and *Prrx1*-lineage cell recruitment can involve primary-cilium-associated signaling.⁹⁰ Together, these adjacent studies provide plausible routes through which printing direction, pore architecture, crosslinking, and the resulting strain field could influence matrix maturation. Adjacent spatiotemporal cue studies also support matching signal timing to tissue maturation.⁹¹ Mechanical regulation is strongly stage-dependent: prolonged unloading can delay collagen organization and mineralization, whereas premature or excessive loading can disrupt immature neotissue.^{92–94} As matrix maturation progresses, moderate loading may increasingly support organization of a load-transmitting architecture, although responses remain dependent on model and maturation stage.

The role of bone-side mineralization depends on its spatial position and timing rather than on total mineral content. Mineral in the native enthesis does not extend uniformly from the bone surface toward the tendon, but increases progressively along the spatial axis and forms a continuous compositional and mechanical gradient together with changing collagen orientation.^{3–5,86,95–97} BdECM, hydroxyapatite, and β -tricalcium phosphate can provide environments for mineral nucleation and osteoconduction, but their effects depend on where, at what concentration, and when mineral is presented. Uniform distribution of mineralization cues throughout a construct, or premature enhancement before unmineralized fibrocartilage has formed, may create a new local stiffness discontinuity and restrict the space available for transition-zone maturation. Conversely, insufficient mineral or mechanical support on the bone side may leave neotendon collagen attached to the bone surface only through mechanically weak fibrous tissue. Mineralization should therefore progress as a controlled front from unmineralized fibrocartilage through mineralized fibrocartilage to bone, limiting mineral expansion toward the tendon while maintaining bone-side collagen anchorage. Differences in local stiffness and cellular responses produced by mineral-content-gradient scaffolds further support the principle that spatial distribution is more important than total mineral amount.⁹⁷

Decellularized extracellular matrix provides a matrix context containing collagen, proteoglycans, and mineralization-related molecules, while printing paths define their initial position and orientation, and the mechanical environment contributes to subsequent reorganization. At implantation, however, the resulting gradient remains a provisional material pattern. If degradation is too rapid, spatial information may dissipate before tissue maturation; if it is too slow or regional boundaries remain persistent, cross-zone matrix integration may be impeded. A material gradient becomes a stable tissue gradient only when the initial compartments are progressively replaced by continuous neotissue ECM comprising aligned collagen, a fibrocartilaginous buffer, and a controlled mineralization front.

3.3.4. Regional perfusion and mechanical adaptation

Direct dECM–TBI evidence currently supports an association between selected constructs and angiogenesis-related responses, but does not establish vascular mediation of interface regeneration.^{45,47,49} In dECM-printed constructs of appreciable thickness and high cell density, regional identity and matrix maturation also depend on whether different regions receive oxygen and nutrients adequate for their metabolic and differentiation requirements. Supportive evidence from broader tendon–bone repair models indicates that inhibition of angiogenesis can compromise interface healing and that local blood flow changes over the course of repair.^{98,99} Vascularization therefore changes dynamically alongside tenogenesis, fibrocartilage formation, and osteogenesis while providing metabolic support for regional maturation.

After decellularization, comminution, enzymatic digestion, and re-gelation, dECM does not preserve native microvascular lumina or their long-range continuity. Instead, its principal role is to provide endothelial adhesion sites, matrix-binding sites, and a remodelable local environment.¹⁰⁰ Building on this matrix context, 3D bioprinting can incorporate sacrificial channels or interconnected pores that shorten diffusion distances and provide geometric routes for endothelialization. General thick-tissue studies show that perfusable channels improve intraconstruct mass transport and cell survival more directly than endothelial-marker expression alone.^{101,102} These findings provide adjacent engineering support and require direct validation in dECM-bioprinted tendon–bone constructs, where the spatial location of vascular ingrowth may also influence regional tissue formation.

Bone-side vascularization supports oxygen and nutrient delivery and may also contribute to osteogenic maturation through endothelial–osteoprogenitor interactions. Bone

studies have identified type-H vessels (CD31^{hi} EMCN^{hi}) associated with osteoprogenitors and have shown that endothelial Notch signaling and blood flow can influence vascular function and osteogenesis.^{103,104} These studies provide adjacent mechanistic rationale for a coupled vascular–osteogenic microenvironment on the bone side, but they derive from models of bone development and homeostasis and do not establish type-H vessels as mediators of dECM-bioprinted tendon–bone regeneration. In contrast, chondrocyte adaptation to hypoxia and hypoxia-inducible factor 1 alpha-dependent survival¹⁰⁵ suggest that the unmineralized fibrocartilaginous region should not simply reproduce a densely vascularized bone-side environment. A plausible design objective is therefore to support its metabolic requirements through adjacent vascular beds and diffusion while avoiding premature vascular ingrowth into unmineralized fibrocartilage. Direct dECM–TBI evidence also shows that angiogenesis-related responses can occur alongside matrix remodeling. In the rabbit rotator cuff model reported by Kim *et al.*⁴⁹, the biologically graded construct showed concurrent changes in type I/III collagen and the vascular markers platelet endothelial cell adhesion molecule 1 and vascular endothelial growth factor (VEGF) at 8 weeks (Figure 5C). However, these parallel histological and angiogenesis-related changes demonstrate association rather than vascular mediation, because vascular function was not selectively perturbed or restored. Supportive non-dECM evidence from Huang *et al.*¹⁰⁶ similarly showed concurrent increases in interface vascularization and improvements in inflammatory, matrix, and mechanical outcomes after exosome treatment in a rat rotator cuff model. Because the intervention simultaneously altered immune and cellular responses, however, this study supports an association between vascularization and improved healing rather than establishing vascular mediation.

Design of pro-angiogenic signaling should therefore consider vascular quality and spatial distribution rather than simply maximizing VEGF dose or total vessel number. Classic studies show that local VEGF concentration influences whether newly formed vessels are organized or aberrant, with excessive local expression producing dilated and structurally abnormal vasculature.¹⁰⁷ Importantly, vascular sprouting is not equivalent to functional vascular maturation. Sequential or combined VEGF and platelet-derived growth factor-BB signaling can promote mural-cell recruitment and vessel stabilization.¹⁰⁸ These studies provide adjacent design principles rather than direct evidence for dECM–TBI constructs. They nevertheless suggest that pro-angiogenic signals should be spatially and temporally controlled—favoring the bone side, construct periphery, or predefined vascular routes while avoiding

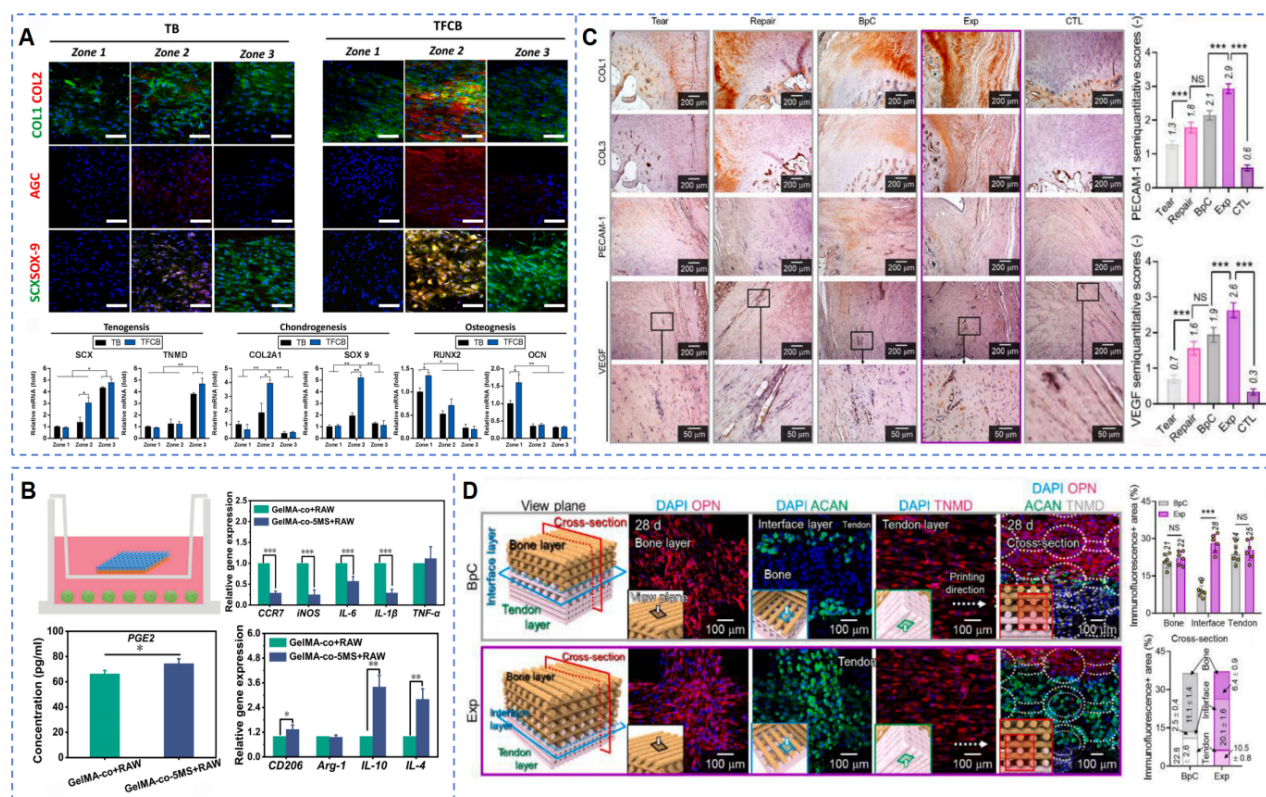


Figure 5. Mechanism-related regional cell–matrix responses and host integration in spatially programmed TB constructs. (A) Region-specific cell–matrix responses in biphasic TB and triphasic TFCB constructs after 14 d of culture. Zone 1 is the bone-directed region, Zone 2 is the fibrocartilage-directed interface region, and Zone 3 is the tendon-directed region. Immunofluorescence images show the spatial distributions of newly formed types I and II collagen, aggrecan, and colocalized SCX and SOX9 proteins. Reverse-transcription quantitative polymerase chain reaction shows regional differences in the transcription of the tenogenic markers SCX and TNMD, the chondrogenic markers COL2A1 and SOX9, and the osteogenic markers RUNX2 and OCN. The intermediate region of the TFCB construct was formed from a mixed tendon- and bone-derived dECM bioink rather than from an independent fibrocartilage-derived dECM. Reprinted with permission from Chae *et al.*⁴⁷ Copyright © 2022, The Authors. (B) Transwell co-culture system comprising a bilayer GelMA multicellular scaffold containing tendon stem/progenitor cells and bone marrow-derived mesenchymal stem cells, together with RAW 264.7 macrophages. This non-dECM model is included as supportive mechanistic evidence for macrophage–stromal paracrine interactions and should not be interpreted as direct evidence of a dECM-specific immune mechanism. Reprinted with permission from Du *et al.*²¹ Copyright © 2024 The Authors. (C) *In vivo* collagen remodeling and angiogenesis-related responses 8 weeks after implantation in a rabbit rotator cuff tear model. The images show immunohistochemical staining for type I collagen, type III collagen, PECAM-1, and VEGF in an untreated tear group (Tear), surgical-repair-only group (Repair), bioprinted control construct group (BpC), biologically graded experimental construct group (Exp), and uninjured control group (CTL), together with semi-quantitative PECAM-1 and VEGF scores. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (D) Regional matrix-related phenotypes in BpC and Exp constructs after 28 d of *in vitro* culture. Positive signals for OPN, ACAN, and TNMD were enriched in the bone-directed, interface, and tendon-directed regions, respectively, and the Exp construct had a greater proportion of ACAN-positive interface area. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors.

Abbreviations: ACAN: Aggrecan; AGC: Aggrecan; Arg-1: Arginase 1; CCR7: C-C motif chemokine receptor 7; CD206: Cluster of differentiation 206; COL1: Type I collagen; COL2: Type II collagen; COL2A1: Collagen type II alpha 1 chain; COL3: Type III collagen; CTL: Control; DAPI: 4',6-Diamidino-2-phenylindole; Exp: Experimental; GelMA: Gelatin methacryloyl; IL-1β: Interleukin 1 beta; IL-4: Interleukin 4; IL-6: Interleukin 6; IL-10: Interleukin 10; iNOS: Inducible nitric oxide synthase; NS: Not significant; OCN: Osteocalcin; OPN: Osteopontin; PECAM-1: Platelet endothelial cell adhesion molecule 1; PGE2: Prostaglandin E2; RUNX2: Runt-related transcription factor 2; SCX: Scleraxis; SOX9: SRY-box transcription factor 9; TB: Tendon–bone; TFCB: Tendon–fibrocartilage–bone; TNF-α: Tumor necrosis factor alpha; TNMD: Tenomodulin; VEGF: Vascular endothelial growth factor.

persistent high-level exposure within the unmineralized fibrocartilaginous region.

Mechanical support and perfusion are likely to interact because scaffold density or channel collapse can simultaneously alter mass transport and the local strain environment. In a rat rotator cuff model, Cai *et al.*¹⁰⁹ linked material-dependent TRPV4/vascular endothelial growth factor receptor 2-associated vascular responses with collagen maturation, fibrocartilage formation, bone formation, and improved interface mechanics. Because this study did not use a dECM-bioprinting model, it provides supportive mechanistic evidence for vascular–mechanical coupling rather than a dECM-specific effect. Overall, current evidence supports coordinated interactions among regional perfusion, matrix formation, and mechanical adaptation, but direct causal validation in dECM-bioprinted tendon–bone constructs remains limited. Representative *in vivo* remodeling, interface maturation, and functional outcomes are summarized in Figure 6.

4. A five-level interpretive framework for evaluating decellularized extracellular matrix-bioprinted tendon–bone interface regeneration

4.1. Framework logic and cross-cutting principles for claim evaluation

Two complementary dimensions are used throughout this review. Evidence provenance describes how directly a study addresses dECM-bioprinted TBI regeneration, whereas the framework level describes the strongest claim justified by the reported evidence. These dimensions should not be conflated. A directly relevant study may remain at an early framework level, whereas supportive or adjacent evidence may provide biological plausibility without independently establishing a higher level of dECM–TBI regeneration.

Across all levels, claim strength should be matched to experimental design. Attribution of tissue-specific dECM effects requires physicochemically matched and, where feasible, source-swapped controls; evaluation of spatial gradients requires appropriately matched homogeneous constructs; mechanistic claims require perturbation, component-subtraction, or rescue experiments rather than parallel biomarker changes; and functional-regeneration claims require evidence linking scaffold degradation with local load transfer and repeated-loading stability. When multiple variables are altered concurrently, improved repair should be attributed to the integrated construct unless the contribution of an individual component has been experimentally isolated.

Predefined spatial sampling, biologically meaningful

Table 3. Key assessment attributes, minimum evidence and claim boundaries from material identity to neotissue-mediated load takeover in dECM-bioprinted tendon–bone constructs

Evidence stage	Core scientific question	Minimum evidence	Recommended assessments	Critical comparators and spatiotemporal design	Common overinterpretation	Claim justified	References
Cross-cutting study design	Are comparators, experimental units, and assessment time points aligned with the scientific claim and sufficient for attribution and reproducibility?	- Prespecified primary claim and primary endpoint - Separation of independent donors, dECM-processing batches, bioink batches, print batches, and animals - Sample-size rationale, randomization, blinding, exclusion criteria, and transparent handling of outliers in animal studies	- Factorial designs, subtraction/rescue experiments, and pathway perturbation - Multilevel or nested statistics with the true biological n explicitly defined - ARRIVE 2.0-compliant animal-study reporting	- Physicochemically matched non-dECM matrix - Homogeneous versus graded constructs matched for total dECM, cell number, mineral content, and initial bulk mechanics - Acellular, scaffold-only, surgical-repair, and intact controls; early, intermediate, and late time points	- Treating multiple constructs from one batch or multiple sections from one animal as independent biological replicates - Inferring an independent dECM, gradient, or pathway effect because an all-in-one construct outperforms an untreated control	Provides an attributable and reproducible evidence base; does not justify a regeneration claim by itself.	110–115

(Cont'd...)

Evidence stage	Core scientific question	Minimum evidence	Recommended assessments	Critical comparators and spatiotemporal design	Common overinterpretation	Claim justified	References
Material identity	Does dECM retain a distinguishable, reproducible, source-dependent molecular identity and biological potency after decellularization, digestion, sterilization, and crosslinking, and printing?	- Joint evidence of cell removal, controlled residual risks, retained ECM/matrisome features, and use-related potency - Variation reported across independent donors and processing batches - Common decellularization reference criteria: dsDNA <50 ng/mg dry ECM, DNA fragments <200 bp, and no visible nuclei	- dsDNA, fragment length, and H&E/DAPI - Residual detergent, endotoxin, potential antigens, sterility, and cytotoxicity - Region-relevant ECM quantification, LC–MS/MS proteomics/glycomics, and use-related potency assays	- Paired native tissue and sequential processing stages - Source-matched, source-swapped, and physicochemically matched non-dECM matrices - Repeated assessment before and after digestion, sterilization, crosslinking, and printing	- Equating the tissue-of-origin label or a DNA reference value with retained tissue specificity, safety, and potency - Assuming that detection of an ECM component proves intact conformation and active binding sites	Material identity and concept validation; does not justify enthesis-like tissue formation.	26–29,62,63,116
	Is the bioink manufactured within a reproducible process window, and do the realized material, cell, and mineral distributions match the design and persist during crosslinking, culture, and degradation?	- Linked pre-print, in-process, and post-print process–quality attributes - Quantified gradient slope, transition-zone width, and batch variation along the tendon–bone axis - Acute and sublethal cell injury plus temporal stability of the spatial pattern	- Viscosity–shear rate, yield stress, thixotropic recovery, and gelation/crosslinking kinetics - Filament width, pore fidelity, interlayer bonding, swelling/shrinkage, and long-term shape stability - Fluorescent tracing, Raman spectroscopy, infrared spectroscopy, and elemental imaging, 3D reconstruction, apoptosis, oxidative stress, and metabolic assays	- CAD model versus realized print - Homogeneous versus graded constructs matched for total composition and bulk properties - Independent print batches; immediate, early-culture, and intermediate-culture assessments	- Treating continuous extrusion, a representative image, or a CAD path as proof of a stable manufactured gradient - Treating immediate post-print Live/Dead staining as proof of long-term cell compatibility	Manufacturing fidelity and concept validation; does not prove translation of a material pattern into a neotissue gradient.	117–122
Regional tissue formation	Is the initial material pattern translated into a tendon–fibrocartilage–bone neotissue with zonal cell states, continuous ECM, and a spatially controlled mineralization front?	- Tenogenic, fibrocartilage-like, and osteogenic states at predefined coordinates with controlled overlap - Continuous aligned collagen, a proteoglycan-rich transition zone, and bone-side mineralization - Quantified transition-zone width, cross-zone continuity, and mineralization-front position	- Coordinate-defined sampling followed by IF/IHC or qPCR, or spatial omics - Polarized-light picrosirius red imaging, second-harmonic generation imaging, and collagen-orientation analysis - Safranin O staining and ACAN/COL II assessment, micro-CT and elemental imaging, and local modulus mapping	- Homogeneous versus graded, biphasic versus triphasic, acellular, and source-swapped controls - Geometry, bulk stiffness, and degradation rate matched as closely as possible - Time series spanning early cell-state specification, intermediate ECM formation, and late mineral maturation	- Equating whole-construct qPCR, a single marker, or increased positive area with organized interface formation - Equating increased cartilage-like matrix or bone volume with a fibrocartilage buffer and controlled mineralization	Enthesis-like tissue formation when zonal states, continuous ECM, and controlled mineralization co-occur; not functional regeneration.	3–6,8,66–71,86,87,123–126

(Cont'd...)

Table 3. (Continued)

Evidence stage	Core scientific question	Minimum evidence	Recommended assessments	Critical comparators and spatiotemporal design	Common overinterpretation	Claim justified	References
Host integration	Does the construct support timely inflammatory resolution, host-cell entry, regionally appropriate perfusion, and constructive remodeling across the host–construct boundary?	• Immune initiation, resolution, and fibrosis assessed as trajectories rather than a static M1/M2 dichotomy • Host-cell infiltration and cross-boundary ECM continuity • Lumen connectivity, host anastomosis, actual perfusion, and region-restricted vascular distribution	• Multiparameter immune phenotyping, single-cell/spatial transcriptomics, and inflammatory and pro-resolving mediators • α-SMA/fibrosis, host-donor tracing, and boundary histology • Perfusion tracing, microvascular imaging, pericyte coverage, and vascular leakage	• Acellular constructs, processing-quality variants, and surgical-repair controls • Region-specific sampling of tendon, transition, and bone zones at multiple early and later time points • Blockade, subtraction, and rescue experiments for causal claims	• Equating increased CD206 with constructive immune integration or CD31/VEGF positivity with mature perfused vessels • Assuming persistent donor-cell fluorescence demonstrates durable tissue contribution	Supports host integration and strengthens enthesis-like tissue formation when combined with regional-tissue evidence; causality requires perturbation.	72–85,98–108,125,127–131
	After substantial scaffold degradation, does neotissue sustain load transfer and remain stable under repeated loading?	• Concurrent quantification of scaffold residue/degradation and neotissue load contribution • Evidence that local strain changes from an interface discontinuity to a continuous distribution • Cyclic durability, viscoelastic behavior, and failure-site transition supported by whole-limb function	• Digital image correlation, ultrasound elastography, or other full-field strain methods • Tension, cyclic fatigue, creep, stress relaxation, hysteresis, and failure-site analysis • Scaffold-degradation quantification with spatial histology–mechanics registration; gait and weight bearing as supportive outcomes	• Intact, repair-only, acellular/scaffold-only, and homogeneous-construct controls • Initial bulk mechanics matched as closely as possible • Multiple late time points spanning substantial scaffold degradation	• Equating a higher maximum failure load, stiffness, or improved gait at one endpoint with functional regeneration • Assuming bulk specimen strengthening identifies neotissue as the load-bearing component	Within this framework, supports a claim of functional regeneration when sustained post-degradation neotissue load transfer, local-strain continuity, cyclic stability, and failure-site transition co-occur.	3–6,86,132–139

Note: Minimum evidence refers to the minimum combination of evidence required to support the stated claim rather than to a universal fixed threshold; established reference criteria are provided only for decellularization. The evidence stages correspond to the cumulative framework shown in **Figure 1** and are not intended as a formal risk-of-bias assessment. Within this framework, functional regeneration requires sustained neotissue-mediated load transfer after substantial scaffold degradation, supported concurrently by local-strain continuity, cyclic durability and failure-site transition.

Abbreviations: α -SMA: Alpha-smooth muscle actin; ACAN: Aggrecan; ARRIVE: Animal Research: Reporting of *In Vivo* Experiments; CAD: Computer-aided design; COL II: Type II collagen; DAPI: 4',6-Diamidino-2-phenylindole; dECM: Decellularized extracellular matrix; dsDNA: Double-stranded DNA; ECM: Extracellular matrix; H&E: Hematoxylin and eosin; IF: Immunofluorescence; IHC: Immunohistochemistry; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; Micro-CT: Micro-computed tomography; qPCR: Quantitative polymerase chain reaction; VEGF: Vascular endothelial growth factor.

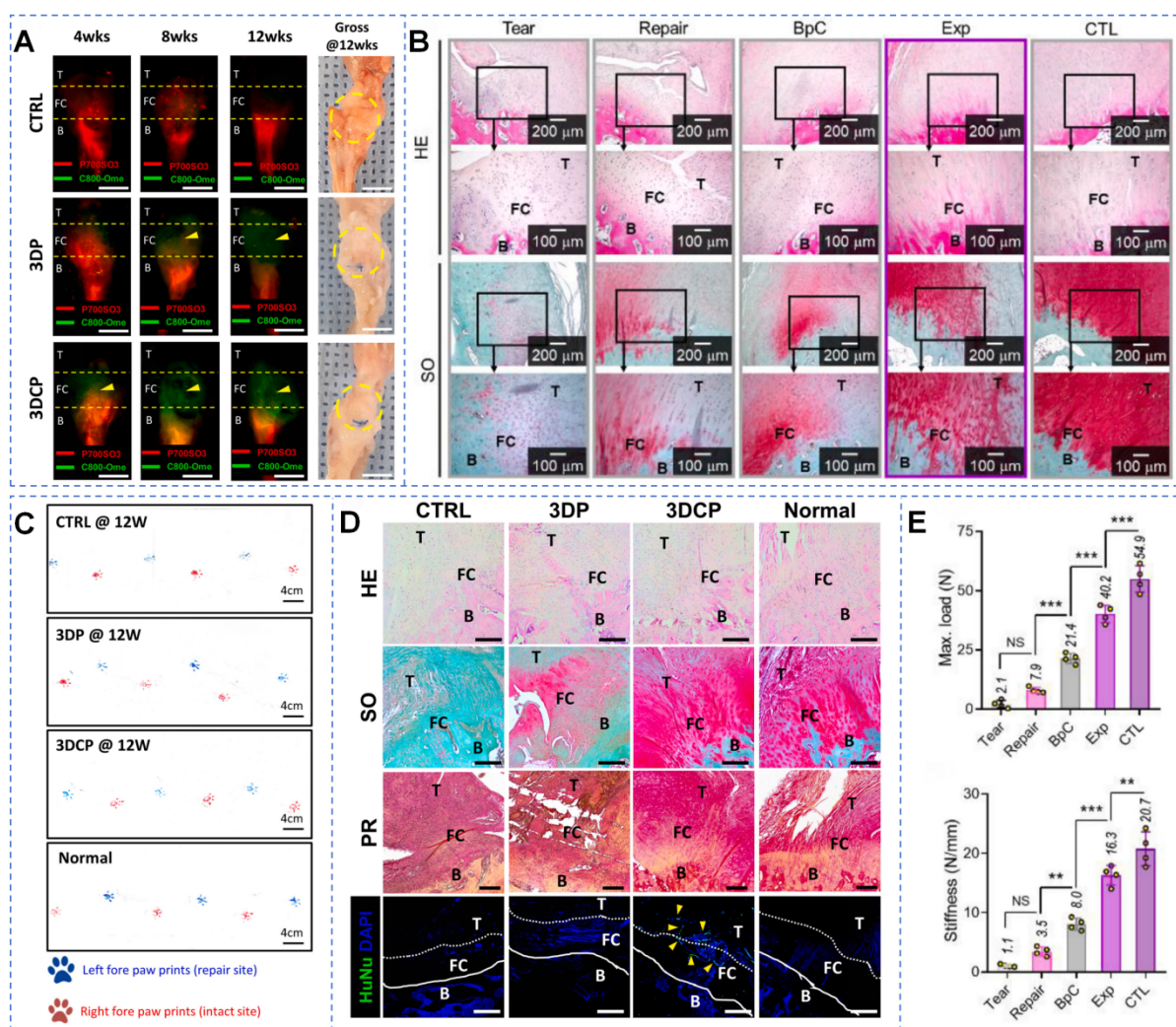


Figure 6. *In vivo* matrix remodeling and functional outcomes associated with spatially organized dECM tendon–bone constructs. (A) Longitudinal dual-channel near-infrared fluorescence imaging at 4, 8, and 12 weeks after repair in a rat model of chronic rotator cuff tear. The CTRL group underwent surgical repair without construct implantation, the 3DP group received a 3DP construct, and the 3DCP group received a 3D cell-printed construct containing human bone marrow-derived mesenchymal stem cells. Red pseudocolor indicates the mineral-associated probe P700SO₃, and green pseudocolor indicates the fibrocartilage- or proteoglycan-associated probe C800–OMe. Yellow arrows indicate regions with C800–OMe-associated signal at the interface, and the yellow circle marks the repair region in the specimen collected at 12 weeks. Reprinted with permission from Chae *et al.*⁴⁷ Copyright © 2022, The Authors. (B) Representative H&E and Safranin O staining 8 weeks after surgery in a rabbit rotator cuff model, comparing an untreated tear group (Tear), surgical repair without a construct (Repair), a bioprinted control construct without a specifically designed biological tendon–bone gradient (BpC), a biologically graded experimental construct (Exp), and an uninjured control (CTL). Black boxes in the low-magnification images indicate the regions shown at higher magnification below. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (C) Representative footprints 12 weeks after repair in the rat model. Blue footprints correspond to the repaired left forepaw, and red footprints correspond to the intact contralateral right forepaw. Reprinted with permission from Chae *et al.*⁴⁷ Copyright © 2022, The Authors. (D) Representative H&E, SO, and PR staining, together with human nuclear antigen/DAPI fluorescence imaging, 12 weeks after repair of the rat tendon–bone interface. Yellow arrows indicate HuNu-positive human cells near the interface and implanted region. Reprinted with permission from Chae *et al.*⁴⁷ Copyright © 2022, The Authors. (E) Maximum load and stiffness of the supraspinatus tendon–humerus complex 8 weeks after surgery in the rabbit rotator cuff model. Data are presented as mean ± standard deviation, with independent biological replicates shown ($n = 4$). The biologically graded Exp construct improved both mechanical measures relative to the untreated Tear, Repair-only, and non-gradient BpC groups, although values remained below those of uninjured CTL tissue. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors.

Abbreviations: 3DCP: Three-dimensional cell-printed construct with bone marrow–derived mesenchymal stem cells; 3DP: Three-dimensional bioprinted construct; B: Bone; BpC: Bioprinted control construct; CTL: Control; CTRL: Control; DAPI: 4',6-Diamidino-2-phenylindole; Exp: Experimental construct; FC: Fibrocartilage; H&E: Hematoxylin and eosin; HuNu: Human nuclei; NS: Not significant; PR: Picrosirius red; SO: Safranin O; T: Tendon.

time points, appropriate identification of independent experimental units, and animal models matched to the scientific question are cross-cutting requirements. The operational criteria, recommended assessments, critical comparators, common forms of overinterpretation, and justified claim boundaries for each evidence level are consolidated in [Table 3](#).

4.2. Level 1—Material identity: Retention and attribution of tissue-specific matrix information

The presence of dECM in a bioink provides only preliminary evidence of material identity; tissue-specific identity and biological potency require independent validation after processing. Material identity should jointly document adequate removal of cellular material and retention of biologically meaningful source-associated ECM information. Conventional criteria based on residual DNA, DNA fragment length, and nuclear staining provide important evidence of decellularization efficiency but do not establish preservation of source-associated matrix information, biological potency, or overall material safety.^{26–28,116} Safety-related residuals should therefore be treated as complementary quality attributes, particularly for xenogeneic or composite dECM systems.

The retained ECM profile should correspond to the intended regional use and may be characterized using targeted tendon-, fibrocartilage-, or bone-relevant matrix markers together with broader matrisome or proteomic profiling. Representative matrix-retention and source-associated potency assessments are illustrated in [Figure 7A](#). Molecular detection alone is insufficient to establish preserved biological activity because processing can alter conformation, binding sites, and cellular recognition. Potency assays should therefore be aligned with the intended regional function, such as tenogenic matrix assembly, spatially confined fibrocartilage-like matrix formation, or controlled bone-side mineralization. Raw-material reproducibility should distinguish independent donors and independently processed tissue batches from repeated constructs derived from the same dECM batch; the latter demonstrate within-batch technical repeatability rather than donor- or processing-batch reproducibility. For composite bioinks, final dECM content should be reported explicitly, and tissue-source effects should, where feasible, be evaluated using source-swapped dECM and physicochemically matched controls. When source, formulation, mechanics, cell composition, or construct geometry cannot be separated experimentally, outcomes should be attributed to the integrated construct rather than to tissue-specific dECM. Within this author-proposed framework, Level 1 is supported when processed dECM

retains a reproducible, distinguishable, and biologically meaningful source-associated identity; this level alone does not establish regional tissue formation.

4.3. Level 2—Manufacturing fidelity: From computer-aided design to persistent spatial gradients

Manufacturing fidelity should be evaluated as a reproducible process window linking material properties, printing conditions, and fabrication outcomes. Relevant preprint, in-process, and postprint quality attributes should establish whether the formulation can be deposited reproducibly while maintaining rheological recovery, construct fidelity, and acceptable cell integrity; detailed parameters are summarized in [Table 3](#).^{117–122} Independent bioink-formulation and printing batches should likewise be distinguished when evaluating manufacturing reproducibility. For gradient tendon–bone constructs, spatial authenticity requires evidence beyond geometric fidelity. A CAD model, multi-nozzle configuration, and initial printing path represent design intent only; they do not establish the intended distributions of different dECM sources, cells, mineral, and local modulus. Gradient slope, transition-zone width, and between-batch deviation should be quantified along the tendon–bone axis using appropriate spatially resolved methods.^{120–122} A continuous gradient should be shown as a signal-versus-distance profile rather than as representative images from only three regions. Spatial fidelity should also be reassessed over time because diffusion, swelling, cell migration, contraction, and differential degradation may alter the initially printed pattern.

Regional distributions should therefore be assessed after fabrication and at subsequent culture or remodeling stages to distinguish the initial material gradient from the emerging neotissue gradient. Immediate post-print viability is an acute manufacturing endpoint and should not substitute for later assessment of sublethal injury, proliferation, or regional matrix formation. The degree of TdECM digestion can alter collagen-fiber formation, extrusion behavior, shear-thinning rheology, and tendon-related gene responses ([Figure 7B](#)). Initial geometric fidelity of aligned filament bundles and longitudinal channels can be verified by concordance among the CAD, support-bath fabrication, and the resulting structure ([Figure 7C](#)).^{117,118,120} Within this framework, Level 2 is supported when the intended material and spatial design is reproducibly translated into a measurable pattern that remains sufficiently stable during early remodeling; manufacturing fidelity alone does not establish that the printed pattern has generated a corresponding neotissue gradient.

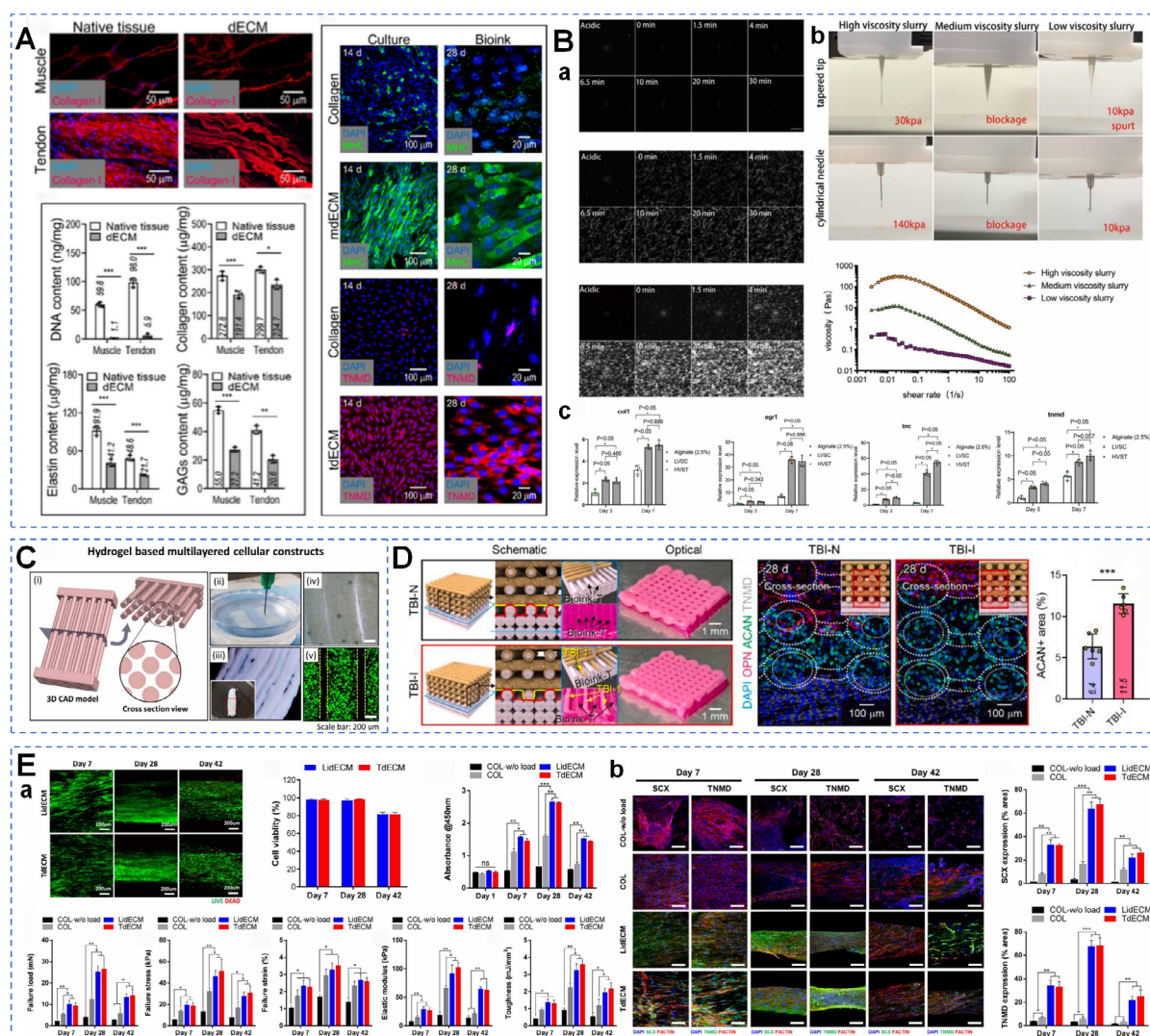


Figure 7. Structure–process–potency relationships among dECM-processing variables, manufacturing fidelity and regional biological potency. (A) Representative assessment of matrix-component retention after decellularization of muscle and tendon tissues and source-associated cellular responses. The analyses include DAPI/type I collagen staining; DNA, collagen, elastin, and GAG contents; and MHC- or TNMD-related responses after 14 d of two-dimensional culture and 28 d of three-dimensional dECM-bioink culture. Reproduced from Kim and Kim.⁶⁵ (B) Collagen-fiber formation, extrusion behavior, shear-thinning rheology, and tendon-related gene expression as functions of the degree of digestion of a tendon-derived dECM bioink. High-, medium-, and low-viscosity slurries were evaluated using conical nozzles or cylindrical needles. Reprinted with permission from Zhao *et al.*³⁸ Copyright © 2020, IOP Publishing Ltd. (C) CAD and support-bath fabrication of a hydrogel-based multilayer, cell-laden tendon/ligament construct containing aligned filament bundles and longitudinal channels. Reprinted with permission from Chae *et al.*⁴⁶ Copyright © 2022, IOP Publishing Ltd. (D) Comparison of a conventional contact tendon–bone interface (TBI-N) with an interdigitated tendon–bone interface (TBI-I). OPN/ACAN/TNMD immunofluorescence and quantification of ACAN-positive area after 28 d of culture show more pronounced fibrocartilage-like matrix specialization in the interdigitated design. Reprinted with permission from Kim *et al.*⁴⁹ Copyright © 2024, The Authors. (E) Time-dependent *in vitro* responses of collagen, LidECM and tendon-derived dECM constructs during 42 d of culture, including cell survival and proliferation, construct-level tensile properties, and SCX and TNMD expression. Reprinted with permission from Chae *et al.*⁴⁶ Copyright © 2022, IOP Publishing Ltd.

Abbreviations: 3D: Three-dimensional; ACAN: Aggrecan; CAD: Computer-aided design; COL1: Type I collagen; DAPI: 4',6-Diamidino-2-phenylindole; dECM: Decellularized extracellular matrix; DNA: Deoxyribonucleic acid; EGR1: Early growth response 1; F-actin: Filamentous actin; GAGs: Glycosaminoglycans; HVSC: High-viscosity slurry printed using a cylindrical needle; HVST: High-viscosity slurry printed using a tapered tip; LidECM: Ligament-derived decellularized extracellular matrix; LVSC: Low-viscosity slurry printed using a cylindrical needle; mdECM: Muscle-derived decellularized extracellular matrix; MHC: Myosin heavy chain; OPN: Osteopontin; SCX: Scleraxis; TBI-I: Tendon–bone interface with an interdigitated contact boundary; TBI-N: Tendon–bone interface with a normal contact boundary; tECM/TdECM: Tendon-derived decellularized extracellular matrix; TNC: Tenascin-C; TNMD: Tenomodulin.

4.4. Level 3—Regional tissue formation: From zonal cell states to continuous neotissue extracellular matrix

At Level 3, biological assessment asks whether the manufactured spatial pattern produces appropriately positioned cellular states and a continuous neotissue architecture along the tendon–fibrocartilage–bone axis, rather than merely increasing lineage-associated markers at the whole-construct level. Tenogenic, fibrocartilage-associated, and osteogenic programs should emerge at predefined positions along the tendon–bone axis, with controlled overlap between adjacent regions. Particular caution is required when interpreting the intermediate zone. A compartment formed from mixed TdECM and BdECM should be described as an intermediate material design strategy and does not, by itself, establish fibrocartilage regeneration. More persuasive evidence requires transitional cellular states, including spatial overlap of tendon- and cartilage-associated programs, along with formation of a spatially confined proteoglycan-rich fibrocartilage-like matrix. Representative regional matrix specialization in an interdigitated interface design is illustrated in [Figure 7D](#).

Regional identity gains regenerative significance only when these cellular states translate into a continuous neotissue architecture. Tendon-side collagen should extend along the principal loading direction, the transition region should develop a proteoglycan-rich fibrocartilaginous matrix that remains continuous with adjacent tendon- and bone-side tissues, and mineralization should progress in a spatially controlled manner toward the bone side. Transition-zone width, cross-zone ECM continuity, and the position and progression of the mineralization front should therefore be evaluated as spatial properties rather than inferred from isolated marker-positive regions. Increases in lineage-associated markers, collagen, cartilage-like matrix, or bone volume alone are insufficient to establish this level. Time-dependent changes in cell survival, construct mechanics, and tenogenic responses provide complementary longitudinal context for tissue maturation ([Figure 7E](#)). Under the author-proposed framework, Level 3 is supported when regionally appropriate cellular states, continuous cross-zone ECM organization, and spatially controlled mineralization are demonstrated concurrently along the intended tendon–fibrocartilage–bone axis.

4.5. Level 4—Host integration: Immune resolution, perfusion, and construct–host remodeling

At Level 4, host integration asks whether regionalized neotissue remains constructively integrated with host tissues under *in vivo* immune, vascular, and remodeling

conditions. Immune assessment should examine the temporal relationship among inflammatory initiation, resolution, and matrix remodeling, rather than assigning macrophages to fixed M1 or M2 categories solely on the basis of CD86, inducible nitric oxide synthase, CD206, or arginase-1. Vascular assessment should distinguish endothelial recruitment from the establishment of a host-connected, functionally perfused network; more persuasive evidence includes lumen continuity, host anastomosis, intraluminal perfusion, and vascular maturation rather than endothelial-marker expression alone.^{127–130} Vascular distribution should also remain regionally appropriate, supporting bone-side remodeling while avoiding premature or excessive invasion of unmineralized fibrocartilage. For cell-laden constructs, short-term retention, long-term survival, direct tissue contribution, and paracrine effects should be distinguished because persistent fluorescence alone does not demonstrate incorporation of implanted cells into neotissue.¹³¹ Evidence of timely inflammatory resolution, cross-boundary ECM continuity, host-cell participation, and functional perfusion strengthens the interpretation of durable host integration, but does not by itself establish functional regeneration. Causal claims regarding immune or vascular mediation require appropriately controlled perturbation or rescue experiments.

4.6. Level 5—Neotissue-mediated load takeover after scaffold degradation

The ultimate mechanical objective is to reconstruct a continuous load-transfer pathway from tendon collagen through fibrocartilage and the mineralized transition into bone. Mechanical assessment should address three sequential questions: whether the construct satisfies early fixation requirements; whether the repaired interface develops an appropriate local load-transfer pattern; and whether neotissue progressively assumes load-bearing function during scaffold degradation and repeated loading.^{3–6,86,133}

Because scaffold materials differ substantially in degradation kinetics and mechanical contribution, substantial scaffold degradation should be defined prospectively for each material system rather than by a universal percentage. It can be demonstrated by longitudinal loss of scaffold mass, volume fraction, or a material-specific imaging signal, together with a measurable decline in scaffold-derived mechanical support. Continuous strain distribution refers to a distance-resolved strain profile across the tendon–transition–bone axis in which the local strain discontinuity is attenuated relative to repair-only controls and approaches that of the intact interface, preferably assessed using full-field methods such as digital

image correlation or ultrasound elastography. Cyclic durability refers to the ability of the repaired interface to retain stiffness and energy-dissipation behavior with limited accumulation of residual deformation during a prespecified, physiologically relevant repeated-loading protocol. These endpoints should be interpreted together with failure-site transition rather than as independent criteria.

Preimplantation assessment primarily determines whether a construct is suitable for surgical handling and early mechanical support. At this stage, higher stiffness or failure load documents initial mechanical stability rather than functional enthesis formation. Because scaffold stiffness and degradation kinetics determine how long the construct continues to carry load, the duration of mechanical support should be interpreted relative to neotissue matrix maturation. Persistent reinforcement may produce stress shielding, whereas overly rapid loss of support may compromise early fixation and geometric stability. A higher single-cycle maximum failure load demonstrates improved bulk load-bearing capacity but does not identify whether the load is carried primarily by residual scaffold, scar tissue, or continuous neotissue. Once *in vivo* repair is underway, assessment should expand beyond ultimate failure load to local load transfer, viscoelastic behavior, cyclic stability, and failure location. Mechanical results should also be spatially correlated with collagen orientation, fibrocartilage width, the mineralization front, and residual scaffold to distinguish improvement caused by an undegraded framework or local bone overgrowth from maturation of neotissue collagen, fibrocartilage, and bone.

Load takeover is explicitly time-dependent, and scaffold degradation should therefore be assessed together with changes in interface mechanics at the same time points. Early stability provided jointly by the printed scaffold, suture fixation, and host tissue is appropriate. As the scaffold degrades and neotissue ECM forms, load should progressively transfer to the regenerating tissue. At each time point, residual scaffold mass, volume fraction, or imaging-based material persistence should be reported together with local interface strain, cyclic stability, and failure location to determine whether improved bulk mechanics still depend on material support. If bulk performance declines as scaffold content decreases, the earlier improvement was probably dominated by material reinforcement. If local strain becomes more continuous, cyclic stability improves and the site of failure shifts from the repair interface toward the tendon substance or bone while the scaffold continues to degrade, the findings support progressive load takeover by neotissue.^{136–138}

Whole-animal functional outcomes can support the global relevance of recovery but cannot replace direct evidence of interface continuity and local load transfer.¹³⁹ Within the author-proposed framework, printability, cell survival, and regional marker expression support proof of concept, whereas regionalized ECM formation without local load-transfer evidence supports only enthesis-like tissue formation. Functional regeneration is justified only when substantial scaffold degradation, continuous load transfer across the interface, cyclic durability, failure-site transition, and maintenance of function are concordant. Functional regeneration is therefore defined here as a verifiable transfer of the principal load-bearing role from the artificial scaffold to spatially continuous regenerated tissue.

5. Manufacturing standardization and regulatory translation of decellularized extracellular matrix bioinks

Translation of dECM-bioprinted tendon–bone constructs requires control of raw-material variability, manufacturing reproducibility, safety, storage, and product-level quality attributes.^{28,116,140,141} Donor species, age, anatomical site, disease status, and processing history can alter dECM composition and biological potency, whereas residual nucleic acids or processing reagents, endotoxin, xenogeneic antigens, and sterilization-related changes may affect matrix integrity, safety, and subsequent host remodeling.^{26–29,85,116,142,143} These variables should therefore be incorporated into predefined critical quality attributes, with batch-release testing addressing decellularization efficiency, residual contaminants, sterility and endotoxin control, source-associated matrix identity, rheological and printing performance, and region-relevant biological potency.^{28,116,140,141}

Translation toward Good Manufacturing Practice-compliant manufacture will additionally require standardized tissue sourcing, decellularization, and formulation, controlled printing and crosslinking, validated aseptic processing or terminal sterilization procedures, packaging, storage, and stability testing.^{28,140–143} Regulatory classification will depend on the final product configuration and jurisdiction and should not be presented as a universal pathway; cell-laden bioprinted constructs introduce additional requirements for cell-source qualification, expansion, viability, sterility, traceability, and manufacturing control beyond those of acellular dECM scaffolds.^{140,141} Surgical handling, fixation compatibility, large-animal validation, and long-term mechanically relevant follow-up should therefore be integrated into translational development rather than treated as post hoc considerations.^{132,140,141}

6. Conclusion and future perspectives

Current studies show that tissue-derived dECM can be formulated into bioinks and incorporated into regionalized tendon–bone constructs. Direct studies associate these constructs with regional matrix formation, fibrocartilage-like transition tissue, and improved interface integration, supporting proof of concept and enthesis-like tissue formation. However, tissue-source information and printed spatial gradients may be altered during processing and remodeling, while improved bulk mechanics may still depend partly on residual scaffold support. Current evidence therefore does not yet establish functional regeneration defined by sustained neotissue-mediated load transfer after substantial material degradation.

At the material and manufacturing levels, priorities are reproducible source characterization, process-linked potency testing, and longitudinal verification that printed dECM, cell, and mineral distributions remain stable during remodeling. Donor and processing-batch variability should be reported explicitly, and tissue-source effects should be linked to region-relevant potency rather than to source labels alone. Biological design should focus on the sequence of interface maturation rather than on maximizing terminal lineage markers. Regionalized constructs should support appropriately overlapping tenogenic, fibrocartilage-like, and osteogenic states while coordinating inflammatory resolution, perfusion, mineralization, degradation, and loading with tissue maturity.

The principal barrier to clinical translation is the absence of long-term, comparable evidence under realistic loading. Chronic-injury and large-animal models should record material persistence, regional tissue maturation, local strain, cyclic durability, and failure location concurrently to determine whether early reinforcement by a construct is converted into stable function after scaffold degradation. Multicenter and interlaboratory studies should also harmonize raw-material batches, bioink formulations, printing parameters, sampling coordinates, and mechanical-testing conditions so that different strategies can be reproduced and compared. The value of dECM bioprinting ultimately lies in a verifiable transition: processed tissue-source information is preserved and accurately positioned; the initial material gradient is progressively replaced by continuous neotissue ECM; and the load path remains stable after substantial scaffold degradation. Evidence of this transition is needed before enthesis-like tissue formation can be described as functional TBI regeneration.

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Conflict of interest

Jincheng Wang is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Further disclosure

During revision of this manuscript, the authors used ChatGPT (OpenAI; model: GPT-5.6 Sol) for language editing and suggestions to improve readability, clarity, and linguistic coherence. The tool was not used to generate experimental data or independently determine scientific conclusions. All AI-assisted text was critically reviewed, verified, and revised by the authors, who take full responsibility for the accuracy, integrity, originality, and final content of the manuscript.

References

1. Lu HH, Thomopoulos S. Functional attachment of soft tissues to bone: development, healing, and tissue engineering. *Annu Rev Biomed Eng.* 2013;15:201–226.
doi: 10.1146/annurev-bioeng-071910-124656

2. Pugliese E, Rossoni A, Zeugolis DI. Enthesis repair-state of play. *Biomater Adv.* 2024;157:213740.
doi: 10.1016/j.bioadv.2023.213740
3. Moffat KL, Sun WH, Pena PE, *et al.* Characterization of the structure–function relationship at the ligament-to-bone interface. *Proc Natl Acad Sci USA.* 2008;105(23):7947–7952.
doi: 10.1073/pnas.0712150105
4. Rossetti L, Kuntz LA, Kunold E, *et al.* The microstructure and micromechanics of the tendon–bone insertion. *Nat Mater.* 2017;16(6):664–670.
doi: 10.1038/nmat4863
5. Genin GM, Kent A, Birman V, *et al.* Functional grading of mineral and collagen in the attachment of tendon to bone. *Biophys J.* 2009;97(4):976–985.
doi: 10.1016/j.bpj.2009.05.043
6. Thomopoulos S, Marquez JP, Weinberger B, Birman V, Genin GM. Collagen fiber orientation at the tendon to bone insertion and its influence on stress concentrations. *J Biomech.* 2006;39(10):1842–1851.
doi: 10.1016/j.jbiomech.2005.05.021
7. Schwartz AG, Pasteris JD, Genin GM, Daulton TL, Thomopoulos S. Mineral distributions at the developing tendon enthesis. *PLoS ONE.* 2012;7(11):e48630.
doi: 10.1371/journal.pone.0048630
8. Blitz E, Sharir A, Akiyama H, Zelzer E. Tendon–bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. *Development.* 2013;140(13):2680–2690.
doi: 10.1242/dev.093906
9. Steltzer SS, Abraham AC, Killian ML. Interfacial tissue regeneration with bone. *Curr Osteoporos Rep.* 2024;22(2):290–298.
doi: 10.1007/s11914-024-00859-1
10. Lei T, Zhang T, Ju W, *et al.* Biomimetic strategies for tendon/ligament-to-bone interface regeneration. *Bioact Mater.* 2021;6(8):2491–2510.
doi: 10.1016/j.bioactmat.2021.01.022
11. Li X, Xie J, Lipner J, Yuan X, Thomopoulos S, Xia Y. Nanofiber scaffolds with gradations in mineral content for mimicking the tendon-to-bone insertion site. *Nano Lett.* 2009;9(7):2763–2768.
doi: 10.1021/nl901582f
12. Font Tellado S, Balmayor ER, Van Griensven M. Strategies to engineer tendon/ligament-to-bone interface: biomaterials, cells and growth factors. *Adv Drug Deliv Rev.* 2015;94:126–140.
doi: 10.1016/j.addr.2015.03.004
13. Pattnaik A, Sanket AS, Pradhan S, *et al.* Designing of gradient scaffolds and their applications in tissue regeneration. *Biomaterials.* 2023;296:122078.
doi: 10.1016/j.biomaterials.2023.122078
14. Chen Y, Li Y, Zhu W, Liu Q. Biomimetic gradient scaffolds for the tissue engineering and regeneration of rotator cuff enthesis. *Biofabrication.* 2024;16(3):032005.
doi: 10.1088/1758-5090/ad467d
15. Liu H, Yang L, Zhang E, *et al.* Biomimetic tendon extracellular matrix composite gradient scaffold enhances ligament-to-bone junction reconstruction. *Acta Biomater.* 2017;56:129–140.
doi: 10.1016/j.actbio.2017.05.027
16. Lei T, Zhang T, Fang T, *et al.* Engineering a stem cell-embedded bilayer hydrogel with biomimetic collagen mineralization for tendon–bone interface healing. *Bioact Mater.* 2025;49:207–217.
doi: 10.1016/j.bioactmat.2025.03.001
17. Dakin SG, Martinez FO, Yapp C, *et al.* Inflammation activation and resolution in human tendon disease. *Sci Transl Med.* 2015;7(311):311ra173.
doi: 10.1126/scitranslmed.aac4269
18. Galatz LM, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am.* 2004;86(2):219–224.
doi: 10.2106/00004623-200402000-00002
19. Brown BN, Londono R, Tottey S, *et al.* Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials. *Acta Biomater.* 2012;8(3):978–987.
doi: 10.1016/j.actbio.2011.11.031
20. Sicari BM, Dziki JL, Siu BF, *et al.* The promotion of a constructive macrophage phenotype by solubilized extracellular matrix. *Biomaterials.* 2014;35(30):8605–8612.
doi: 10.1016/j.biomaterials.2014.06.060
21. Du L, Wu J, Han Y, Wu C. Immunomodulatory multicellular scaffolds for tendon-to-bone regeneration. *Sci Adv.* 2024;10(10):eadk6610.
doi: 10.1126/sciadv.adk6610
22. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater.* 2009;5(1):1–13.
doi: 10.1016/j.actbio.2008.09.013
23. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science.* 2009;326(5957):1216–1219.
doi: 10.1126/science.1176009

24. Naba A. Mechanisms of assembly and remodelling of the extracellular matrix. *Nat Rev Mol Cell Biol.* 2024;25(11):865-885.
doi: 10.1038/s41580-024-00767-3
25. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev.* 2016;97:4-27.
doi: 10.1016/j.addr.2015.11.001
26. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials.* 2006;27(19):3675-3683.
doi: 10.1016/j.biomaterials.2006.02.014
27. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials.* 2011;32(12):3233-3243.
doi: 10.1016/j.biomaterials.2011.01.057
28. Golebiowska AA, Intravaia JT, Sathe VM, Kumbar SG, Nukavarapu SP. Decellularized extracellular matrix biomaterials for regenerative therapies: advances, challenges and clinical prospects. *Bioact Mater.* 2024;32:98-123.
doi: 10.1016/j.bioactmat.2023.09.017
29. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. *Acta Biomater.* 2017;49:1-15.
doi: 10.1016/j.actbio.2016.11.068
30. Pati F, Jang J, Ha DH, *et al.* Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat Commun.* 2014;5(1):3935.
doi: 10.1038/ncomms4935
31. Groll J, Burdick JA, Cho DW, *et al.* A definition of bioinks and their distinction from biomaterial inks. *Biofabrication.* 2019;11(1):013001.
doi: 10.1088/1758-5090/aaec52
32. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol.* 2014;32(8):773-785.
doi: 10.1038/nbt.2958
33. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol.* 2016;34(3):312-319.
doi: 10.1038/nbt.3413
34. Hinton TJ, Jallerat Q, Palchesko RN, *et al.* Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci Adv.* 2015;1(9):e1500758.
doi: 10.1126/sciadv.1500758
35. Lee A, Hudson AR, Shiwardski DJ, *et al.* 3D bioprinting of collagen to rebuild components of the human heart. *Science.* 2019;365(6452):482-487.
doi: 10.1126/science.aav9051
36. Levato R, Jungst T, Scheuring RG, Blunk T, Groll J, Malda J. From shape to function: the next step in bioprinting. *Adv Mater.* 2020;32(12):e1906423.
doi: 10.1002/adma.201906423
37. Toprakhisar B, Nadernezhad A, Bakirci E, Khani N, Skvortsov GA, Koc B. Development of bioink from decellularized tendon extracellular matrix for 3D bioprinting. *Macromol Biosci.* 2018;18(10):e1800024.
doi: 10.1002/mabi.201800024
38. Zhao F, Cheng J, Sun M, *et al.* Digestion degree is a key factor to regulate the printability of pure tendon decellularized extracellular matrix bio-ink in extrusion-based 3D cell printing. *Biofabrication.* 2020;12(4):045011.
doi: 10.1088/1758-5090/aba411
39. Zhao F, Cheng J, Zhang J, *et al.* Comparison of three different acidic solutions in tendon decellularized extracellular matrix bio-ink fabrication for 3D cell printing. *Acta Biomater.* 2021;131:262-275.
doi: 10.1016/j.actbio.2021.06.026
40. Park W, Gao G, Cho DW. Tissue-specific decellularized extracellular matrix bioinks for musculoskeletal tissue regeneration and modeling using 3D bioprinting technology. *Int J Mol Sci.* 2021;22(15):7837.
doi: 10.3390/ijms22157837
41. Kim BS, Das S, Jang J, Cho DW. Decellularized extracellular matrix-based bioinks for engineering tissue- and organ-specific microenvironments. *Chem Rev.* 2020;120(19):10608-10661.
doi: 10.1021/acs.chemrev.9b00808
42. Abaci A, Guvendiren M. Designing decellularized extracellular matrix-based bioinks for 3D bioprinting. *Adv Healthc Mater.* 2020;9(24):e2000734.
doi: 10.1002/adhm.202000734
43. De Santis MM, Alsafadi HN, Tas S, *et al.* Extracellular-matrix-reinforced bioinks for 3D bioprinting human tissue. *Adv Mater.* 2021;33(3):e2005476.
doi: 10.1002/adma.202005476
44. Zhang H, Wang Y, Zheng Z, *et al.* Strategies for improving the 3D printability of decellularized extracellular matrix bioink. *Theranostics.* 2023;13(8):2562-2587.
doi: 10.7150/thno.81785
45. Chae S, Sun Y, Choi YJ, Ha DH, Jeon I, Cho DW. 3D cell-printing of tendon–bone interface using tissue-derived extracellular matrix bioinks for chronic rotator cuff repair. *Biofabrication.* 2021;13(3):035005.
doi: 10.1088/1758-5090/abd159

46. Chae S, Choi YJ, Cho DW. Mechanically and biologically promoted cell-laden constructs generated using tissue-specific bioinks for tendon/ligament tissue engineering applications. *Biofabrication*. 2022;14(2):025013.
doi: 10.1088/1758-5090/ac4fb6
47. Chae S, Yong U, Park W, *et al.* 3D cell-printing of gradient multi-tissue interfaces for rotator cuff regeneration. *Bioact Mater*. 2023;19:611-625.
doi: 10.1016/j.bioactmat.2022.05.004
48. Zhang X, Song W, Han K, *et al.* Three-dimensional bioprinting of a structure-, composition-, and mechanics-graded biomimetic scaffold coated with specific decellularized extracellular matrix to improve the tendon-to-bone healing. *ACS Appl Mater Interfaces*. 2023;15(24):28964-28980.
doi: 10.1021/acsami.3c03793
49. Kim WJ, Kwon DR, Lee H, *et al.* 3D bioprinted multi-layered cell constructs with gradient core-shell interface for tendon-to-bone tissue regeneration. *Bioact Mater*. 2025;43:471-490.
doi: 10.1016/j.bioactmat.2024.10.002
50. Du L, Qin C, Zhang H, *et al.* Multicellular bioprinting of biomimetic inks for tendon-to-bone regeneration. *Adv Sci*. 2023;10(21):e2301309.
doi: 10.1002/advs.202301309
51. Cao Y, Yang S, Zhao D, *et al.* Three-dimensional printed multiphasic scaffolds with stratified cell-laden gelatin methacrylate hydrogels for biomimetic tendon-to-bone interface engineering. *J Orthop Transl*. 2020;23:89-100.
doi: 10.1016/j.jot.2020.01.004
52. Bai L, Han Q, Meng Z, *et al.* Bioprinted living tissue constructs with layer-specific, growth factor-loaded microspheres for improved enthesis healing of a rotator cuff. *Acta Biomater*. 2022;154:275-289.
doi: 10.1016/j.actbio.2022.10.058
53. Jiang X, Kong Y, Kuss M, *et al.* 3D bioprinting of multilayered scaffolds with spatially differentiated ADMSCs for rotator cuff tendon-to-bone interface regeneration. *Appl Mater Today*. 2022;27:101510.
doi: 10.1016/j.apmt.2022.101510
54. Fang F, Xiao Y, Zelzer E, Leong KW, Thomopoulos S. A mineralizing pool of Gli1-expressing progenitors builds the tendon enthesis and demonstrates therapeutic potential. *Cell Stem Cell*. 2022;29(12):1669-1684.e6.
doi: 10.1016/j.stem.2022.11.007
55. Schwartz AG, Long F, Thomopoulos S. Enthesis fibrocartilage cells originate from a population of Hedgehog-responsive cells modulated by the loading environment. *Development*. 2015;142(1):196-206.
doi: 10.1242/dev.112714
56. Killian ML, Thomopoulos S. Scleraxis is required for the development of a functional tendon enthesis. *FASEB J*. 2016;30(1):301-311.
doi: 10.1096/fj.14-258236
57. Dymment NA, Hagiwara Y, Matthews BG, Li Y, Kalajzic I, Rowe DW. Lineage tracing of resident tendon progenitor cells during growth and natural healing. *PLoS One*. 2014;9(4):e96113.
doi: 10.1371/journal.pone.0096113
58. Felsenthal N, Rubin S, Stern T, *et al.* Development of migrating tendon–bone attachments involves replacement of progenitor populations. *Development*. 2018;145(24):dev165381.
doi: 10.1242/dev.165381
59. Schweitzer R, Chyung JH, Murtaugh LC, *et al.* Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. *Development*. 2001;128(19):3855-3866.
doi: 10.1242/dev.128.19.3855
60. Schwartz AG, Galatz LM, Thomopoulos S. Enthesis regeneration: a role for Gli1+ progenitor cells. *Development*. 2017;144(7):1159-1164.
doi: 10.1242/dev.139303
61. Thomopoulos S, Parks WC, Rifkin DB, Derwin KA. Mechanisms of tendon injury and repair. *J Orthop Res*. 2015;33(6):832-839.
doi: 10.1002/jor.22806
62. Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. The extracellular matrix: tools and insights for the omics era. *Matrix Biol*. 2016;49:10-24.
doi: 10.1016/j.matbio.2015.06.003
63. Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. *Mol Cell Proteomics*. 2012;11(4):M111.014647.
doi: 10.1074/mcp.M111.014647
64. Malda J, Visser J, Melchels FPW, *et al.* 25th anniversary article: engineering hydrogels for biofabrication. *Adv Mater*. 2013;25(36):5011-5028.
doi: 10.1002/adma.201302042
65. Kim WJ, Kim GH. A bioprinted complex tissue model for myotendinous junction with biochemical and biophysical cues. *Bioeng Transl Med*. 2022;7(3):e10321.
doi: 10.1002/btm2.10321
66. Liu Q, Yu Y, Reisdorf RL, *et al.* Engineered tendon-fibrocartilage-bone composite and bone marrow-derived mesenchymal stem cell sheet augmentation promotes rotator cuff healing in a non-weight-bearing canine model.

- Biomaterials*. 2019;192:189–198.
doi: 10.1016/j.biomaterials.2018.10.037
67. Shukunami C, Takimoto A, Oro M, Hiraki Y. Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes. *Dev Biol*. 2006;298(1):234–247.
doi: 10.1016/j.ydbio.2006.06.036
68. Murchison ND, Price BA, Conner DA, *et al*. Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. *Development*. 2007;134(14):2697–2708.
doi: 10.1242/dev.001933
69. Lefebvre V, Behringer RR, de Crombrughe B. L-Sox5, Sox6 and Sox9 control essential steps of the chondrocyte differentiation pathway. *Osteoarthritis Cartilage*. 2001;9(Suppl A):S69–S75.
doi: 10.1053/joca.2001.0447
70. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell*. 1997;89(5):747–754.
doi: 10.1016/S0092-8674(00)80257-3
71. Nakashima K, Zhou X, Kunkel G, *et al*. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002;108(1):17–29.
doi: 10.1016/S0092-8674(01)00622-5
72. Sugg KB, Lubardic J, Gumucio JP, Mendias CL. Changes in macrophage phenotype and induction of epithelial-to-mesenchymal transition genes following acute Achilles tenotomy and repair. *J Orthop Res*. 2014;32(7):944–951.
doi: 10.1002/jor.22624
73. Howell KL, Kaji DA, Li TM, *et al*. Macrophage depletion impairs neonatal tendon regeneration. *FASEB J*. 2021;35(6):e21618.
doi: 10.1096/fj.202100049R
74. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med*. 2012;18(7):1028–1040.
doi: 10.1038/nm.2807
75. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*. 2016;44(3):450–462.
doi: 10.1016/j.immuni.2016.02.015
76. Ackerman JE, Geary MB, Orner CA, Bawany F, Loiselle AE. Obesity/type II diabetes alters macrophage polarization resulting in a fibrotic tendon healing response. *PLoS One*. 2017;12(7):e0181127.
doi: 10.1371/journal.pone.0181127
77. Younesi FS, Miller AE, Barker TH, Rossi FMV, Hinz B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. *Nat Rev Mol Cell Biol*. 2024;25(8):617–638.
doi: 10.1038/s41580-024-00716-0
78. Murray PJ, Allen JE, Biswas SK, *et al*. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41(1):14–20.
doi: 10.1016/j.immuni.2014.06.008
79. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials*. 2009;30(8):1482–1491.
doi: 10.1016/j.biomaterials.2008.11.040
80. Valentin JE, Stewart-Akers AM, Gilbert TW, Badylak SF. Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds. *Tissue Eng Part A*. 2009;15(7):1687–1694.
doi: 10.1089/ten.tea.2008.0419
81. Sadtler K, Singh A, Wolf MT, Wang X, Pardoll DM, Elisseff JH. Design, clinical translation and immunological response of biomaterials in regenerative medicine. *Nat Rev Mater*. 2016;1(7):16040.
doi: 10.1038/natrevmats.2016.40
82. Julier Z, Park AJ, Briquez PS, Martino MM. Promoting tissue regeneration by modulating the immune system. *Acta Biomater*. 2017;53:13–28.
doi: 10.1016/j.actbio.2017.01.056
83. Sridharan R, Cameron AR, Kelly DJ, Kearney CJ, O'Brien FJ. Biomaterial based modulation of macrophage polarization: a review and suggested design principles. *Mater Today*. 2015;18(6):313–325.
doi: 10.1016/j.mattod.2015.01.019
84. Sadtler K, Estrellas K, Allen BW, *et al*. Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. *Science*. 2016;352(6283):366–370.
doi: 10.1126/science.aad9272
85. Stahl EC, Bonvillain RW, Skillen CD, *et al*. Evaluation of the host immune response to decellularized lung scaffolds derived from α -Gal knockout pigs in a non-human primate model. *Biomaterials*. 2018;187:93–104.
doi: 10.1016/j.biomaterials.2018.09.038
86. Deymier AC, An Y, Boyle JJ, *et al*. Micro-mechanical properties of the tendon-to-bone attachment. *Acta Biomater*. 2017;56:25–35.
doi: 10.1016/j.actbio.2017.01.037
87. Han WM, Heo SJ, Driscoll TP, *et al*. Microstructural heterogeneity directs micromechanics and mechanobiology

- in native and engineered fibrocartilage. *Nat Mater*. 2016;15(4):477–484.
doi: 10.1038/nmat4520
88. Ker EDF, Nain AS, Weiss LE, *et al*. Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. *Biomaterials*. 2011;32(32):8097–8107.
doi: 10.1016/j.biomaterials.2011.07.025
89. Bian X, Liu X, Zhou M, *et al*. Mechanical stimulation promotes fibrochondrocyte proliferation by activating the TRPV4 signaling pathway during tendon–bone insertion healing: CCN2 plays an important regulatory role. *Burns Trauma*. 2024;12:tkae028.
doi: 10.1093/burnst/tkae028
90. Xiao H, Zhang T, Li C, *et al*. Mechanical stimulation promotes enthesis injury repair by mobilizing Prrx1+ cells via ciliary TGF- β signaling. *eLife*. 2022;11:e73614.
doi: 10.7554/eLife.73614
91. Freeman FE, Pitacco P, van Dommelen LHA, *et al*. 3D bioprinting spatiotemporally defined patterns of growth factors to tightly control tissue regeneration. *Sci Adv*. 2020;6(33):eabb5093.
doi: 10.1126/sciadv.abb5093
92. Hettrich CM, Gasinu S, Beamer BS, *et al*. The effect of mechanical load on tendon-to-bone healing in a rat model. *Am J Sports Med*. 2014;42(5):1233–1241.
doi: 10.1177/0363546514526138
93. Peltz CD, Sarver JJ, Dourte LM, Würgler-Hauri CC, Williams GR, Soslowsky LJ. Exercise following a short immobilization period is detrimental to tendon properties and joint mechanics in a rat rotator cuff injury model. *J Orthop Res*. 2010;28(7):841–845.
doi: 10.1002/jor.21059
94. Schwartz AG, Lipner JH, Pasteris JD, Genin GM, Thomopoulos S. Muscle loading is necessary for the formation of a functional tendon enthesis. *Bone*. 2013;55(1):44–51.
doi: 10.1016/j.bone.2013.03.010
95. Phillips JE, Burns KL, Le Doux JM, Guldberg RE, García AJ. Engineering graded tissue interfaces. *Proc Natl Acad Sci USA*. 2008;105(34):12170–12175.
doi: 10.1073/pnas.0801988105
96. Sugimoto Y, Takimoto A, Akiyama H, *et al*. Scx+/Sox9+ progenitors contribute to the establishment of the junction between cartilage and tendon/ligament. *Development*. 2013;140(11):2280–2288.
doi: 10.1242/dev.096354
97. Spalazzi JP, Doty SB, Moffat KL, Levine WN, Lu HH. Development of controlled matrix heterogeneity on a triphasic scaffold for orthopedic interface tissue engineering. *Tissue Eng*. 2006;12(12):3497–3508.
doi: 10.1089/ten.2006.12.3497
98. Takayama K, Kawakami Y, Mifune Y, *et al*. The effect of blocking angiogenesis on anterior cruciate ligament healing following stem cell transplantation. *Biomaterials*. 2015;60:9–19.
doi: 10.1016/j.biomaterials.2015.03.036
99. Fealy S, Adler RS, Drakos MC, *et al*. Patterns of vascular and anatomical response after rotator cuff repair. *Am J Sports Med*. 2006;34(1):120–127.
doi: 10.1177/0363546505280212
100. Taylor DA, Sampaio LC, Ferdous Z, Gobin AS, Taite LJ. Decellularized matrices in regenerative medicine. *Acta Biomater*. 2018;74:74–89.
doi: 10.1016/j.actbio.2018.04.044
101. Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. *Proc Natl Acad Sci USA*. 2016;113(12):3179–3184.
doi: 10.1073/pnas.1521342113
102. Miller JS, Stevens KR, Yang MT, *et al*. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater*. 2012;11(9):768–774.
doi: 10.1038/nmat3357
103. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature*. 2014;507(7492):323–328.
doi: 10.1038/nature13145
104. Ramasamy SK, Kusumbe AP, Schiller M, *et al*. Blood flow controls bone vascular function and osteogenesis. *Nat Commun*. 2016;7(1):13601.
doi: 10.1038/ncomms13601
105. Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS. Hypoxia in cartilage: HIF-1 α is essential for chondrocyte growth arrest and survival. *Genes Dev*. 2001;15(21):2865–2876.
doi: 10.1101/gad.934301
106. Huang Y, He B, Wang L, *et al*. Bone marrow mesenchymal stem cell-derived exosomes promote rotator cuff tendon–bone healing by promoting angiogenesis and regulating M1 macrophages in rats. *Stem Cell Res Ther*. 2020;11(1):496.
doi: 10.1186/s13287-020-02005-x
107. Ozawa CR, Banfi A, Glazer NL, *et al*. Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *J Clin Invest*. 2004;113(4):516–527.
doi: 10.1172/JCI18420

108. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol.* 2001;19(11):1029–1034.
doi: 10.1038/nbt1101-1029
109. Cai Z, Qu C, Song W, *et al.* Hierarchical chiral calcium silicate hydrate films promote vascularization for tendon-to-bone healing. *Adv Mater.* 2024;36(31):e2404842.
doi: 10.1002/adma.202404842
110. Landis SC, Amara SG, Asadullah K, *et al.* A call for transparent reporting to optimize the predictive value of preclinical research. *Nature.* 2012;490(7419):187–191.
doi: 10.1038/nature11556
111. Ioannidis JPA, Greenland S, Hlatky MA, *et al.* Increasing value and reducing waste in research design, conduct, and analysis. *Lancet.* 2014;383(9912):166–175.
doi: 10.1016/S0140-6736(13)62227-8
112. Munafò MR, Nosek BA, Bishop DVM, *et al.* A manifesto for reproducible science. *Nat Hum Behav.* 2017;1(1):0021.
doi: 10.1038/s41562-016-0021
113. Lazic SE, Clarke-Williams CJ, Munafò MR. What exactly is ‘N’ in cell culture and animal experiments? *PLoS Biol.* 2018;16(4):e2005282.
doi: 10.1371/journal.pbio.2005282
114. Macleod MR, Lawson McLean A, Kyriakopoulou A, *et al.* Risk of bias in reports of in vivo research: a focus for improvement. *PLoS Biol.* 2015;13(10):e1002273.
doi: 10.1371/journal.pbio.1002273
115. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol.* 2020;18(7):e3000410.
doi: 10.1371/journal.pbio.3000410
116. Hussey GS, Dziki JL, Badylak SF. Extracellular matrix-based materials for regenerative medicine. *Nat Rev Mater.* 2018;3(7):159–173.
doi: 10.1038/s41578-018-0023-x
117. Hölzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A. Bioink properties before, during and after 3D bioprinting. *Biofabrication.* 2016;8(3):032002.
doi: 10.1088/1758-5090/8/3/032002
118. Ouyang L, Yao R, Zhao Y, Sun W. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication.* 2016;8(3):035020.
doi: 10.1088/1758-5090/8/3/035020
119. Paxton N, Smolan W, Böck T, Melchels F, Groll J, Jungst T. Proposal to assess printability of bioinks for extrusion-based bioprinting and evaluation of rheological properties governing bioprintability. *Biofabrication.* 2017;9(4):044107.
doi: 10.1088/1758-5090/aa8dd8
120. Schwab A, Levato R, D’Este M, Piluso S, Eglin D, Malda J. Printability and shape fidelity of bioinks in 3D bioprinting. *Chem Rev.* 2020;120(19):11028–11055.
doi: 10.1021/acs.chemrev.0c00084
121. Ribeiro A, Blokzijl MM, Levato R, *et al.* Assessing bioink shape fidelity to aid material development in 3D bioprinting. *Biofabrication.* 2018;10(1):014102.
doi: 10.1088/1758-5090/aa90e2
122. Hospodiuk M, Dey M, Sosnoski D, Ozbolat IT. The bioink: a comprehensive review on bioprintable materials. *Biotechnol Adv.* 2017;35(2):217–239.
doi: 10.1016/j.biotechadv.2016.12.006
123. Campagnola PJ, Loew LM. Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms. *Nat Biotechnol.* 2003;21(11):1356–1360.
doi: 10.1038/nbt894
124. Chen X, Nadiarynkh O, Plotnikov S, Campagnola PJ. Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure. *Nat Protoc.* 2012;7(4):654–669.
doi: 10.1038/nprot.2012.009
125. Xue J, Schmidt SV, Sander J, *et al.* Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity.* 2014;40(2):274–288.
doi: 10.1016/j.immuni.2014.01.006
126. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res.* 2010;25(7):1468–1486.
doi: 10.1002/jbmr.141
127. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature.* 2011;473(7347):298–307.
doi: 10.1038/nature10144
128. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell.* 2011;21(2):193–215.
doi: 10.1016/j.devcel.2011.07.001
129. Skylar-Scott MA, Uzel SGM, Nam LL, *et al.* Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. *Sci Adv.* 2019;5(9):eaaw2459.
doi: 10.1126/sciadv.aaw2459
130. Grigoryan B, Paulsen SJ, Corbett DC, *et al.* Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science.* 2019;364(6439):458–464.
doi: 10.1126/science.aav9750

131. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell*. 2011;9(1):11-15.
doi: 10.1016/j.stem.2011.06.008
132. Derwin KA, Baker AR, Iannotti JP, McCarron JA. Preclinical models for translating regenerative medicine therapies for rotator cuff repair. *Tissue Eng Part B Rev*. 2010;16(1):21-30.
doi: 10.1089/ten.TEB.2009.0209
133. Butler DL, Goldstein SA, Guilak F. Functional tissue engineering: the role of biomechanics in reparative medicine. *J Biomech Eng*. 2000;122(6):570-575.
doi: 10.1115/1.1318906
134. Huang CY, Wang VM, Pawluk RJ, *et al*. Inhomogeneous mechanical behavior of the human supraspinatus tendon under uniaxial loading. *J Orthop Res*. 2005;23(4):924-930.
doi: 10.1016/j.orthres.2004.02.016
135. Huang CY, Wang VM, Flatow EL, Mow VC. Temperature-dependent viscoelastic properties of the human supraspinatus tendon. *J Biomech*. 2009;42(4):546-551.
doi: 10.1016/j.jbiomech.2008.11.013
136. Fung DT, Wang VM, Andarawis-Puri N, *et al*. Early response to tendon fatigue damage accumulation in a novel in vivo model. *J Biomech*. 2010;43(2):274-279.
doi: 10.1016/j.jbiomech.2009.08.039
137. Andarawis-Puri N, Sereysky JB, Jepsen KJ, Flatow EL. The relationships between cyclic fatigue loading, changes in initial mechanical properties, and the in vivo temporal mechanical response of the rat patellar tendon. *J Biomech*. 2012;45(1):59-65.
doi: 10.1016/j.jbiomech.2011.10.008
138. Neviaser A, Andarawis-Puri N, Flatow EL. Basic mechanisms of tendon fatigue damage. *J Shoulder Elbow Surg*. 2012;21(2):158-163.
doi: 10.1016/j.jse.2011.11.014
139. Galatz LM, Sandell LJ, Rothermich SY, *et al*. Characteristics of the rat supraspinatus tendon during tendon-to-bone healing after acute injury. *J Orthop Res*. 2006;24(3):541-550.
doi: 10.1002/jor.20067
140. Taylor S, Mueller E, Jones LR, Makela AV, Ashammakhi N. Translational aspects of 3D and 4D printing and bioprinting. *Adv Healthc Mater*. 2024;13(27):e2400463.
doi: 10.1002/adhm.202400463
141. Agarwal T, Onesto V, Banerjee D, *et al*. 3D bioprinting in tissue engineering: current state-of-the-art and challenges towards system standardization and clinical translation. *Biofabrication*. 2025;17(4):042003.
doi: 10.1088/1758-5090/ade47a
142. Dearth CL, Keane TJ, Carruthers CA, *et al*. The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold. *Acta Biomater*. 2016;33:78-87.
doi: 10.1016/j.actbio.2016.01.038
143. de Wit RJJ, van Dis DJ, Bertrand ME, *et al*. Scaffold-based tissue engineering: supercritical carbon dioxide as an alternative method for decellularization and sterilization of dense materials. *Acta Biomater*. 2023;155:323-332.
doi: 10.1016/j.actbio.2022.11.028