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

Running title: dECM Bioinks for Tendon–Bone Bioprinting

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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. After adult injury, 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 pro-reparative factor, but 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, printability, increased lineage-marker expression, or a higher ultimate failure load alone cannot 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} This spatial organization emerges through coordinated programs of cell lineage specification, ECM deposition, mineralization, and mechanical loading that are only incompletely re-established after adult injury.^{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 solve 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 are not readily reproduced by single-component hydrogels.^{19–29} Tendon-, cartilage-, and bone-derived dECM are therefore of particular interest for reconstructing regionally distinct microenvironments across the tendon–bone interface. 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 remains reproducible and biologically meaningful after processing. 3D bioprinting complements dECM by enabling controlled spatial placement of materials, cells, and bioactive components.^{30–36} Multimaterial deposition, multiple-nozzle printing, and related biofabrication approaches can organize distinct formulations into predefined regions or continuous gradients, providing a manufacturing route for translating tissue-derived matrix 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 decellularized extracellular matrix (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. The Review is organized accordingly, progressing from the native enthesis and dECM processing to spatial construct design, biological maturation, evidence grading, and translational readiness.

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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	References
Lei <i>et al.</i> , 2021	Biomimetic strategies for TBI	Partial	Yes	Yes	No	No	10
Park <i>et al.</i> , 2021	Tissue-specific dECM bioinks for musculoskeletal applications	Yes	Yes	Partial	No	No	40
Kim <i>et al.</i> , 2020	dECM-based bioinks and tissue-specific microenvironments	Yes	Yes	Not TBI-specific	No	No	41
Abaci & Guvendiren, 2020	Design/rheology of dECM bioinks	Yes	Yes	No	No	No	42
De Santis <i>et al.</i> , 2021	ECM-enriched bioinks	Yes	Yes	No	No	No	43
Zhang <i>et al.</i> , 2023	Strategies to improve dECM printability	Yes	Yes	No	No	No	44
Chen <i>et al.</i> , 2024	Biomimetic gradient scaffolds for rotator cuff enthesis	Limited/not central	Yes	Yes	No	No	14
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; TBI, tendon–bone interface.

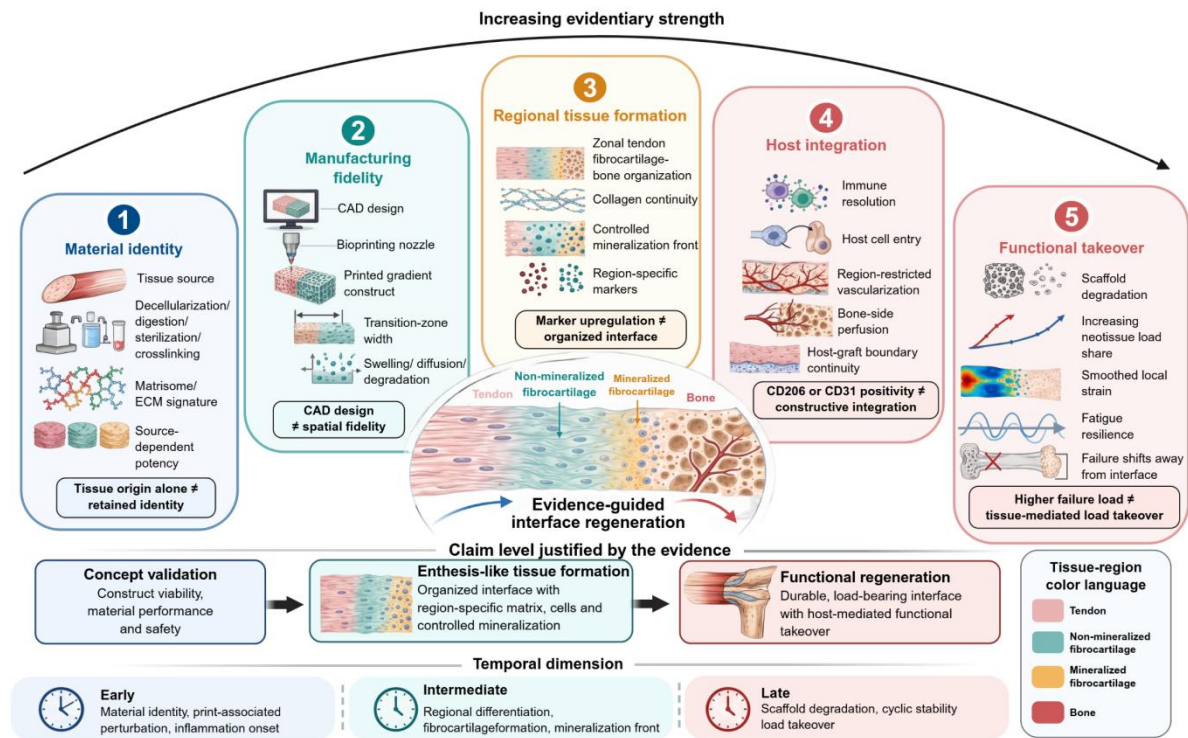


Figure 1. Author-proposed cumulative five-level evidence framework for evaluating dECM-bioprinting-mediated tendon-bone interface regeneration. The framework comprises five sequential levels of evidence—material identity, manufacturing fidelity, regional tissue formation, host integration and load takeover—with increasing evidentiary strength from left to right. The central schematic depicts the continuous tissue transition from tendon through unmineralized and mineralized fibrocartilage 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>

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 tendon–bone interface generally comprises a tendon region, an unmineralized fibrocartilage region, a mineralized fibrocartilage 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 fibrocartilage 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 fibrocartilage 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 tendon–bone interface does not arise from the simple juxtaposition of four zones, but from the continuously changing spatial order between them. Using multiscale imaging and micromechanical analysis, Rossetti et al. 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,C).⁴ Genin et al. further showed that functional gradients in mineral and collagen help reduce mechanical mismatch between compliant and rigid tissues.⁵ The native tendon–bone interface can therefore be regarded as a biologically functional graded 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 is formed as a continuous attachment unit rather than by late-stage joining of mature tendon and bone. Scx/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 the tendon–bone interface. 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 tendon–bone interface 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 tendon–bone interface injury is not a reactivation of the embryonic enthesis-development program, but a reparative response prioritizing 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 tendon–bone interface.

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 rather than 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 tendon–bone interface, the relevant regenerative requirement is therefore timely inflammatory resolution rather than complete suppression of early inflammation.

The central obstacle during the proliferative phase is likewise not an insufficient number of cells or inadequate collagen deposition, but the absence 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. 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.³ Failure of the proliferative phase therefore reflects not an inadequate repair response, but 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 recreate 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 tendon–bone interface, 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.

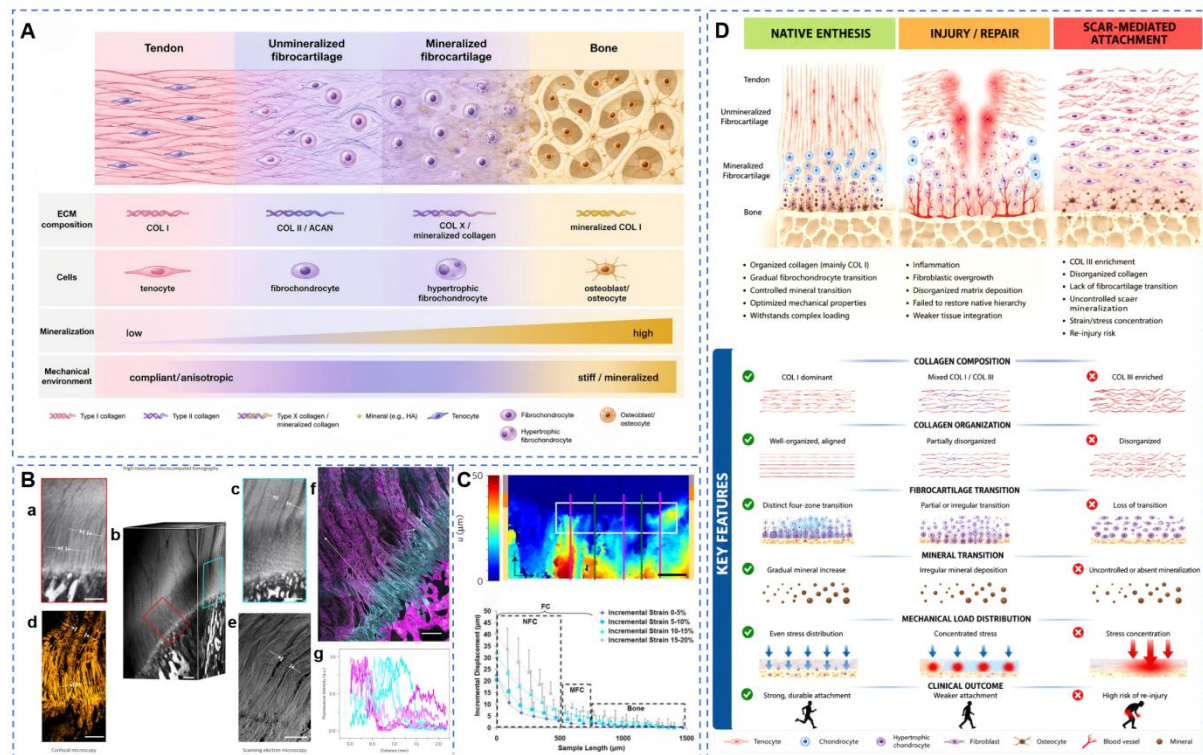


Figure 2. Multiscale structure, micromechanics and scar-mediated healing of the tendon–bone enthesis. (A) Idealized tissue organization of a fibrocartilaginous tendon–bone enthesis along the tendon-to-bone axis, comprising tendon, unmineralized fibrocartilage, mineralized fibrocartilage and bone regions. 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 type I collagen-rich tendon matrix to type II collagen-rich interface matrix. Reprinted with permission from Rossetti et al.⁴ Copyright © 2017, Macmillan Publishers Limited, part of Springer Nature. All rights reserved. (C) Representative displacement maps and incremental displacement curves during stepwise tensile loading show spatially heterogeneous deformation and progressive load transfer across unmineralized fibrocartilage, mineralized fibrocartilage and bone. Reprinted with permission from Rossetti et al.⁴ Copyright © 2017, Macmillan Publishers Limited, part of Springer Nature. All rights reserved. (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>

3. From dECM Material Information to Spatially Programmed Tendon–Bone Constructs

3.1 Preservation of Tissue-Source Information during dECM 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 identity and tissue-relevant potency should be supported by residual ECM composition, molecular structure, and cellular assays rather than inferred from tissue source alone. Different tissue sources of dECM have plausible source-associated relevance to distinct regions of the tendon–bone interface. Tendon-derived dECM 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 more suitable for reproducing the tendon-side microenvironment. Bone-derived dECM contains mineralization-related matrix proteins and osteogenic cues and may provide a more native bone-matrix-like inductive environment for bone-side integration. Cartilage-derived dECM 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. Its value 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. emphasized that the biological

function of dECM hydrogels is closely related to tissue source, preparation method and gel architecture.^{29,62,63} In tendon–bone bioprinting, dECM is best viewed as a processed material whose value depends on retaining tissue-specific matrix cues. Whether these cues are subsequently translated 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, source-associated responses should be interpreted at the integrated-construct level unless dECM-specific potency is isolated.

3.1.2 Preservation of Matrix Information during Bioink Formulation and Printing

3D 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. demonstrated that tissue-derived dECM can retain measurable source-associated biological activity after solubilization, gelation, and printing (Figure 3B,C,F).³⁰ 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} Tendon-dECM 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 tendon–bone interface 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,D,E). The purpose of these additions is not to maximize construct complexity, but to convert tissue-derived matrix cues into spatially locatable and verifiable signals while maintaining adequate printability and 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.

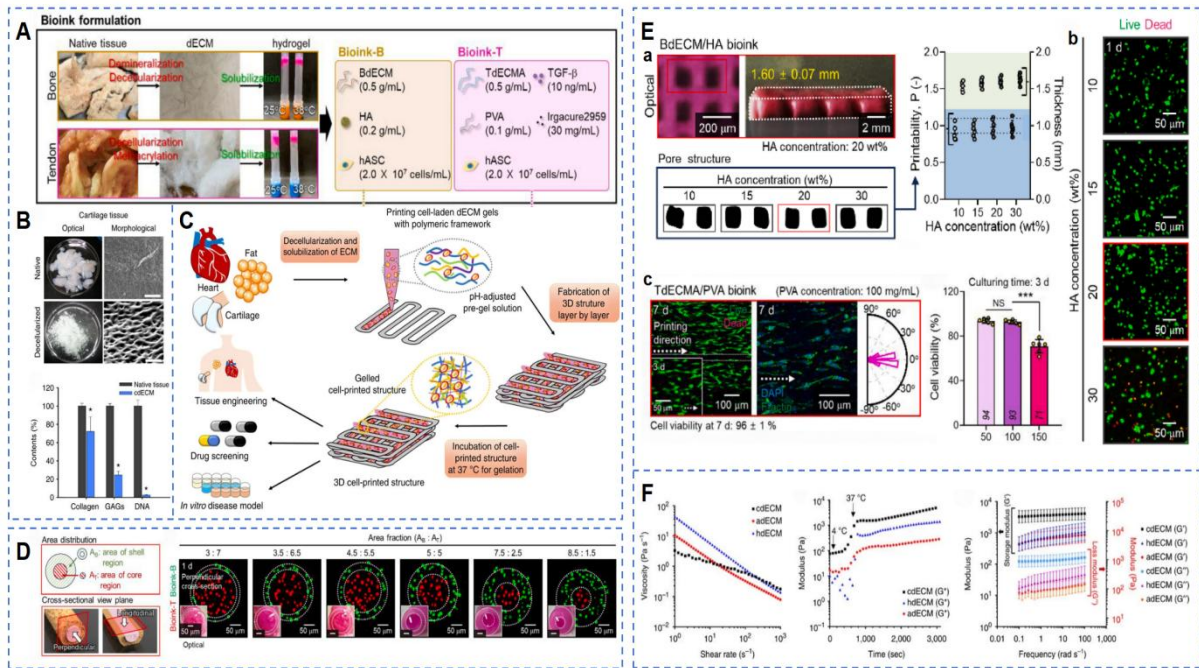


Figure 3. Manufacturing workflow, structure–process relationships and critical quality attributes of tissue-derived dECM bioinks for tendon–bone interface biofabrication. (A) Representative preparation and formulation of bone- and tendon-directed dECM bioinks. Bone-derived decellularized extracellular matrix (BdECM) was combined with hydroxyapatite and human adipose-derived stem cells, whereas methacrylated tendon-derived dECM (TdECMA) was combined with poly(vinyl alcohol), transforming growth factor- β , a photoinitiator and human adipose-derived stem cells. Reprinted with permission from Kim et al.⁴⁹ Copyright © 2024, The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (B) Representative morphological and biochemical quality-control assessment of cartilage-derived dECM, 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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 hydroxyapatite 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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.

3.2 Spatial Translation of dECM Information into Tendon–Bone Interface Architecture

3.2.1 Single-Source dECM 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. Tendon-derived dECM 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. Tendon-derived dECM 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 tendon–bone interface is not a single local tissue, but 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 tendon–bone interface. 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 dECM 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 tendon–bone interface regeneration. A useful working design principle is source-to-zone matching. Tendon-derived dECM is assigned to the tendon-side region to provide tenogenic matrix cues, whereas cartilage- or fibrocartilage-derived dECM may provide more directly relevant matrix information for the transition zone. Bone-derived dECM is assigned to the bone-side region, optionally together with mineralized components, to support osseous integration. The critical feature of this strategy is not the simple stacking of different materials, but the use of source-dependent matrix differences to confer an appropriate tissue identity on each region within the same construct. Chae et al. used tissue-derived ECM bioinks for 3D cell printing of a tendon–bone interface construct for chronic rotator cuff repair, demonstrating that dECM from different tissues can create regionalized interface microenvironments.⁴⁵ In a later study of a graded multitissue interface, Chae et al. 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 transitional architecture of the tendon–bone interface.⁴⁷ 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 requires separation 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 the regional boundary is an abrupt layer interface or a continuous transition generated through 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., in which tendon-, cartilage-, and bone-related dECM coatings were integrated with structural, compositional, and mechanical gradients (Figure 4C).⁴⁸ 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 but participate in establishing regional identity and remodeling the local matrix. MSCs, TSPCs, 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 this 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 a separate cell- or drug-delivery strategy. 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 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.

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.

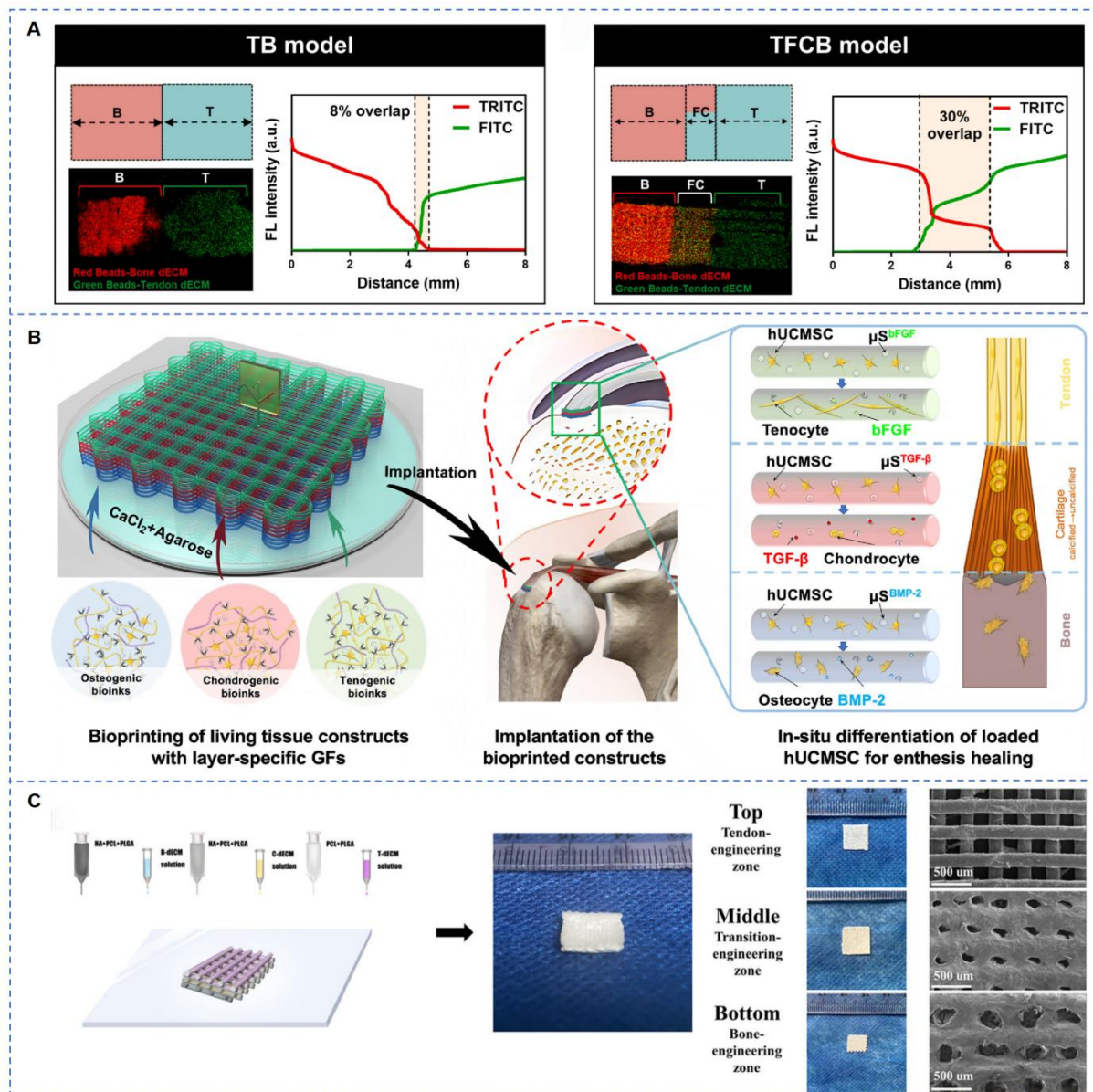


Figure 4. Spatial and spatiotemporal programming strategies for tendon–bone interface engineering constructs. (A) Representative comparison of a biphasic tendon–bone (TB) model and a triphasic tendon–fibrocartilage–bone (TFCB) model fabricated by 3D cell printing. Red fluorescent microspheres label bone-derived decellularized extracellular matrix (BdECM), and green fluorescent microspheres label tendon-derived decellularized extracellular matrix (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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (B) Schematic of a construct containing human umbilical-cord mesenchymal stem cells and layer-specific growth-factor-loaded microspheres. Microspheres in different regions contain basic fibroblast growth factor, transforming growth factor- β or bone morphogenetic protein-2, with the aim of sustaining local biochemical presentation 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. Published by Elsevier Ltd. All rights reserved. (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-tissue-engineering, intermediate-transition-tissue-engineering and bone-tissue-engineering regions, respectively. Reprinted with permission from Zhang et al.⁴⁸ Copyright © 2023, American Chemical Society.

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	Ref.
<i>Direct evidence—dECM-integrated tendon–bone interface studies</i>						
Chae et al., 2021	Spatially graded hBMMSC-laden TdECM/mixed dECM/BdECM zones integrated with a PU/PCL support patch; in vitro, 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 / entheses-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.	45
Chae et al., 2023	Biphasic tendon–bone construct versus a triphasic tendon–fibrocartilage–bone construct containing a mixed TdECM/BdECM intermediate zone and hBMMSCs; in vitro, 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 tendon- and bone-derived dECM 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 / entheses-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.	47
Zhang et al., 2023	3D-printed structure-, composition-, and mechanics-graded scaffold with zone-specific tendon-, cartilage-, and bone-related dECM coatings; in vitro 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 / entheses-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.	48
Kim et al., 2025	Gradient core–shell construct combining BdECM/HAp and TdECMA/PVA/TGF- β bioinks with hASCs to generate bone-, interface-, and tendon-directed regions; in vitro, 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 in vivo follow-up.	49
<i>Supportive evidence—dECM bioinks and related interface models</i>						
Pati et al., 2014	Adipose-, cartilage-, and cardiac-derived dECM bioinks containing tissue-relevant cells, deposited by extrusion and stabilized by thermal gelation; in vitro tissue analogues, 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: in vitro, non-TBI model without orthotopic or mechanically relevant validation.	30

Study	Construct / model & follow-up	Key comparator(s) / confounder(s)	Key evidence supporting claim	Claim level / highest claim supported	Missing decisive evidence / translational limitation	Ref.
Zhao et al., 2021	Tendon dECM bioinks prepared using three acidic solubilization systems to compare formulation-dependent gelation, rheology, and biological performance; in vitro 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 in vitro study with no long-term manufacturing or in vivo validation.	39
Chae et al., 2022	Tendon- and ligament-specific dECM bioinks used for support-bath printing of aligned, longitudinally channeled cell-laden constructs; in vitro, 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 physiologic tendon–bone loading.	46
Kim and Kim, 2022	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 non-matched ECM contexts. The model addressed a muscle–tendon rather than a tendon–bone interface.	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: in vitro non-TBI interface model.	65
Supportive evidence—tendon–bone design or mechanism studies without dECM						
Cao et al., 2020	Multiphasic PCL/PCL–TCP scaffold with stratified GelMA-encapsulated fibroblasts, BMSCs, and osteoblasts to emulate tendon, fibrocartilage, and bone regions; in vitro, 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 in vivo.	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.	51
Bai et al., 2022	Bioprinted living construct containing layer-specific bFGF-, TGF- β -, and BMP-2-loaded microspheres for tendon-, fibrocartilage-, and bone-directed signaling; in vitro 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.	52
Jiang et al., 2022	Multilayer scaffold containing spatially pre-differentiated autologous ADMSCs and a melt-electrowritten mechanical support structure; in vitro 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.	53
Liu et al., 2019	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.	66

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.

3.3 Biological Maturation of Regionalized dECM 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. Tendon-derived dECM retains matrix components associated with tenogenic microenvironments and can support SCX/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} Cartilage-derived 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 Bone-derived dECM, mineralized components, and osteogenic cues can support RUNX2/SP7-associated osteogenic responses.^{70,71} These source-associated effects do not map one-to-one onto terminal lineages but instead 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. Chae et al.^{45,47} demonstrated regionally distinct microenvironments and tissue responses in dECM-integrated constructs, while gradient core–shell and multicellular printing studies further showed that coordinated cellular, material, and geometric patterning can promote multiregional organization (Figure 5A, D).^{49,50} 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 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 the kinetics and products of degradation.^{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 an additional 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 tendon–bone interface, the preferred trajectory may also differ regionally: tendon-side repair requires inflammatory resolution without persistent fibrosis, the unmineralized transition should avoid excessive vascular–inflammatory invasion and premature mineralization, and bone-side maturation requires compatible inflammatory resolution and angiogenic–osteogenic coupling. A uniformly M2-oriented immune 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 improvements in 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. 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. Digestion degree 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. Tissue-specific dECM-printing studies 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. Tendon-derived dECM 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 fibrocartilage-cell responses through TRPV4-related mechanosensing,⁸⁹ while TGF- β 1 activation and *Prrxl*-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} Bone-derived dECM, hydroxyapatite and β -TCP 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. Mineral reconstruction should therefore establish a controlled front progressing from unmineralized fibrocartilage through mineralized fibrocartilage to bone, thereby limiting expansion of mineral 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

dECM 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 matched to 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, native microvascular lumina and their long-range continuity are generally not preserved in dECM. Its principal role is instead 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 HIF-1 α -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 PECAM-1 and 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 PDGF-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 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/VEGFR2-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.

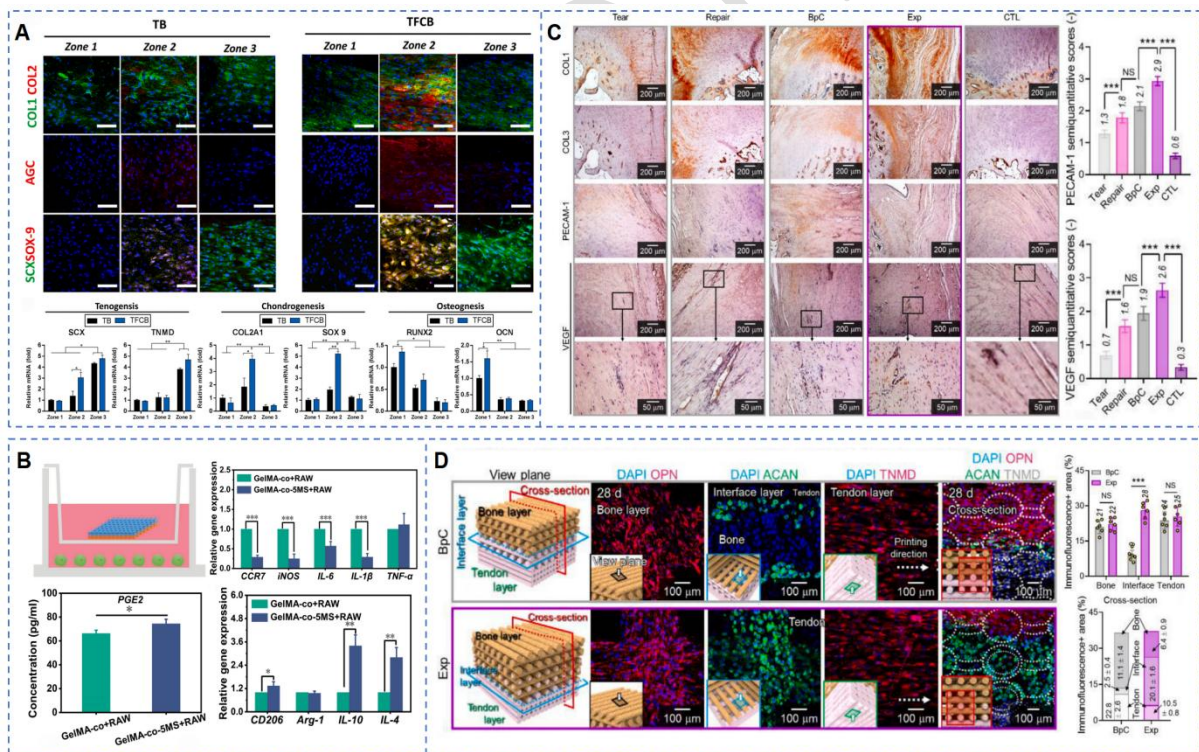


Figure 5. Mechanism-related regional cell–matrix responses and host integration in spatially programmed tendon–bone constructs. (A) Region-specific cell–matrix responses in biphasic tendon–bone (TB) and triphasic tendon–fibrocartilage–bone (TFCB) constructs after 14 d of culture. Zone 1 is the bone-directed 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. Quantitative reverse-transcription polymerase chain reaction shows regional differences in the transcription of the tenogenic genes *SCX* and *TNMD*, the chondrogenic genes *COL2A1* and *SOX9*, and the osteogenic genes *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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (B) Transwell co-culture system comprising a bilayer GelMA multicellular scaffold containing tendon stem/progenitor cells and bone-marrow 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; exclusive licensee American Association for the Advancement of Science. (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, platelet endothelial cell adhesion molecule-1 and vascular endothelial growth factor 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd.

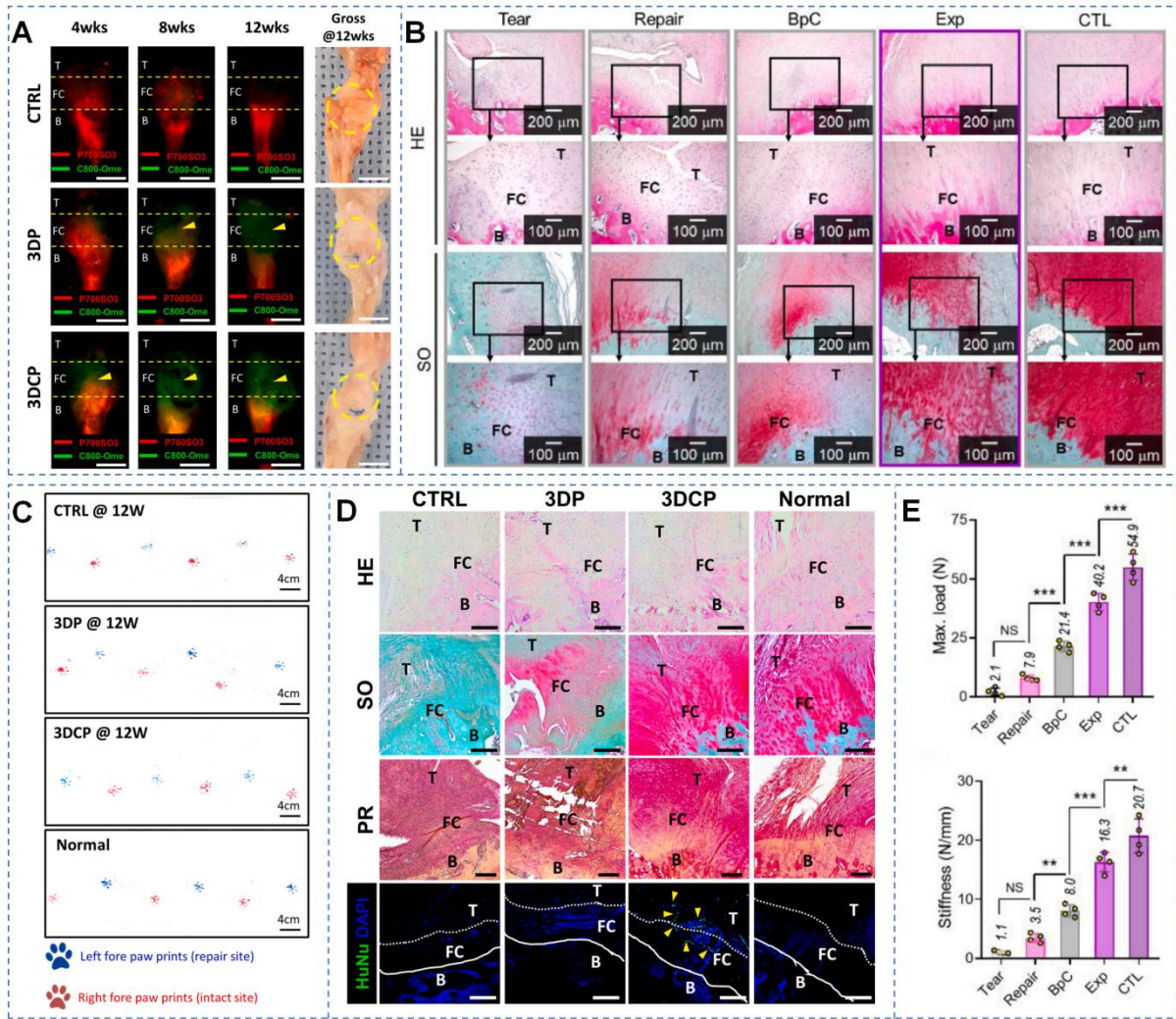


Figure 6. In vivo matrix remodeling and functional outcomes associated with spatially organized dECM tendon–bone constructs. (A) Longitudinal dual-channel near-infrared fluorescence imaging at 4, 8 and 12 weeks after repair in a rat model of chronic rotator cuff tear. The CTRL group underwent surgical repair without construct implantation, the 3DP group received an acellular 3D-printed 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (D) Representative H&E, Safranin O and Picrosirius Red 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (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 $\pm$ 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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd.

4. A Five-Level Interpretive Framework for Evaluating dECM-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 tendon–bone interface regeneration, whereas 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, subtractive, 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 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..

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
Material identity	Does dECM retain a distinguishable, reproducible, source-dependent molecular identity and biological potency after decellularization, digestion, sterilization, crosslinking, and printing?	• Joint evidence of cell removal, controlled residual risks, retained ECM/matrix features, and use-related potency • Variation reported across independent donors and processing batches • Common decellularization references: 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
Manufacturing fidelity	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/infrared and elemental imaging, 3D reconstruction, apoptosis, oxidative stress, and metabolic assays	• CAD design 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 IF/IHC, qPCR, or spatial omics • Polarized Picrosirius Red, second-harmonic generation imaging, and collagen-orientation analysis • Safranin O/ACAN/COL2, micro-CT/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
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-resolution 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

Evidence stage	Core scientific question	Minimum evidence	Recommended assessments	Critical comparators and spatiotemporal design	Common overinterpretation	Claim justified	References
Neotissue-mediated load takeover	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/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: Here, minimum evidence denotes the minimum combination of evidence required for the stated claim rather than a universal fixed threshold; common reference ranges are provided only for established decellularization criteria. The evidence stages correspond to the cumulative framework in Figure 1 and do not constitute a formal risk-of-bias rating. 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: ACAN, aggrecan; ARRIVE, Animal Research: Reporting of In Vivo Experiments; CAD, computer-aided design; dECM, decellularized extracellular matrix; DAPI, 4',6-diamidino-2-phenylindole; DIC, digital image correlation; 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; SHG, second-harmonic generation; α -SMA, alpha-smooth muscle actin.

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 residual-DNA, DNA-fragment, and nuclear-staining criteria 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 CAD Design to Persistent Spatial Gradients

Manufacturing fidelity should be evaluated as a reproducible process window linking material properties, printing conditions, and fabrication outcomes. Relevant pre-, in-, and post-print 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, multiple-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 tendon-dECM 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 design, 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.

4.4 Level 3—Regional Tissue Formation: From Zonal Cell States to Continuous Neotissue ECM

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 tendon- and bone-derived dECM 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, together 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 acquires regenerative significance only when these cellular states are translated 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 establishment of a host-connected and 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.

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. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. (E) Time-dependent in vitro responses of collagen, ligament-derived dECM 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.

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 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 under way, assessment should expand beyond bulk ultimate 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, spatially continuous load transfer, 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 dECM 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 GMP-compatible manufacture will additionally require standardized tissue sourcing, decellularization and formulation, controlled printing and crosslinking, validated aseptic or sterilization procedures, packaging, storage, and stability testing.^{28,140–143} Regulatory classification should be described as dependent on the final product configuration and jurisdiction rather than 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. Conclusions 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 material clearance. Evidence of this transition is needed before enthesis-like tissue formation can be described as functional tendon–bone interface regeneration.

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Conflict of interest

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Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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

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