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Collagen-Based Bioactive Materials for Tissue Engineering: From Fundamental Properties to Clinical Translation and Future Horizons

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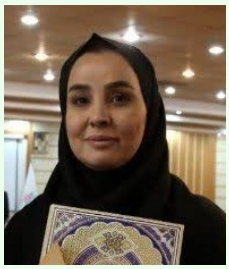

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Abstract

Collagen, the predominant structural protein in the extracellular matrix (ECM), has gained significant attention as a natural bioactive material for tissue engineering. Its intrinsic biocompatibility, biodegradability, and low immunogenicity, together with cell-recognition motifs such as Arg-Gly-Asp (RGD), enable specific interactions with integrins and growth factors that regulate cell adhesion, migration, and differentiation. Recent progress in collagen-based scaffold design including hydrogels, sponges, nanofibers, and composite matrices, has expanded its use across multiple tissue systems such as bone, cartilage, skin, vascular, corneal, and neural regeneration. Incorporation of synthetic polymers (e.g., polycaprolactone, polylactic acid, polyethylene glycol) and inorganic bioactives (e.g., hydroxyapatite, bioactive glass, silica nanoparticles) has enhanced the mechanical performance and degradation control of collagen-based constructs, addressing the limitations of native collagen. Nevertheless, batch variability, rapid enzymatic degradation, and limited long-term stability continue to constrain clinical applications. Emerging directions, including recombinant and marine-derived collagens, nanocomposite reinforcement, gene-activated matrices, and 3D/4D bioprinting technologies, are opening new pathways toward personalized, scalable, and immunocompatible tissue-engineered products. This review synthesizes two decades of progress, arguing that the convergence of recombinant sourcing, smart composite design, and advanced biofabrication is poised to overcome historical limitations and unlock the full potential of collagen for patient-specific regenerative therapies.

Keywords: Collagen; Tissue Engineering; Bioactive Materials; Extracellular Matrix; Scaffolds; Regenerative Medicine; 3D Bioprinting; Wound Healing.

1. Introduction

Tissue engineering and regenerative medicine have emerged as rapidly advancing interdisciplinary fields aiming to restore, maintain, or improve the function of damaged tissues through the combined use of cells, biomaterials, and bioactive signaling molecules (1,2). Among the various classes of biomaterials investigated, natural polymers have attracted significant attention due to their intrinsic biocompatibility, biodegradability, and ability to mimic the native extracellular matrix (ECM) microenvironment (3). Synthetic polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and polycaprolactone (PCL) offer favorable mechanical strength and processing versatility; however, they often lack the biological recognition sites necessary for effective cell adhesion and signaling (4,5). In contrast, natural biomacromolecules such as collagen, chitosan, gelatin, and hyaluronic acid possess bioactive motifs that promote cellular attachment, proliferation, and differentiation (6,7).

Collagen is the most abundant structural protein in the ECM, accounting for approximately 25–30% of total body protein in mammals (8). It provides mechanical support and biochemical cues that are essential for tissue integrity and homeostasis. The collagen molecule is characterized by a right-handed triple-helical conformation composed of three polypeptide α-chains arranged in repeating Gly–X–Y sequences, where X and Y are frequently proline and hydroxyproline (9). This unique molecular architecture not only confers mechanical stability but also exposes specific amino acid motifs, such as Arg–Gly–Asp (RGD), which mediate cell–matrix interactions via integrin binding (10,11). To date, at least 29 distinct collagen types have been identified, with Type I comprising nearly 90% of the total collagen content and being predominant in bone, skin, tendon, and ligament tissues (12).

Owing to these properties, collagen has been extensively used as a bioactive scaffold material in diverse tissue engineering applications, including skin substitutes, bone grafts, corneal implants, and nerve conduits (13-15). Its inherent biocompatibility, low immunogenicity, and biodegradability make it an ideal candidate for scaffold design and drug delivery systems (16,17). However, native collagen exhibits several limitations, such as poor mechanical strength, rapid enzymatic degradation, and source-dependent variability, which can hinder reproducibility and long-term functionality (18,19). These drawbacks have driven intensive research efforts toward developing composite and hybrid collagen-based biomaterials, wherein collagen is blended or crosslinked with synthetic polymers (e.g., PCL, PEG, PLA) or inorganic fillers such as hydroxyapatite (HAp) and bioactive glass to enhance structural integrity and biological performance (20-22).

Advances in fabrication technologies, including electrospinning, freeze-drying, self-assembly, and three-dimensional (3D) or four-dimensional (4D) bioprinting, have further expanded the design possibilities for collagen-based scaffolds, allowing precise control over architecture, porosity, and degradation behavior (23-26). Furthermore, recent developments in recombinant and marine-derived collagen have provided sustainable, pathogen-free alternatives to traditional bovine or porcine sources, offering improved purity, molecular uniformity, and reduced immunogenicity (27,28).

This review provides a comprehensive overview of collagen-based bioactive materials in tissue engineering, emphasizing their sources, extraction and purification techniques, structural and functional characteristics, and their diverse biomedical applications. Particular attention is given to recent innovations in hybrid and nanocomposite collagen systems, emerging fabrication technologies, and the translational potential of next-generation collagen-based scaffolds for personalized regenerative therapies.

2. Collagen: Source and Extraction

Collagen is ubiquitously distributed in multicellular organisms and serves as the primary component of connective tissues. Its source and extraction method play critical roles in determining physicochemical properties, purity, and biological performance of the final biomaterial (29,30). Historically, animal-derived collagen from mammalian sources such as bovine dermis and porcine tendon has dominated biomedical applications due to its high yield and well-characterized molecular structure (31). However, the risk of zoonotic disease transmission (e.g., bovine spongiform encephalopathy) and religious or ethical restrictions have motivated exploration of alternative sources, including marine, avian, and recombinant systems (32-34).

2.1 Natural Sources

2.1.1 Mammalian Collagen

Bovine and porcine collagens are the most extensively used owing to their high structural similarity to human collagen and established purification protocols (35). Bovine Type I collagen, extracted primarily from hide or Achilles tendon, exhibits robust fibril-forming ability, whereas porcine collagen is often preferred for soft-tissue regeneration because

of its fine fibrillar architecture (36,37). Despite their biological advantages, mammalian collagens may provoke mild immunogenic responses if telopeptide regions remain intact (38).

2.1.2 Marine Collagen

Marine organisms—including fish skin, scales, bones, jellyfish, and sponges—provide an abundant, low-risk alternative to terrestrial animals (39-41). Marine collagen offers additional advantages such as lower antigenicity, avoidance of zoonotic concerns, and extraction under milder conditions due to lower denaturation temperature (42,43). However, its reduced thermal stability compared to mammalian collagen may limit applications in load-bearing tissues, necessitating stabilization via crosslinking or blending with synthetic polymers (44).

2.1.3 Avian, Plant, and Recombinant Collagen

Avian sources, such as chicken sternal cartilage, yield collagen types II and IX valuable for cartilage engineering (45). Recombinant collagen, produced through yeast (*Pichia pastoris*), bacterial (*E. coli*), and plant systems, provides a highly controllable, pathogen-free alternative that allows for sequence customization and functional peptide incorporation (46-48). Advances in recombinant expression and gene-edited systems now permit scalable production of human-identical collagen with consistent molecular weight and purity suitable for clinical use (49,50).

2.2 Extraction and Purification Methods

Collagen extraction is commonly performed through acid, enzymatic, or combined approaches that disrupt crosslinked telopeptide regions and solubilize fibrillar collagen (51). The choice of extraction method affects molecular integrity, fibrillogenesis capability, and bioactivity.

2.2.1 Acid-Solubilized Collagen (ASC)

Acid extraction using dilute organic acids (e.g., acetic, citric, or lactic acid) disrupts non-covalent interactions, yielding acid-solubilized collagen (ASC) with intact telopeptides (52,53). ASC maintains native helical conformation but may contain antigenic epitopes associated with immunogenicity (54).

2.2.2 Enzymatic Extraction and Atelocollagen

Enzymatic digestion most commonly with pepsin—removes telopeptide regions, producing atelocollagen, which exhibits reduced antigenicity while retaining triple-helix integrity (55,56). Other proteolytic enzymes such as collagenase or papain have also been used to enhance yield and solubility (57). Pepsin-solubilized collagen is particularly suitable for medical implants and injectable formulations because of its improved biocompatibility (58).

2.2.3 Alkaline and Salt Extraction

Mild alkaline treatment can remove non-collagenous proteins and pigments prior to solubilization (59). Salt precipitation (e.g., with NaCl or ammonium sulfate) assists in selective recovery of collagen from crude extracts, improving purity and fibril assembly capability (60).

2.2.4 Marine Collagen Extraction

Marine collagen is generally extracted at lower temperatures (4–10 °C) using acid and enzyme combinations to prevent thermal denaturation (61,62). Optimization of pH, ionic strength, and extraction duration is essential to maximize yield while maintaining molecular integrity (63).

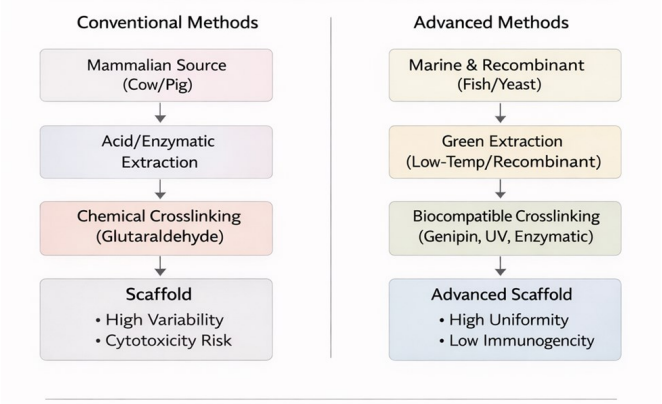
2.2.5 Purification and Characterization

Purification typically involves dialysis, centrifugation, and lyophilization to remove residual salts and acids. Analytical confirmation through SDS-PAGE, FTIR, and DSC ensures retention of triple-helical structure and thermal stability (64-66).

2.3 Crosslinking and Stabilization

Native collagen exhibits rapid degradation and low mechanical resilience; hence, crosslinking is critical to enhance stability and tune degradation kinetics (67). Physical methods such as dehydrothermal (DHT) and ultraviolet (UV) irradiation create intermolecular covalent bonds without toxic residues (68). Chemical crosslinkers including glutaraldehyde, carbodiimide (EDC/NHS), and genipin provide tunable mechanical reinforcement but require careful control to avoid cytotoxicity (69-71). In recent years, natural and enzymatic crosslinkers such as tannic acid, riboflavin, and microbial transglutaminase—have emerged as biocompatible

alternatives for medical-grade scaffolds (72-74). Crosslinked collagen scaffolds demonstrate improved mechanical integrity, reduced swelling, and slower enzymatic degradation while preserving bioactivity,



making them ideal for load-bearing and long-term regenerative applications (75) (Fig 1).

Figure 1: Conventional versus advanced collagen processing strategies. Schematic comparison of traditional mammalian-derived collagen processing using acid/enzymatic extraction and chemical crosslinking versus advanced marine and recombinant approaches employing green extraction and biocompatible crosslinking. Advanced methods result in scaffolds with improved uniformity and reduced immunogenicity, enhancing their suitability for biomedical applications.

3. Modification and Functionalization of Collagen

While native collagen offers favorable biological properties, its inherent limitations such as poor mechanical strength, rapid enzymatic degradation, and low thermal stability often necessitate modification to meet the demanding requirements of tissue engineering applications. Strategic functionalization not only addresses these drawbacks but also introduces new bioactive, mechanical, and physical functionalities, enabling tailored interactions with cells and tissues (76).

3.1 Chemical Modification

Chemical modification of collagen involves the introduction of functional groups or covalent bonding of bioactive molecules to enhance stability, bioactivity, or degradation profiles.

3.1.1 Amino Group Modification

The abundant ε-amino groups of lysine and hydroxylysine residues are common sites for chemical conjugation. **Carbodiimide chemistry (EDC/NHS)** is widely used to crosslink collagen or to conjugate peptides, growth factors, and glycosaminoglycans (GAGs) without introducing cytotoxic by-products (77,78).

3.1.2 Carboxyl Group Activation

Glutamic and aspartic acid residues can be activated for coupling with amine-containing molecules, enabling the introduction of cell-adhesive motifs (e.g., RGD, GFOGER) or therapeutic agents (79).

3.1.3 Thiolation

The introduction of thiol groups (-SH) via reagents such as 2-iminothiolane (Traut's reagent) allows for disulfide bond formation or Michael-type addition with maleimide-functionalized biomolecules, enhancing scaffold stability and enabling controlled drug release (80).

3.1.4 Photo-crosslinkable Groups

Methacrylation or acrylation of collagen (producing Col-MA or Col-AA) introduces polymerizable groups that enable UV- or visible-light-initiated crosslinking, facilitating the fabrication of shape-stable hydrogels with tunable stiffness and degradation rates (81,82).

3.2 Physical and Enzymatic Crosslinking

Crosslinking remains a cornerstone of collagen modification, directly influencing mechanical properties, swelling behavior, and degradation kinetics.

3.2.1 Physical Methods

Dehydrothermal (DHT) treatment and UV irradiation induce inter- and intramolecular crosslinks through non-enzymatic glycation and radical-mediated reactions, respectively, without introducing exogenous chemicals (83). Plasma treatment can introduce reactive oxygen and nitrogen species that modify surface

chemistry and enhance wettability and cell adhesion (84).

3.2.2 Enzymatic Crosslinking

Microbial transglutaminase (mTG) catalyzes the formation of ε-(γ-glutamyl)lysine isopeptide bonds between collagen molecules, resulting in stable, biocompatible networks (85). Lysyl oxidase (LOX)-mediated crosslinking mimics the natural maturation of collagen in vivo, improving mechanical resilience and biological integration (86).

3.3 Biofunctionalization

Biofunctionalization aims to enhance the biological performance of collagen scaffolds by incorporating bioactive molecules that guide cell behavior.

3.3.1 Peptide Conjugation

Short peptide sequences, such as RGD (for integrin binding) (Arginine-Glycine- Aspartic Acid) IKVAV (for neural adhesion) (Isoleucine-Lysine-Valine-Alanine-Valine)or KRSR (for osteoblast attachment) (Lysine-Arginine-Serine-Arginine,) can be covalently grafted to collagen to promote specific cellular responses (87,88).

3.3.2 Growth Factor Immobilization

Covalent tethering of growth factors (e.g., VEGF, BMP-2, FGF-2) to collagen matrices prolongs their local bioavailability and reduces off-target effects compared to simple adsorption (89).

3.3.3 Glycosaminoglycan (GAG) Integration

Blending or conjugating GAGs such as chondroitin sulfate, hyaluronic acid, or heparin enhances water retention, modulates growth factor release, and mimics the native ECM microenvironment (90,91).

3.3.4 ECM-Mimetic Hybridization

Collagen can be combined with other natural polymers (e.g., elastin, fibronectin, laminin) to better replicate the biochemical complexity of specific tissues (92,93).

3.4 Nanocomposite Integration

The incorporation of nanoscale fillers into collagen matrices imparts enhanced mechanical, electrical, or antimicrobial properties.

3.4.1 Inorganic Nanoparticles

Hydroxyapatite (nano-HAp), bioactive glass nanoparticles, and silica nanoparticles improve osteoconductivity, stiffness, and mineralization capacity (94-96).

3.4.2 Carbon-Based Nanomaterials

Graphene oxide (GO) and carbon nanotubes (CNTs) introduce electrical conductivity, enhance tensile strength, and can modulate oxidative stress responses, benefiting neural and cardiac tissue engineering (97).

3.4.3 Metallic Nanoparticles

Silver, gold, or ceria nanoparticles provide antimicrobial, anti-inflammatory, or antioxidant functionalities, respectively (98).

3.5 Smart and Responsive Collagen Systems

Recent advances have led to the development of "smart" collagen materials that respond to physiological or external stimuli.

3.5.1 Thermo-responsive Collagen-Gelatin Systems

Leveraging the sol–gel transition of gelatin, hybrid formulations enable injectable, in situ-gelling scaffolds for minimally invasive delivery (99).

3.5.2 pH- and Enzyme-Sensitive Hydrogels

Incorporation of pH-labile linkers or MMP-cleavable peptides allows for degradation in response to local microenvironmental cues, facilitating cell-driven remodeling (100).

3.5.3 4D Bioprinting

Collagen-based bioinks that change shape or stiffness over time in response to hydration, temperature, or chemical signals enable the fabrication of dynamic constructs that better mimic native tissue development (101,102).

3.6 Characterization of Modified Collagen

Rigorous characterization is essential to validate the success of functionalization and ensure reproducible performance.

3.6.1 Structural Integrity

Circular dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC) confirm retention of the triple-helical conformation post-modification (103-105).

3.6.2 Mechanical Testing

Uniaxial tensile tests, compression assays, and rheology evaluate changes in modulus, strength, and viscoelasticity (106).

3.6.3 Biological Validation

In vitro cell studies (adhesion, proliferation, differentiation) and in vivo implantation assess biocompatibility, bioactivity, and integration (107).

4. Structure–Property Relationship

The unique biological performance of collagen arises from its highly conserved molecular architecture and hierarchical assembly. Understanding these structure–property relationships is essential for optimizing collagen-based biomaterials in tissue engineering, regenerative medicine, and drug delivery (108).

4.1 Molecular Architecture

Collagen molecules are characterized by their triple-helical structure, composed of three polypeptide α-chains coiled into a right-handed helix. Each α-chain contains repeating Gly–X–Y triplets, where X is frequently proline (Pro) and Y is typically hydroxyproline (Hyp). Glycine, the smallest amino acid, occupies the central core of the helix, enabling tight packing, while Pro and Hyp confer rigidity and thermal stability through steric constraints and hydrogen bonding (109). Hydroxyproline content is directly correlated with the helix's denaturation temperature and is one reason marine collagens have lower thermal stability than mammalian sources (110)(Fig 2).

4.2 Hierarchical Organization

Collagen's mechanical and biological properties emerge from its hierarchical structure, which spans multiple length scales (111):

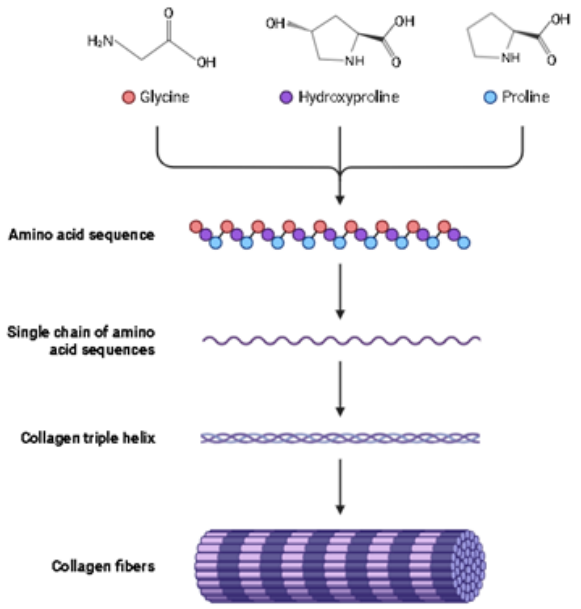


*Figure 2 illustrates the triple helix and key cell-binding motifs. (*DOI:10.7759/cureus.102052

1. Molecule (tropocollagen): ~300 nm triple-helical unit
2. Fibril: Lateral assembly of molecules with characteristic D-banding periodicity (~67 nm)
3. Fiber: Bundles of fibrils providing tensile strength
4. Tissue: Higher-order alignment supporting load-bearing functions in tendon, skin, and bone

This hierarchical organization enables anisotropic behavior, efficient load distribution, and high tensile modulus in mature tissues (112).

4.3 Collagen Classes and Functional Diversity

Collagen comprises a family of 28+ isoforms with distinct architectures:

- Fibrillar collagens (Types I, II, III): Form rope-like fibrils critical for structural integrity of skin, tendon, ligament, bone, and vasculature (113)
- Network-forming collagens (Type IV): Create sheet-like assemblies in basement membranes, guiding filtration and cell polarity (114)

FACIT collagens (Fibril-Associated Collagens with Interrupted Triple Helices; Types IX, XII): Bind to the surface of fibrils and regulate fibrillogenesis, intermolecular crosslinking, and ECM organization (115)(Table 1)

4.4 Bioactive Motifs and Cell Interactions

In addition to structural roles, collagen provides biochemical cues through integrin-binding domains (131):

RGD (Arg-Gly-Asp) motifs, although not abundant in native Type I collagen, appear in denatured regions and other collagen types (132)

Table 1: Comparison of Major Collagen Types in Tissue Engineering

Coll. Type	Primary Tissue Distribution	Key Applications in TE	Advantages	Limitations	Ref
I	Skin, bone, tendon, ligament, cornea, dentin	Bone grafts Skin substitutes, vascular grafts Corneal implants, Wound dressings	- High tensile strength - Most abundant & well-characterized - Excellent biocompatibility - Supports osteogenesis and fibroblast proliferation	- Low elasticity - Rapid degradation - Batch variability (animal sources) - May elicit immunogenic response if telopeptides intact	(6,8,70,72,75)
II	Articular cartilage, vitreous humor, nucleus pulposus	Cartilage repair, intervertebral disc regeneration, ocular tissue engineering	- Maintains chondrocyte phenotype - Promotes GAG synthesis - Good compressive properties - Low immunogenicity when purified	- Poor mechanical strength alone - Limited sourcing (mainly cartilage) - Requires crosslinking or blending for load-bearing use	(63,64,81,83)
III	Skin, blood vessels, uterus, granulation tissue, reticular fibers	Vascular engineering, soft tissue repair, skin regeneration, hybrid scaffolds	- High elasticity and flexibility - Promotes cell migration and angiogenesis - Often co-localized with Type I in healing tissues	- Weaker than Type I - Faster degradation - Less studied in isolation - Can be pro-fibrotic in excess	(78,79,81,112)
IV	Basement membranes, kidney glomeruli, lens capsule	Basement membrane mimics, kidney tissue engineering, vascular lining, corneal limbus scaffolds	- Forms sheet-like networks - Supports epithelial and endothelial cell polarization - Low immunogenicity - Regulates filtration and cell signaling	- Poor mechanical integrity - Difficult to isolate in pure form - Not suitable for load-bearing applications - Limited availability	(4,62,81,112)

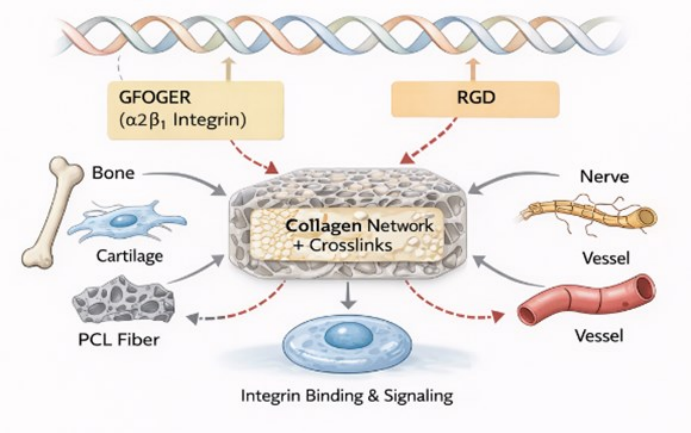
These domains promote osteogenic differentiation, fibroblast attachment, and angiogenic responses, making collagen intrinsically instructive for tissue engineering (134).

4.5 Solubility, Denaturation, and Environmental Sensitivity

Collagen solubility is governed by pH, ionic strength, and temperature. In acidic environments, collagen remains soluble due to reduced intermolecular interactions, while neutral pH promotes fibrillogenesis and gelation (135). At elevated temperatures, the triple helix unwinds irreversibly, forming gelatin, a denatured state with distinct rheological and bioactive properties. Denaturation sensitivity varies across species and tissue sources, reflecting differences in hydroxyproline content and crosslink density (136).

4.6 Mechanical and Degradation Properties

Collagen exhibits high tensile strength, low compressive resistance, and viscoelastic behavior, allowing tissues to withstand dynamic loading (137). Mechanical properties depend on fibril alignment, crosslinking (enzymatic or non-enzymatic), and molecular integrity. Enzymatic degradation by collagenases and matrix metalloproteinases (MMPs) regulates remodeling but also limits biomaterial longevity. Controlled crosslinking (physical, chemical, or enzymatic) modulates degradation rates, stiffness, and elasticity, enabling application-specific tuning (138)(Fig 3).



GFOGER (Gly-Phe-Hyp-Gly-Glu-Arg) is a high-affinity motif for α2β1 integrins, mediating cell adhesion, migration, and mechanotransduction (133)

Figure 3, Collagen triple helix architecture and bioactive binding motifs. Illustration of the collagen triple helix highlighting key integrin-binding motifs, including GFOGER and RGD sequences, which regulate cell adhesion, mechanotransduction, and intracellular signaling. These interactions play a central role in controlling cellular behavior and tissue regeneration.

5. Bioactivity and Mechanisms of Action

Collagen plays a central role in regulating cellular behavior through biochemical signaling, integrin-mediated interactions, and dynamic cross-talk with other extracellular matrix (ECM) components. Its bioactivity is essential not only for structural integrity but also for modulation of adhesion, migration, proliferation, inflammation, and tissue regeneration (139).

5.1 Integrin-Mediated Cell Adhesion and Migration

Cells primarily interact with collagen via α1β1 and α2β1 integrins, which recognize specific amino acid motifs, including GFOGER sequences within the triple helix (140). These interactions trigger focal adhesion assembly, cytoskeletal reorganization, and downstream activation of FAK/MAPK signaling, ultimately promoting cell adhesion, spreading, and migration essential for wound healing (141-143). Collagen-integrin binding also influences cell polarity and directional motility, enabling guided tissue regeneration and matrix remodeling (144).

5.2 Regulation of Cell Proliferation and Lineage-Specific Differentiation

Collagen substrates support the proliferation of mesenchymal, endothelial, and epithelial cell populations by enhancing integrin clustering and mechanotransduction signals (145,146). In stem cell biology, collagen scaffolds have been shown to promote osteogenic differentiation by enhancing RUNX2, ALP, and OCN expression, while also supporting chondrogenic commitment through SOX9 and aggrecan upregulation (147,148). The hierarchical fibrillar architecture provides mechanical cues that synergize with biochemical signaling to drive lineage specificity (149).

5.3 Modulation of Angiogenic Signaling

Collagen matrices contribute to neovascularization by modulating VEGF, FGF-2, and other pro-angiogenic mediators (150). Degradation fragments of collagen type I and III can act as matrikines, further stimulating endothelial cell migration and tubulogenesis (151). In engineered tissues, collagen hydrogels provide a permissive environment for endothelial sprouting, lumen formation, and stabilization of nascent vessels (152).

5.4 Hemostatic, Anti-Inflammatory, and Immunomodulatory Roles

Collagen triggers platelet adhesion and activation via GPVI and α2β1 integrins, making it intrinsically hemostatic and useful in wound dressings and surgical sealants (153). Collagen-based biomaterials also exhibit anti-inflammatory effects by modulating macrophage phenotype, often promoting a shift from pro-inflammatory M1 to pro-regenerative M2 states (154). Additionally, collagen fragments can regulate cytokine production and leukocyte recruitment, contributing to immunomodulation and tissue homeostasis (155).

5.5 Cross-Talk with ECM Macromolecules

Collagen forms complex interactions with fibronectin, glycosaminoglycans (GAGs), proteoglycans, and elastin, supporting matrix integrity and controlling the bioavailability of growth factors (156). These interactions stabilize the ECM network, regulate cell traction forces, and modulate biochemical gradients essential for morphogenesis and regeneration (157).

6. Collagen-Based Materials in Tissue Engineering

Collagen has emerged as one of the most versatile biomaterials in regenerative medicine due to its biocompatibility, hierarchical organization, integrin-binding motifs, tunable degradation, and ease of chemical modification (158). It functions as a structural scaffold, bioactive signaling matrix, and delivery vehicle for cells and morphogens. Across musculoskeletal, dermal, vascular, and neural applications, collagen-based constructs demonstrate significant translational success, with several commercial products already approved for clinical use (159).

6.1 Cartilage Tissue Engineering

Articular cartilage is characterized by an ECM rich in Type II collagen and glycosaminoglycans (GAGs), including chondroitin sulfate and hyaluronic acid, which collectively contribute to tensile strength, compressive stiffness, and osmotic swelling pressure (160). Collagen-based hydrogels replicate this biochemical microenvironment and provide a supportive niche for chondrocytes and mesenchymal stem cells (MSCs) (161).

Collagen–GAG hydrogels, particularly those incorporating chondroitin sulfate or hyaluronic acid, enhance chondrocyte proliferation and maintain a hyaline-like phenotype by upregulating COL2A1 and aggrecan expression (162,163). Atelocollagen, produced by enzymatic removal of telopeptides, reduces immunogenicity and supports formation of cartilage-like matrices in vitro and in vivo (164). Injectable atelocollagen hydrogels offer minimally invasive approaches for cartilage repair, with thermosensitive crosslinking systems enabling in situ gelation and defect conformability (165). In parallel, 3D bioprinted composite constructs, such as collagen–alginate bioinks, enable precise deposition of zonal cartilage architectures and improved mechanical integrity (166).

Several collagen-based cartilage therapies have progressed to clinical translation. MACI® (Matrix-Induced Autologous Chondrocyte Implantation) utilizes a collagen I/III membrane seeded with autologous chondrocytes and demonstrates long-term durability in repairing full-thickness defects (167). CaReS® (Cartilage Regeneration System) employs Type I collagen matrices to support chondrocyte expansion, while NeoCart®, a collagen type II scaffold matured in a bioreactor, has shown superior outcomes compared to microfracture techniques in Phase II trials (168).

6.2 Bone Tissue Engineering

Type I collagen constitutes ~90% of the organic bone matrix, serving as a template for hydroxyapatite nucleation and providing structural integrity (169). Thus, collagen-based biomaterials closely mimic the natural bone ECM.

Collagen–hydroxyapatite (HAp) and nano-HAp composites replicate the mineralized fibrous architecture of bone, enhancing osteoconductivity and mechanical strength (170). The nanoscale surface topography of HAp promotes integrin binding, osteoblast adhesion, and early mineral deposition (171). Hybrid scaffolds combining collagen with polycaprolactone (PCL) or bioactive glass exhibit improved tensile strength, controlled degradation, and enhanced osteogenesis by releasing ions such as Si, Ca, and P (172).

Collagen also serves as an FDA-approved carrier for growth factors, most notably in InFuse®, a collagen sponge delivering recombinant human BMP-2 for spinal fusion and long-bone nonunion treatment (173). The collagen matrix enables sustained release and protects the bioactivity of BMP-2, while simultaneously promoting cell infiltration.

Furthermore, collagen scaffolds guide mineralization templating, organizing apatite crystals along fibrillar axes and enhancing osteoinductive potential. MSCs cultured on these scaffolds demonstrate increased ALP activity, RUNX2 expression, and matrix mineralization (174).

6.3 Skin and Wound Healing

Collagen plays essential roles in skin regeneration by supporting keratinocyte migration, fibroblast proliferation, angiogenesis, and ECM remodeling (175). Because of its biodegradability and hemostatic prop-

erties, collagen is a key component of a wide array of wound dressings.

Collagen–gelatin–chitosan composite sponges and hydrogels exhibit synergistic effects: gelatin improves cell adhesion, while chitosan provides antibacterial activity and enhances tensile strength (176). Electrospun collagen nanofibers mimic the dermal ECM and support organized fibroblast infiltration (177).

Several clinically approved skin substitutes rely on collagen matrices:

- Integra® (collagen–chondroitin-6-sulfate) provides a dermal regeneration template for burns and trauma, reducing hypertrophic scarring (178)
- Biobrane®, composed of collagen-polydioxanone mesh with a silicone epidermal layer, accelerates re-epithelialization in partial-thickness burns (179)
- Apligraf® and Dermagraft®, both containing collagen-based scaffolds seeded with living cells, provide bioactive signals that enhance angiogenesis and regulate inflammation (180)

Collagen matrices also promote scarless healing by supporting M2 macrophage polarization, attenuating TGF-β1-driven fibrosis, and facilitating organized collagen deposition (181).

6.4 Vascular and Soft Tissue Engineering

Given its mechanical compliance and bioactivity, collagen is widely used for engineering vascular and soft tissue grafts (182). Collagen–elastin tubular scaffolds exhibit physiological elasticity and burst pressures nearing native vessels, supporting smooth muscle cell alignment and contractility (183).

Decellularized collagen-rich matrices, derived from small intestinal submucosa (SIS) or pericardium, provide native ECM architecture conducive to endothelialization and integration following implantation (184). The preserved basement membrane supports endothelial cell attachment via α1β1 and α2β1 integrins, promoting rapid luminal coverage.

Mechanical tuning of collagen—via crosslinking intensity, fiber alignment, and elastin incorporation—enables customization of compliance to match native arteries, reducing intimal hyperplasia associated with mechanical mismatch (185).

6.5 Corneal and Periodontal Regeneration

In ophthalmology, recombinant human collagen type III (RHC-III) has shown remarkable success as a biosynthetic corneal substitute (186). RHC-III constructs exhibit transparency, low immunogenicity, and strong stromal integration, supporting epithelial cell adhesion and nerve regeneration (187). Clinical trials demonstrate stable vision outcomes and minimal complications compared to donor grafts (188).

In dentistry, collagen–hyaluronic acid (HA) and collagen–chitosan membranes are widely used in periodontal regeneration for guided tissue and bone regeneration (GTR/GBR) (189). These membranes maintain space for periodontal ligament formation, support angiogenesis, and block epithelial downgrowth. Crosslinked collagen membranes provide enhanced longevity and tensile strength, improving outcomes in intrabony defects and furcation lesions (190).

6.6 Intervertebral Disc, Tendon, Nerve, and Ligament Engineering

Collagen's versatility extends across multiple load-bearing and neural tissues.

In intervertebral disc regeneration, collagen–HA composite hydrogels mimic the nucleus pulposus, providing hydration, viscoelasticity, and proteoglycan-like osmotic support (191). These materials support nucleus pulposus cell phenotype and restore disc height in animal models. Collagen meniscus implants (CMI®) offer a bioresorbable Type I collagen scaffold for partial meniscectomy repair, facilitating fibrocartilage regeneration and reducing progression of osteoarthritis (192).

In peripheral nerve repair, collagen nerve conduits, such as NeuraGen® and NeuroMatrix®, guide axonal regrowth by providing a permissive ECM environment with aligned fibrillar structure and appropriate degradation kinetics (193). Aligned collagen fibers also serve as excellent scaffolds for tendon and ligament regeneration, promoting tenocyte alignment, matrix synthesis, and load transfer. Mechanical anisotropy replicates native tissue biomechanics and improves functional integration (194)(Table 2).

Table 2. Chronological Summary of Collagen-Based Tissue Engineering Applications

Year	Application	Material System	In Vitro/In Vivo	Key Findings	Ref.
2014	Cartilage	Collagen–HA hydrogel	In vitro	Improved chondrocyte phenotype &	(162)
2015	Bone	Collagen–nano-HAp composite	In vivo (rat)	Enhanced osteogenesis & mineraliza-	(170)
2016	Skin	Collagen–chitosan scaffold	In vivo	Accelerated wound closure, reduced	(176)
2017	Nerve	NeuraGen® collagen conduit	Clinical	Successful sensory recovery in digital	(193)
2018	Cartilage	Collagen–alginate bioprinting	In vitro	Zonal cartilage formation	(166)
2019	Cornea	RHC-III corneal implant	Clinical	Transparent, stable graft integration	(187)
2020	Bone	InFuse® (BMP-2/collagen)	Clinical	Spinal fusion success >90%	(173)
2022	Ligament	Aligned collagen fibers	In vivo	Improved tensile strength & fibro-	(194)
2023	Vascular	Collagen–elastin graft	In vivo (sheep)	Rapid endothelialization & patency	(183)
2025	IVD	Collagen–HA hydrogel	In vivo	Restoration of disc biomechanics	(191)

7. Composite and Hybrid Collagen Materials

Hybrid collagen materials combine collagen with polymers, inorganic phases, and nanostructures to overcome mechanical limitations and to introduce tunable biological functions (195). Polymer–polymer hybrids, such as collagen blended with polycaprolactone (PCL), polylactic acid (PLA), polyethylene glycol (PEG), or chitosan, provide enhanced tensile strength, elasticity, and degradation control (196). Collagen–PCL electrospun scaffolds exhibit increased stiffness while preserving cell-adhesive RGD motifs, making them suitable for tendon, ligament, and vascular tissue engineering (197,198). Chitosan–collagen formulations also provide antimicrobial activity and improved hemostasis, supporting wound healing and cartilage regeneration (199).

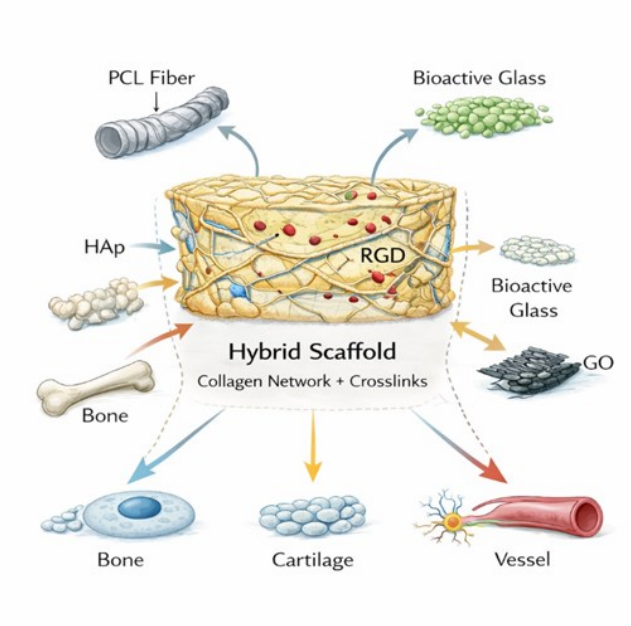


Figure 4 : Hybrid collagen-based bioactive scaffold system for tissue engineering. Schematic representation of a multifunctional collagen-based scaffold integrated with synthetic polymers and bioactive components, enabling tunable mechanical, biological, and biochemical properties. The hybrid system supports regeneration of multiple tissues, including bone, cartilage, nerve, and vascular structures.

Inorganic composites, including collagen–hydroxyapatite (HAp), collagen–bioactive glass, and collagen–graphene oxide (GO), mimic the hierarchical organization of mineralized tissues (200). Collagen–HAp scaffolds promote osteoblast differentiation and mineral nucleation by providing a biomimetic microenvironment (201). Bioactive glass–collagen hybrids release Si and Ca ions that stimulate angiogenesis and osteogenesis (202). GO-containing collagen systems introduce electrical conductivity, enhance mechanical modulus, and modulate ROS-dependent cellular responses relevant for neural and musculoskeletal repair (203).

Nanocomposites integrate nanoparticles, such as silica, ceria, silver nanoparticles, or carbon nano tubes, within the collagen matrix to provide targeted functionalities, including antimicrobial effects, enhanced stiffness, or controlled bioactive molecule release (204).

Crosslinking strategies remain essential in hybrid systems to tailor degradation and mechanical resilience. Chemical (EDC/NHS, genipin), physical (UV, DHT), and enzymatic (transglutaminase) crosslinkers are used to stabilize composite matrices, balance bioactivity with durability, and regulate remodeling kinetics (205)(Fig 4).

8. Fabrication Technologies

A wide range of fabrication techniques are used to engineer collagen constructs with customized architectures and mechanical properties (206). Freeze-drying is widely used to generate porous 3D scaffolds with interconnected pore networks that facilitate nutrient diffusion and cellular infiltration (207). Pore size can be controlled by freezing rate and collagen concentration, enabling fabrication of sponges for bone, skin, and cartilage engineering (208). Electrospinning produces collagen-based nanofiber mats that closely mimic the ECM's fibrillar architecture (209). Aligned nanofibers enhance cell orientation and anisotropic mechanical behavior, making them suitable for tendon, nerve, and ligament tissue engineering (210). 3D and 4D bioprinting offer precise spatial control over collagen deposition (211). Collagen-based bioinks, often combined with alginate, gelatin, or fibrin, allow printing of personalized constructs with patient-specific geometries (212). Emerging 4D technologies incorporate dynamic hydrogels that change shape or stiffness in response to physiological stimuli (213).

Self-assembly leverages collagen's intrinsic tendency to form fibrils under controlled pH and temperature conditions, producing highly organized matrices without external processing (214). Decellularization and recellularization techniques generate biologically relevant scaffolds by removing cellular components while preserving the collagen-rich ECM of donor tissues (215).

Microfluidic and layer-by-layer fabrication enable the generation of vascularized constructs, multilayered tissues, and gradient scaffolds. These methods mimic native tissue interfaces and allow precise delivery of growth factors, cytokines, or cells (216).

9. Challenges and Limitations

Despite significant advancements in collagen-based biomaterials and their successful translation into numerous clinical products, several critical challenges continue to constrain their widespread adoption and long-term therapeutic efficacy (217). These limitations span biological, mechanical, manufacturing, and regulatory domains, necessitating continued interdisciplinary innovation (218).

9.1 Source-Dependent Variability and Immunogenicity

Collagen derived from animal sources—predominantly bovine, porcine, and ovine—exhibits inherent batch-to-batch variability influenced by species, age, anatomical origin, and extraction conditions (219). This variability affects critical quality attributes including fibril diameter, crosslinking density, mechanical strength, degradation kinetics, and immunogenicity (220). Although telopeptide removal via pepsin digestion produces atelocollagen with reduced antigenicity, residual immunogenic epitopes may still elicit foreign body responses in susceptible patients, particularly with repeated or long-term implantation (221,222). Marine-derived collagens, while offering lower zoonotic risk and broader bio-

compatibility, exhibit reduced thermal stability and mechanical strength due to lower hydroxyproline content, limiting their utility in load-bearing applications without extensive crosslinking or hybridization (223,224). Recombinant collagens address many of these concerns by providing human-identical sequences with consistent molecular weight and purity, yet they remain cost-prohibitive and lack the complex post-translational modifications present in native mammalian collagen (225,226).

9.2 Rapid Enzymatic Degradation and Mechanical Insufficiency

Native collagen scaffolds undergo rapid degradation in vivo due to enzymatic cleavage by matrix metalloproteinases (MMPs) and collagenases, often resulting in premature loss of structural integrity before adequate host tissue remodeling occurs (227). While chemical crosslinking with agents such as glutaraldehyde, EDC/NHS, or genipin effectively prolongs scaffold persistence, it may also compromise bioactivity, reduce cell infiltration, and introduce cytotoxic residuals if not rigorously purified (228,229). Physical crosslinking methods (DHT, UV) avoid chemical toxicity but often provide insufficient stabilization for long-term applications (230). Furthermore, even crosslinked collagen scaffolds typically lack the mechanical strength required for load-bearing orthopedic, tendon, or cardiovascular applications. This limitation necessitates reinforcement via synthetic polymers (PCL, PLA, PGA) or inorganic fillers (HAp, bioactive glass), which introduces additional complexity in manufacturing and regulatory approval (231-233).

9.3 Sterilization Challenges

Sterilization represents a critical and often underappreciated hurdle in the clinical translation of collagen-based biomaterials (234). Collagen's sensitivity to heat, radiation, and chemical sterilants poses significant risks of denaturation, crosslinking disruption, or loss of bioactivity (235).

- Gamma and Electron Beam Irradiation**:** While effective for terminal sterilization, high-energy radiation generates free radicals that cleave collagen polypeptide chains, reduce mechanical integrity, and accelerate degradation kinetics. Low-temperature or reduced-dose protocols may mitigate damage but require extensive validation (236).
- Ethylene Oxide (EtO): EtO sterilization, though compatible with many collagen devices, carries risks of toxic residual by-products and requires prolonged aeration periods. Incomplete degassing may lead to cytotoxicity or inflammatory responses in vivo (237).
- Supercritical CO_2 ($scCO_2$): Emerging as a promising low-temperature alternative, $scCO_2$ sterilization preserves collagen structure and bioactivity while achieving high sterility assurance levels. However, widespread adoption remains limited by equipment costs and regulatory unfamiliarity (238).
- Aseptic Processing: Many commercial collagen products circumvent sterilization damage by employing aseptic manufacturing techniques. This approach, however, demands stringent facility controls, extensive process validation, and carries higher contamination risks compared to terminal sterilization (239).

9.4 Regulatory Hurdles and Standardization

Collagen-based medical devices are classified as Class III (high-risk) or Class II/III (moderate-to-high risk) devices by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), subjecting them to rigorous premarket approval (PMA) or 510(k) clearance processes (240). Regulatory pathways demand comprehensive demonstration of safety, efficacy, and manufacturing consistency, yet several collagen-specific challenges complicate this process:

- Lack of Standardized Characterization Protocols: Unlike synthetic polymers with well-defined physicochemical specifications, collagen's biological origin and structural complexity hinder the establishment of universal quality control metrics. Parameters such as molecular weight distribution, triple-helix integrity, fibril morphology, and endotoxin levels vary widely across laboratories and manufacturers (241,242).
- Batch-to-Batch Reproducibility: Regulatory agencies require evidence of consistent product quality across manufacturing lots. The inherent variabil-

ity of animal-derived collagen makes this requirement particularly challenging, often necessitating extensive in-process testing, pooling strategies, and tightened raw material specifications (243).

- Immunogenicity Assessment: Current regulatory frameworks lack standardized, predictive in vitro assays for evaluating collagen immunogenicity. Animal models remain the gold standard but are costly, time-consuming, and imperfect predictors of human responses (244).

- Recombinant Collagen Approval Pathways: While recombinant human collagen offers superior purity and consistency, it is regulated as a new biomaterial rather than a naturally derived substance, requiring de novo classification and full toxicological assessment. This regulatory burden, combined with high production costs, has slowed market entry despite clear technical advantages (245,246).

9.5 Cost-Effectiveness: Recombinant vs. Animal-Derived Collagen

Economic considerations profoundly influence the clinical adoption and commercial viability of collagen-based therapeutics (247). A sharp dichotomy exists between traditional animal-derived collagen and emerging recombinant alternatives (248).

Animal-Derived Collagen: The low raw material cost and mature supply chain of bovine and porcine collagen underpin its continued dominance in commercial products such as absorbable hemostats, dermal fillers, and bone graft substitutes (249,250). However, hidden costs associated with rigorous pathogen screening, batch release testing, and immunogenicity risk mitigation substantially increase final product pricing. Additionally, ethical and religious objections restrict market access in certain populations (251).

Recombinant Collagen: Despite substantially higher production costs—attributable to fermentation infrastructure, purification complexity, and low expression yields recombinant collagen offers compelling economic advantages in specific high-value applications (252,253). These include:

Table 3: Comparative Cost-Effectiveness of Animal-Derived vs. Recombinant Collagen

Parameter	Animal-Derived Collagen	Recombinant Collagen
Raw Material Cost	Low ($1–10/g)	High ($100–1,000+/g)
Scalability	Established, large-scale industrial production	Limited; capital-intensive fermentation infrastructure
Batch Consistency	Variable; dependent on source and extraction	High; precisely controlled production system
Purity	Moderate; requires extensive purification	High; pathogen-free, defined composition
Immunogenicity Risk	Low-to-moderate (telopeptide-dependent)	Minimal (human-identical sequence)
Regulatory Burden	Well-established pathways; predicate devices exist	De novo classification; limited regulatory precedent
Clinical Adoption	Widespread; numerous FDA-approved products	Emerging; few approved products (e.g., RHC-III cornea)
Sustainability	Environmental concerns (land use, methane emissions)	Renewable; lower ecological footprint

- Reduced Failure Costs: Superior batch consistency lowers the risk of costly manufacturing deviations and product recalls
- Expanded Market Access: Freedom from zoonotic disease concerns and religious restrictions enables global distribution
- Personalized Medicine: Sequence customization permits patient-specific or indication-specific collagen variants, enabling premium pricing strategies
- Intellectual Property: Novel recombinant sequences and production methods offer patent protection and market exclusivity

Cost-effectiveness analyses suggest that recombinant collagen is becoming increasingly competitive as fermentation yields improve and purification costs decline. Plant-based expression systems (e.g., tobacco, barley) and gene-edited yeast strains are anticipated to further reduce production costs by 50–80% over the coming decade (254,255).

9.6 Long-Term Stability and Host Integration

Beyond initial implantation success, collagen-based scaffolds must support long-term tissue regeneration while undergoing controlled degradation and remodeling (256). Persistent challenges include:

- Chronic Inflammation and Fibrosis: Residual crosslinkers, endotoxin contamination, or degradation products may provoke sustained foreign body responses, leading to fibrotic encapsulation and implant failure (257)
- Incomplete Vascularization: Thick, dense collagen scaffolds often exhibit poor nutrient and oxygen diffusion, resulting in central necrosis and incomplete host integration. Pre-vascularization strategies and angiogenic factor delivery remain active research priorities (258)
- Mechanical Mismatch: Scaffolds that degrade too rapidly lose load-bearing capacity, while those that persist excessively may impede new tissue formation and alter local biomechanics, contributing to adjacent tissue degeneration (259,260)

9.7 Emerging Solutions and Future Outlook

Addressing these multifaceted challenges requires integrated strategies spanning materials science, manufacturing technology, and regulatory innovation (261). Promising directions include:

- Precision Fermentation: Advances in synthetic biology and metabolic engineering are rapidly reducing recombinant collagen production costs while enabling customized post-translational modifications (262,263)
- Machine Learning for Quality Control: AI-driven image analysis and spectroscopic methods enable real-time, non-destructive assessment of collagen scaffold quality, facilitating process analytical technology (PAT) implementation for regulatory compliance (264)
- Sterilization Innovation: Broader adoption of supercritical CO_2 and nitrogen dioxide sterilization technologies offers denaturation-free terminal sterilization compatible with sensitive collagen formulations (265)
- Harmonized Regulatory Standards: International collaboration among regulatory agencies, academic consortia, and industry stakeholders is underway to establish standardized characterization protocols and immunogenicity assessment frameworks specific to collagen-based biomaterials (266)

While substantial barriers remain, the convergence of recombinant sourcing, smart composite design, advanced biofabrication, and evolving regulatory pathways positions collagen-based biomaterials for expanded clinical impact in the coming decade (267).

10. Conclusion

Collagen continues to serve as the benchmark natural biomaterial in tissue engineering due to its hierarchical structure, biocompatibility, and intrinsic bioactivity (268). Its ability to support cell adhesion, ECM remodeling, and tissue-specific signaling underpins its success across musculoskeletal, dermal, vascular, ocular, and neural applications (269). Recent innovations in hybrid materials, recombinant production, smart responsive systems, and AI-driven fabrication are expanding collagen's capabilities beyond traditional scaffolds (270). As next-generation regenerative therapies evolve toward personalized and adaptive designs, collagen-based constructs will remain central to the de-

velopment of clinically translatable solutions for tissue repair and organ reconstruction (271).

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