Engineered Living Systems based on Gelatin: Design, Manufacturing, and Applications

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Abstract

Engineered living systems (ELSs) represent purpose-driven assemblies of living components, encompassing cells, biomaterials, and active agents, intricately designed to fulfill diverse biomedical applications. Gelatin and its derivatives have been used extensively in ELSs owing to their mature translational pathways, favorable biological properties, and adjustable physicochemical characteristics. This review explores the intersection of gelatin and its derivatives with fabrication techniques, offering a comprehensive examination of their synergistic potential in creating ELSs for various applications in biomedicine. It offers a deep dive into gelatin, including its structures and production, sources, processing, and properties. Additionally, the review emphatically explores various fabrication techniques employing gelatin and its derivatives, including generic fabrication techniques, microfluidics, and various three-dimensional printing methods. Furthermore, it discusses the applications of ELSs based on gelatin in regenerative engineering as well as in cell therapies, bioadhesives, biorobots, and biosensors. Future directions and challenges in gelatin fabrication are also examined, highlighting emerging trends and potential areas for improvements and innovations. This comprehensive review underscores the significance of gelatin-based ELSs in advancing biomedical engineering and lays the groundwork for guiding future research and developments within the field.

Keywords: engineered living systems; gelatin; biofabrication; 3D printing; hydrogels

1 Introduction

Global public health faces unprecedented challenges from chronic and infectious diseases.[1] Chronic conditions like cardiovascular diseases and diabetes account for 71% of global deaths,[2, 3] while emerging infections, such as COVID-19, expose vulnerabilities in health systems.[4] The aging population, projected to reach 2.1 billion by 2050,[5] further strains resources, particularly in low-income regions with fewer than one doctor per 100,000 people.[6] These issues underscore the urgent need for equitable healthcare distributions and innovative solutions to address accessibility and quality gaps.

In this context, engineered living systems (ELSs) are gaining increasing attention as a cutting-edge medical technology. These sophisticated, purpose-driven assemblies integrate living components such as cells with biomaterials and other active agents to perform specific functions in biomedical applications.[7] ELSs provide innovative solutions to complex biomedical challenges, effectively addressing the pressing needs of modern public health.[8, 9] At the core of these systems are biomaterials, which play a crucial role in providing structural support and facilitating interactions between cells and other components.[10, 11] These biocompatible materials are meticulously designed to mimic the natural environments of tissues, promoting cell growth, differentiation, and functions.[12-14] By combining these biomaterials with natural or engineered cells, ELSs can perform complex tasks, such as tissue regeneration, cell therapy, and biosensing.[15, 16] The versatility and adaptability of biomaterials within ELSs make them a promising approach for addressing a wide range of medical challenges, from treating chronic diseases to enhancing personalized medicine.[17]

Gelatin, a well-known biomaterial in both daily life and research usage, has been widely used as support or functional materials in ELSs, due to its ability to mimic the extracellular matrices of human tissues. For instance, gelatin-based scaffolds can support cell growth, differentiation, and tissue regeneration, serving broad applications in tissue engineering. Gelatin can also be functionalized with bioactive molecules, enhancing its role in promoting specific cellular responses and improving cell therapy outcomes. Its ease of modification, biocompatibility, and biodegradability make it almost ideal for creating customizable structures to achieve various utilities in biomedicine, such as biosensors and biorobots. As research in ELSs advances, the versatility and adaptability of gelatin will remain crucial in advancing biomedical innovations. Its

integration into these systems not only enhances existing methods but also paves the way for novel solutions in biomedical applications.

This review highlights the role of gelatin in ELSs, exploring its intersection with fabrication techniques to unlock its synergistic potential for various biomedical applications (**Figure 1**). We provide an in-depth examination of gelatin, covering its structure, production, sources, processing, and properties. The review delves into a range of fabrication techniques adopting gelatin and its derivatives, including traditional fabrication methods, microfluidics, and advanced three-dimensional (3D) (bio)printing technologies. Additionally, it discusses the applications of gelatin-based ELSs in tissue engineering, cell-based therapy devices, bioadhesives, biorobots, and biosensors. The review also addresses future directions and challenges in gelatin biofabrication, emphasizing emerging trends and potential areas for innovation. Overall, our comprehensive analyses underscore the importance of gelatin-based ELSs in advancing biomedical engineering and set the stage for future research and developments in the field.

2 Overview of Gelatin

2.1 Structures

Gelatin is primarily derived from the connective tissues of animals, particularly collagen found in the skin, bones, and cartilage (**Figure 2**).[18] Collagen has a complex structure, consisting of three polypeptide chains woven into a sturdy triple helix, making it a key component of the extracellular matrices.[19] Gelatin is produced by partially hydrolyzing collagen, breaking it down into smaller peptides.[20] Depending on the hydrolysis process, gelatin is classified as Type A (acidic) or Type B (alkaline).[21] After hydrolysis, gelatin partially loses its triple-helix structure, possessing more random coils, which increases its water-solubility and broadens its application range.[22] Gelatin's chemical structure is composed of amino acids such as glycine, proline, and hydroxyproline, forming chains of varying molecular weights. The primary molecular weights include α chains (80-125 kDa), β chains (160-250 kDa), and γ chains (240-375 kDa).[23] Glycine constitutes 27-35 wt% of gelatin, while proline and hydroxyproline make up 20-24 wt%.[23, 24] A higher content of β-chains enhances the strength of gelatin-based gels and films by more effectively mimicking the natural collagen structure. The structure of gelatin can be simplified as $(Gly\text{-}X\text{-}Y)_n$, where Gly represents glycine, and X and Y are typically proline or hydroxyproline.

Gelatin is categorized into type A and type B based on the pretreatment method used during production,[25] which is determined by the raw material source and processing techniques.[26, 27] Type-A gelatin, derived from pig skin and fish, undergoes short acid treatment (pH 1-3), preserving more collagen structure and resulting in a higher isoelectric point (pH 7-9) and greater transparency. Type-B gelatin, from cowhide or bones, involves longer alkaline treatment (pH 12-13), modifying the collagen and producing a lower isoelectric point (pH 4-5) with higher gel strength. These distinctions make Type-A gelatin ideal for applications in food and cosmetics, particularly in neutral pH environments,[28] while type-B gelatin is better suited for pharmaceuticals and engineering materials due to its superior mechanical properties.[29]

Acting as a polyampholyte, ions and pH levels significantly impact the electrostatic interactions within gelatin gels.[30] Changes in salt concentration affect gelatin's swelling behaviors due to the formation of ion pairs between its charged network and counterions.[31] Adjusting pH and salt concentration can alter the mechanical properties of gelatin by influencing the electrostatic interactions between its chains.[32] Gelatin contains RGD tripeptide sequences that facilitate cell interactions and can be enzymatically degraded by metalloproteinases like collagenase, allowing for cellular remodeling.[33] Its safety profile is strengthened by the acidic or alkaline treatment during production, which reduces immunogenicity and lowers the risk of pathogen transmission. This safety, combined with its functional properties, has earned gelatin approval by the United States Food and Drug Administration (FDA) for widespread use in the food and pharmaceutical industries.

2.2 Sources

The characteristics of gelatin are determined by the raw materials from which it is extracted. The source of gelatin influences its chemical structure, which in turn affects its properties and potential applications.[21] Gelatin is commonly derived from various animal by-products,[19] with the most prevalent sources being cattle and pigs. Specifically, bovine skin and bones contribute 29.4% and 23.1% of gelatin production, respectively, while pig skin accounts for 46%. Fish, a less common source, contributes to around 1.5% of the gelatin supply. It is worth noting that while porcine gelatin is widely used, concerns about potential impurities or pathogens remaining during production may raise safety and health issues, prompting the exploration of alternatives.[34] Fish gelatin, on the other hand, tends to cause fewer allergic reactions, and fish pathogens and viruses

are less likely to be transmitted to humans, enabling a safer choice for certain applications, such as gelatin extracted from fish scales and skin.[35] While gelatin can also be sourced from other animals (e.g., poultry, camels, and amphibians),[36] the cost-effectiveness and production efficiency of mammalian-based gelatin, particularly that from pigs, remains unmatched due to the rapid reproduction of these animals and the large volumes of raw materials they provide at relatively low costs.

The properties of gelatin, such as its amino acid composition and molecular weight, vary depending on the source, which directly affects its stability and strength (**Table 1**).[37] For example, gelatin is rich in glycine, proline, and hydroxyproline, amino acids crucial for forming the triple-helix structure of collagen.[38] However, the relative abundance of these amino acids can differ among sources.[39] Fish gelatin, for instance, typically contains less proline and hydroxyproline compared to gelatin from pigs and cattle, resulting in less stable triple-helix structures and a softer gels.[40] Additionally, the molecular weight of gelatin varies with the source;[41] fish gelatin often has lower molecular weights due to the shorter collagen chains in fish, leading to softer gels with lower melting points. The shorter chains in fish gelatin result in a lower viscosity of the solution at the same concentration, compared to bovine and porcine gelatin, which require higher concentrations of fish gelatin for applications involving viscous solutions.[42] This makes fish gelatin ideal for products designed to dissolve at or below body temperature. In contrast, mammalian gelatins, with their more stable triple helix structures, have higher melting points and stronger gel strengths, making them suitable for applications requiring durable materials. Also, the viscosity of gelatin solutions typically differs among diverse sources at the same concentrations and temperatures. Generally, porcine and bovine gelatins exhibit higher viscosities compared to fish gelatin.[23]

Table 1. Comparison of gelatins from the various sources.

Source	Solubility	Strength (Bloom)	Molecular Weight (kDa)	Melting Point at 5 wt% (°C)	Amino Acid Composition
Bovine	Soluble in hot water	150-280	20-100	30-35	Similar amino acid profile to porcine gelatin

Porcine	Soluble in hot water	150-280	20-100	30-35	Rich in glycine (nearly 23 wt%), proline, and hydroxyproline
Fish	Soluble in cold water	50-150	5-30	10-15	Contains glycine, proline, and other amino acids; lower molecular weight
Poultry	Soluble in hot water	150-280	20-100	30-35	Shares similarities with bovine gelatin in amino acid composition

Gelatin strength is commonly measured by the "Bloom" index, which indicates the force in grams needed to depress a standard plunger into a gelatin gel to a depth of 4 mm at a specific concentration and temperature. The Bloom number ranges from 50 to 300, with higher numbers signifying stronger, firmer gels. Variations in the chemical structure and properties of gelatin from various sources result in different Bloom numbers, affecting gelation capacity, texture, transparency, and biological activity. Therefore, selecting the appropriate gelatin source is crucial to achieving the desired properties for specific applications.

2.3 Production

Each year, over 300,000 metric tons of gelatin are produced worldwide, with research on its properties and applications dating back to the early 20th century,[43] though it has been used in food for much longer.[44] As a byproduct of the meat industry, gelatin production also offers significant economic benefits. The typical production process involves several key steps (**Figure 3**). First, collagen is extracted from animal sources such as pig skin, cattle bones, or fish scales. This collagen is then purified to remove fats and other impurities. The purified collagen undergoes partial hydrolysis, which breaks down the long collagen chains into smaller peptide fragments. Hydrolysis can be achieved through acidic (dilute HCl, H_2SO_4, or H_3PO_4), alkaline (dilute aqueous solution of NaOH, KOH, or $Ba(OH)_2$), or enzymatic (proteolytic enzymes, including papain, alcalase, pepsin, or plant proteases) methods. The enzymatic approach being preferred due to its milder conditions, which help preserve the integrity of amino acids and other substances in the collagen.[45] After hydrolysis, the gelatin solution is filtered to remove any remaining particulates, concentrated, and then dried into powder or sheets for various target applications.[23]

One of the key advancements in gelatin research, particularly in ELSs, is the development and commercialization of gelatin-based bioinks. Gelatin methacryloyl (GelMA) bioinks, in particular, have recently gained significant attentions due to their desirable properties. Companies such as Bioink Solutions Inc., Cellink Life Sciences, and Advanced Biomatrix have developed specialized GelMA bioinks for applications such as soft tissue and bone regeneration. For instance, Bioink Solutions offers Gel4Cell and a peptide-functionalized variant optimized for ultraviolet (UV) crosslinking. Cellink's GelMA series includes formulations like GelMA A and GelXA BONE, which combine GelMA with other materials such as alginate and xanthan gum, utilizing photocrosslinking and ionic gelation for diverse tissue engineering utilities. These innovations highlight the versatility of GelMA and its essential role in advancing the 3D bioprinting technology for medical research and regenerative medicine.

2.4 Processing

2.4.1 Modification Strategies

While gelatin possesses many outstanding properties, yet inevitably, it also has limitations that may not meet certain specific applicational requirements. To address these limitations, modifications are often necessary to enhance or acquire new properties and performances. Gelatin can typically be modified through two primary strategies: physical methods and chemical methods.

2.4.1.1 Physical Methods

Physical modifications mainly involve altering certain properties of gelatin without the addition of any additives, by modifying its inherent structure. Gelatin is well-known to exist in its products in the form of collagen-like helical and coiled structures.[46] The proportion of these structures significantly influences the performance of gelatin products. As briefed above, gelatin exists in two primary forms: native (crystalline) and denatured (amorphous). When heated above its melting temperature, native parts denature into a random-coil configuration, forming denatured chains. However, during the preparation of gelatin films, if the solution is dried below the helix-coil transition temperature, the denatured chains partially re-form the native structure.[47] Such a phenomenon is known as renaturation, referring to the process by which denatured gelatin molecules regain their original structure and present some features of collagen.[48] For example, when a gelatin solution or film is left for a certain period, the molecular conformation of gelatin

changes, forming a highly helical structure.[49] The reformed solution or film normally presents insolubility in water and higher strength.[50]

In addition to renaturation, blending introduces other components into the gelatin network, physically altering its chemical composition and structure to achieve desired modifications. The components involved in blending can be categorized into low- and high-molecular-weight compounds. When gelatin is used as a dry film, it often suffers from brittleness and easy breakage, which can be improved with plasticizers. These plasticizers penetrate the gelatin molecules, breaking existing bonds and forming new hydrogen bonds, thereby increasing the toughness of the gelatin film. Low-molecular-weight plasticizers, such as glycerin,[51] ethylene glycol,[52] or even water,[53] reduce the glass-transition temperature of gelatin films, lower the film modulus, and enhance toughness.[54] High-molecular-weight agents, including natural and synthetic polymers, further modify the properties of gelatin. Natural polymers such as alginate are effective in rapidly forming a film with gelatin, significantly enhancing its toughness.[55] Other natural polymers, such as soya protein,[56] carrageenan,[57] pectin, chitosan,[58] silk protein,[59] and hyaluronic acid,[60] can modify gelatin properties according to specific application needs when blended with it.[61] Synthetic polymers also play a crucial role in improving the performance of gelatin products and introducing new characteristics to meet various application requirements.[62] For example, polyvinyl alcohol can reduce the brittleness and humidity sensitivity of gelatin films,[63] while poly butyl acrylate or poly acrylonitrile can enhance the strength of blended films.[64, 65] Polyacrylamide has been used to improve the coverage of silver in photosensitive layers and prevent fogging in silver halide emulsions when gelatin is used as a photosensitive film base.[66] The blending process is versatile, allowing for the combination of multiple compounds to integrate various properties into a single film.[67]

In addition, ion processing can enhance the performance of gelatin, particularly in gelatin hydrogels. The introduction of polyvalent metal ions, such as zirconium (Zr^{4+}) or ferric (Fe^{3+}) ions, has been shown to facilitate coordination with the residual carboxyl and amino groups, thereby forming a stable crosslinked network.[68] Achieving strong coordination requires an acidic pH environment and higher valence of ions, which favors gelatinization. For crosslinking of gelatin solution, Zr^{4+} ions exhibit a broader effective pH range (1.5-6.0) compared to Fe^{3+}ions (3.5-5.5) at the same concentration. On the contrary, the cross-linking effect of divalent copper ions (Cu^{2+}) on

gelatin can be ignored. Another ion processing strategy is salting-out, also known as the Hofmeister effect, which involves changes in protein solubility due to neutral electrolyte cosolutes.[69] This method has been used to significantly enhance the mechanical properties of gelatin hydrogels.[70]A detailed discussion of this approach is provided in Section 2.5.

2.4.1.2 Chemical Methods

Gelatin chains are rich in functional groups, such as -NH_2 and -COOH, which make them highly amenable to chemical modifications. These modifications primarily involve adding new functional groups to the gelatin chains, enabling crosslinking according to specific applicational requirements (**Figure 4**).

The most renowned gelatin derivative is perhaps GelMA,[71, 72] an engineered biomaterial created by methacryloyl-modification the gelatin backbone.[73, 74] GelMA retains the favorable inherent properties of gelatin and can be covalently crosslinked to form stable hydrogels when combined with initiators and activated by stimuli such as heat, visible light, or UV light.[75] Unlike physically crosslinked gelatin hydrogels, which rely on the reversible hydrogen bonds formed from natural cooling, chemically crosslinked GelMA hydrogels maintain structural stability at body temperature for extended periods.[76] Typically, the preparation process of GelMA is mainly achieved by dissolving gelatin in a phosphate-buffered solution (pH 7.5) at 50 °C, followed by the slow addition of methacrylic anhydride with vigorous stirring for varying durations. During this process, the amino group of gelatin reacts with the anhydride group, forming GelMA. The reaction is terminated by diluting with an additional buffer solution, and the mixture is dialyzed against deionized water at 40 °C for days.[77] The resulting product is then freeze-dried into a white solid, where the degree of substitution is determined by quantifying the percentage of converted amino groups. By adjusting the amount of methacrylic anhydride, GelMA derivatives with varying levels of substitution can be prepared, allowing for the crosslinking of gelatin into hydrogels with different mechanical properties, such as storage modulus, tailored to specific applications. The degree of substitution levels also influence the biodegradation rates of the corresponding GelMA hydrogels.[78]

Recently, the carbonate-bicarbonate buffer solution has been used for GelMA synthesis due to its high reaction efficiency. GelMA produced using carbonate-bicarbonate buffer solution exhibits superior free amino deprotonation and buffering capabilities compared to those synthesized with

phosphate-buffered solution.[79] In the carbonate-bicarbonate buffer solution, the amount of methacrylic anhydride required for GelMA synthesis is reduced, making the synthesis both environmentally friendly and cost-effective. Another similar derivative is gelatin acryloyl, which shares a comparable structure, preparation method, and properties with GelMA. The absence of a methyl group near the vinyl group in gelatin acryloyl leads to generation of more active free radicals during polymerization, enhancing the curing kinetics and resulting in a stronger hydrogel with a shorter curing time compared to GelMA at the same concentration.[80] Styrenation is another method to obtain chemically crosslinkable gelatin, achieved through the condensation reaction of gelatin with 4-vinylbenzoic acid.[81] Styrenated gelatin can form stable hydrogels under visible-light irradiation when combined with a water-soluble camphorquinone photoinitiator.[82]

The common feature of the aforementioned examples is that they rely on ethylene-based functional groups for crosslinking gelatin chains through polymerization. However, there is evidence that radical-based polymerization can be potentially toxic to sensitive cell types.[83] This type of polymerization primarily occurs through chain-growth mechanisms, resulting in the generation of high concentrations of propagating free radicals. These free radicals can disrupt cellular functions, leading to oxidative stress and potential damages to cellular structures.[84, 85] As a result, the presence of these free radicals poses a significant risk to some cells, particularly those that are more vulnerable, such as stem cells or differentiated cells in specific tissue environments.[86] Low concentrations of free radicals for polymerization are sensitive to dissolved oxygen, potentially delaying the gelation of gelatin solutions. In contrast, thiol-ene click reactions, in particular those involving norbornenes, are not inhibited by oxygen and exhibit rapid crosslinking efficiencies, superior to ethylene-based polymerization even at equivalent monomer concentrations.[87] The orthogonal reactivity between thiol and ene allows for the modular synthesis of both synthetic and naturally derived macromolecules.[88]

The first reported norbornene-modified gelatin (GelNB) was synthesized through a nucleophilic acylation reaction between gelatin and cis-5-norbornene-endo-2,3-dicarboxylic anhydride.[89] In this process, the primary amine from gelatin nucleophilically attacks the carbonyl group of the anhydride, forming an amide bond with a carboxylic acid chain attached to the norbornene group. To enhance the degree of substitution, the reaction is catalyzed by triethylamine, which promotes the deprotonation of the primary amine groups. The degree of substitution of norbornene can be

altered by adjusting the amount of anhydride added to the reaction. Alternatively, GelNB can be synthesized by reacting gelatin with various norbornene derivatives, including 5-norbornene-2-carboxylic acid, 5-norbornene-2-succinimidyl succinate, or 5-norbornene-2-acetic acid succinimidyl ester.[90-92] In addition, GelNB can be synthesized by reacting gelatin with 5-norbornene-2-methylamine, which reacts with the carboxylic acid groups on gelatin.[93] The 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) system, pre-reacted with succinic anhydride, is used to activate the carboxylic acid groups on gelatin, facilitating subsequent functionalization with norbornene-amine.[93, 94] For the thiolation of gelatin, it is typically prepared by dissolving gelatin in carbonate buffer, followed by the addition of EDTA and *N*-acetyl-homocysteine thiolactone, with the reaction conducted under argon at 40 °C.[95] By mixing GelNB with thiolated gelatin, ultrafast curing photoclick hydrogels can be produced. Compared to GelMA, the GelNB and thiolated gelatin mixture requires significantly lower doses of photoinitiator, down to 0.03% (wt/vol), and reduces curing time to merely 1-2 seconds.[96] Although GelMA has been considered the gold standard for many years, thiol-ene hydrogel systems based on norbornene-functionalized gelatin and thiol crosslinkers are gaining increasing popularity.[97, 98] Nonetheless, more convenient and safer preparation methods need to be developed.[99, 100]

Another type of click chemistry, the Diels-Alder reaction, has also been employed for chemical crosslinking of gelatin.[101] This reaction offers advantages such as the absence of byproducts and mild synthesis conditions, making it particularly suitable for biomedical applications.[102] A typical example involves the combination of furan-modified gelatin and maleimide-modified gelatin.[103, 104] In this system, gelatin is functionalized with either furan or maleimide groups, which can then react with a crosslinking agent containing the complementary group to form a hydrogel or directly with gelatin carrying the corresponding group to create a gelatin hydrogel. Furan-modified gelatin is prepared by reacting furfuryl glycidyl ether with the free amino groups in gelatin.[105] Maleimide-modified gelatin is synthesized by reacting gelatin with 3-(maleimido)propionic acid *N*-hydroxysuccinimide ester in a dimethyl sulfoxide and deionized water mixture, followed by purification through dialysis and lyophilization to obtain the final product.[106, 107]

2.4.2 Non-modification

In addition to the previous methods of modifying gelatin, it can also be directly crosslinked into networks using a crosslinker, without the need for prior modification (**Figure 5**).[108] Traditional

crosslinking methods often use aldehyde compounds such as glutaraldehyde, which form covalent bonds with the amino groups in gelatin.[109] The concentration of these amino groups allows precise control over the crosslinking density. However, the degradation products of aldehyde compounds can be cytotoxic, immunogenic, and potentially inflammatory, making them unsuitable for applications involving cell encapsulation.[110] Owing to the coexistence of carboxyl and amine groups within gelatin chains, EDC/NHS is commonly employed to crosslink gelatin by activating the reaction between carboxyl and amine groups on gelatin chains, further forming the network by covalent bonds.[111] EDC, a zero-length crosslinker, activates carboxylic acid groups to generate an unstable O-acylisourea intermediate, which is subsequently stabilized by NHS to form an activated ester that reacts with primary amine groups, resulting in stable amide bonds.[112] Similarly, epoxy compounds and diisocyanates are used as crosslinking agents for gelatin, reacting with its amino groups to form stable covalent bonds. Epoxy compounds such as epichlorohydrin and polyepoxy,[113] while effective, require careful handling due to their toxicity.[114, 115] Diisocyanates, such as isophorone diisocyanate, form urea bonds through reactions with amino groups, providing stable crosslinking but necessitating strict safety precautions due to their toxicity and irritation.[116] Consequently, the use of these crosslinkers in cell-based applications is often restricted. In contrast, natural crosslinkers like genipin, derived from plants, are considered less cytotoxic than aldehydes and can effectively crosslink gelatin at lower concentrations.[117] However, even natural crosslinkers can pose some toxicity risks, requiring careful dosage control.

Although these crosslinkers enhance the mechanical stability of gelatin gels, their notable cytotoxicity limits their use in biomedical applications. To address this, researchers have explored more cell-friendly crosslinking methods,[118] such as enzyme-mediated crosslinking using transglutaminase or tyrosinase.[119] These enzymes can crosslink gelatin under physiological conditions with lower cytotoxicity, preserving cell viability while improving the stability and mechanical properties of the gelatin matrix. Transglutaminase catalyzes crosslinking between glutamine and lysine residues, forming a stable gelatin network,[120] while tyrosinase catalyzes the oxidation of tyrosine residues to create crosslinked structures.[121] However, enzyme-mediated systems often offer limited customization in hydrogel design, making it challenging to control crosslink density and tailor mechanical properties precisely. This limitation arises from the specificity of enzymes, which restricts the range of chemical modifications and desired variations. Furthermore, the variability in enzyme-catalyzed reaction rates, reliance on substrate availability,

and influence of biological factors can impede precise control over the hydrogel network, resulting in inconsistencies in the final properties.[122]

In fact, the use of functionalized gelatin seems to be a more popular method compared to direct crosslinking. By introducing functional groups into the gelatin molecules, it is feasible to achieve more precise control over the crosslinking process and optimize the properties of resulting hydrogels. Functionalized gelatin allows for adjustments in crosslink density and mechanical properties based on specific application needs, enhancing its suitability for various biomedical applications. Generically speaking, functionalized gelatin not only improves hydrogel performances but also provides greater design flexibility for a range of biomaterial applications.

2.5 Mechanical Properties

Due to the diversity of gelatin's applications, the discussions on its mechanical properties here focus on gelatin-based hydrogels, instead of the films of gelatin in the dry state, to confine the scope. Gelatin hydrogels, widely used in engineered living systems (ELSs), are 3D networks capable of retaining significant amounts of water and closely replicating the mechanical properties of natural tissues, making them ideal for simulating human tissue or facilitating transitions from hard substrates to soft tissues.[123] Gelatin offers substantial benefits in engineering hydrogels with a broad spectrum of mechanical properties. By simply adjusting the gelatin type, concentration, and crosslinking density,[118] gelatin hydrogels can be precisely tailored to replicate various human tissues, ranging from soft to hard. For example, GelMA hydrogels with compressive moduli ranging from 5 to 180 kPa can be obtained by varying the precursor solution concentration from 5 to 20 wt%.[124] This range encompasses the modulus of various human tissues, including the brain (0.5-10 kPa), heart (10-30 kPa), and skin (50-100 kPa).[125] To further increase the modulus of gelatin hydrogels for mimicking harder tissues such as the cartilage (500-1000 kPa),[126] the incorporation of external compounds or higher concentrations is required. This section explores strategies to enhance the mechanical properties of gelatin hydrogels.

Incorporating gelatin into other hydrogels may improve their strength and toughness. In typical double-network hydrogels, gelatin functions as the first network, distributing energy, while the synthesized polymer network serves as the second network, maintaining the hydrogel's overall structural stability.[127, 128] The thermo-reversible and physically crosslinked gelatin network can dissipate energy upon external loading, thereby effectively increasing the strength and toughness

of the hydrogels.[129] For example, the optimized gelatin/polyacrylamide double-network hydrogels can achieve a strength of 268 kPa and a toughness of 6 MJ m^{-3}, which were both much higher than those of the single-network polyacrylamide hydrogels (**Figure 6a**).[127]

Furthermore, as a protein, gelatin can form strong hydrogels on its own by tuning the interactions between protein chains.[130, 131] The Hofmeister effect, which involves changes in protein solubility due to neutral electrolyte cosolutes,[132] has been used to create tough gelatin hydrogels.[133, 134] Strong and tough gelatin hydrogels can be prepared by simply soaking the original cold gelatin gel in an ammonium sulfate solution (**Figure 6b**).[135] This treatment allowed the polymer chains in the covalent, non-crosslinked network to move freely, facilitating even stress distribution. Additionally, the highly hydrophilic ammonium sulfate ions significantly enhanced hydrophobic interactions and chain bundling within the gelatin gel by the Hofmeister effect. Consequently, the treated hydrogels exhibited exceptional ultimate strength, with compressive and tensile strains exceeding 99% and 500%, respectively, and corresponding strengths of 12 MPa and 3 MPa. These properties were superior to those of ordinary protein gels. The physical crosslinking introduced by the Hofmeister effect quickly absorbed energy and maintained large deformations through de-crosslinking and dissociation, resulting in effective energy dissipation. Combined with the Hofmeister effect, mechanical training was additionally introduced to be applied to gelatin hydrogels inspired by the training of human muscles.[136] After placing the gelatin hydrogel into a salt solution, axial cyclic stress was applied several times at room temperature. The trained hydrogels were then immersed in phosphate-buffered saline. By repeating this process multiple times, the gelatin hydrogels achieved a tensile strength of up to 6.67 MPa, which is 145 times higher than their initial strength.

Despite the advancements in gelatin-based hydrogels, their mechanical properties still fall short compared to natural tendons.[137] The primary reason for this difference is that the protein chains in synthetic gelatin hydrogels are not aligned, unlike those in natural tendons. In tendons, the alignment of protein chains promotes the formation of more crystalline structures, which efficiently dissipate energy and enhance strength and toughness.[138] To address this limitation, a three-step method known as “salting out-alignment-locking” was developed to produce high-strength gelatin hydrogels (**Figure 6c**).[139] First, weak gelatin gels were treated with a high-concentration Na_3Cit solution (30 wt%) to induce partial chain associations, forming hard domains.

The salting-out effect, due to the Hofmeister effect, created numerous physical crosslinking points in the gelatin network through hydrophobic and ionic interactions. Next, the physically crosslinked gelatin hydrogels were stretched to align these hard domains, mimicking the natural alignment of protein fibers in tendons. Finally, the aligned gelatin hydrogels were immersed in a salt solution, which introduces additional hydrophobic associations and locks the aligned hard domains into an anisotropic structure. This three-step process transformed weak the gelatin hydrogel into a high-strength hydrogel with an anisotropic arrangement. After this treatment, the strength of the gelatin hydrogel increased from 0.011 MPa to 10.12 MPa, a 940-fold improvement. The toughness increased from 0.0085 MJ m^{-3} to 9.14 MJ m^{-3}, a 1,075-fold rise, and the modulus improved from 0.012 MPa to 34.26 MPa, an approximate 2830-fold enhancement.

In conclusion, gelatin-based hydrogels with facile network designs can present good mechanical performances, making them feasible for mimicking the mechanical characteristics of natural tissues. However, each of these techniques has its own limitations. For instance, the double-network strategy typically involves chemically synthesized polymer networks, and the residual monomers and initiators after gelation often require cumbersome removal procedures, which can hinder their applications in biomedicine. While the salting-out method can yield hydrogels with high mechanical properties, the ultra-high ionic strength during the salting-out notably limits the multifunctionality of gelatin hydrogels. Also, the stability of salting-out hydrogels in low-ion environments, such as prolonged immersion in culture media, requires further improvements. Thus, continued exploration and optimizations of these approaches will pave the way for developing advanced gelatin hydrogels with superior mechanical properties and diverse functionalities.

2.6 Biodegradability

The biodegradability of gelatin is one of its most remarkable and eco-friendly features. It naturally degrades both *in vivo* and *in vitro* through enzymatic or chemical activities. *In vivo*, gelatin can be enzymatically degraded by matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which are crucial for tissue remodeling and extracellular matrix degradation. These gelatinases hydrolyze gelatin into smaller fragments that can cross cell membranes and be utilized by the organisms.[140] This property makes gelatin favorable for biomedical applications, including wound dressings and controlled-release drug systems.[141] *In vitro*, gelatin is hydrolyzed by bacterial gelatinases, causing it to liquefy.[142] In environmental settings, microbial activity drives

gelatin degradation, with soil and water bacteria and fungi secreting enzymes that break it down into smaller organic molecules, which are then converted into carbon dioxide, water, and other substances.[143, 144] This natural process prevents gelatin from accumulating, reducing its ecological impact. Its biodegradability makes gelatin an eco-friendly material for industrial uses such as packaging and agricultural films.[145] Compared to many synthetic polymers that are difficult to degrade, renewable and biodegradable nature of gelatin supports sustainable development and underscores its environmental benefits and biocompatibility.[146]

Gelatin also undergoes thermal degradation.[29] Exposure to high temperatures causes gelatin molecules to degrade, leading to a reduction in average molecular weight and subsequently affecting its mechanical properties. For example, overheating in a microwave oven can cause gelatin to degrade and not retain its original strength even after cooling. Other chemical factors, such as strong acid or strong alkaline environments, can also accelerate the breakage of amide bonds, leading to rapid degradation of gelatin.[147]

The rapid degradation of gelatin can be a challenge in specific biomedical applications (such as long-term tissue-regeneration), necessitating strategies to enhance its stability. Chemical crosslinking with agents like genipin or glutaraldehyde creates durable networks with adjustable degradation rates,[148, 149] while blending gelatin with natural polymers such as chitosan or alginate results in hybrid materials with prolonged stability.[150] Additionally, blending with synthetic polymers like polyethylene glycol or poly(lactic-co-glycolic acid) improves enzymatic resistance, offering slow biodegradation.[151] Further precise customization can be achieved through chemical modifications. For instance, hydrogels based on GelMA exhibit slow degradation, enabling the creation of longer-lasting scaffolds. Factors such as crosslinking density and gelatin concentration can also be fine-tuned to meet specific degradation speed, ensuring optimal performance in diverse biomedical contexts.[152, 153]

3 Fabrication Techniques Utilizing Gelatin

Due to the favorable performances of gelatin and its derivatives, constructing them into well-defined architectures is highly desired to extend their functionality into various applications. According to the status of gelatin, such as solution, film, and powder, many distinct techniques

can be selected and applied. In this section, the fabrication techniques for gelatin and its derivatives are introduced and analyzed, which will mainly focus on the applicability.

3.1 General Fabrication Techniques

The general fabrication methods for gelatin include dipping, spinning, spraying, and mold-casting (**Figure 7**). Each method offers distinct advantages depending on the desired applications and the final required properties of the gelatin products. All these methods are also available for many other natural polymers that can be dissolved with suitable solvents.

3.1.1 Dipping

Dipping in a gelatin solution is a simple and adaptable method for creating gelatin coatings or films on various substrates.[154] This technique involves submerging an object or substrate into a gelatin solution and then allowing it to dry or solidify. The process begins with preparing a gelatin solution at the appropriate concentration, typically by dissolving gelatin at 60-70 °C to ensure complete dissolution. Before dipping, the surface of the samples must be thoroughly cleaned and pretreated (such as with plasma treatment or acid-base activation) to enhance adhesion.

Once prepared, the item is immersed in the gelatin solution for a suitable period, usually between 1 to 10 minutes. For materials with 3D micropores, vacuum treatment can be applied to ensure that the gelatin solution fully penetrates the pores. During dipping, it is crucial to maintain the solution's temperature within the optimal range for gelatin. After dipping, the item should be cured in a drying environment at 40-60 °C. The final coating thickness depends on the concentration and type of gelatin solution used. This process is straightforward and allows for uniform coating over complex shapes, making it suitable for applications such as coating medical devices or food products.[155]

In biomedical applications, gelatin dipping is widely used for the surface modification of porous scaffolds. For example, in bone tissue engineering, scaffolds based on bioactive glass particles have been coated with gelatin, significantly enhancing the mechanical properties of the whole scaffold, such as toughness and strength.[156] Additionally, incorporating bioactive nanoparticles (such as copper nanoparticles, microRNAs, and insulin),[156-158] into the gelatin solution can gain the scaffolds new functionalities, including antibacterial properties and controlled drug release. Such a dipping technique is available on different material-based scaffolds, including but not

limited to porous polycaprolactone,[157, 159] metal-organic framework,[158] and polylactic acid,[160] among others.

3.1.2 Spinning

Spin-coating is also a widely used technique for applying thin, uniform layers of materials onto substrates.[161] This process involves depositing a liquid coating solution onto the center of a rotating substrate, where centrifugal force spreads the liquid evenly across the surface. In the case of spin-coating of gelatin, the gelatin solution must be prepared at an optimal concentration, typically between 1 wt% and 10 wt%, and dissolved at 60-70 °C to ensure a clear and uniform mixture. The concentration of gelatin influences the viscosity of the solution, with lower viscosity making the spin-coating process easier. Before coating, the substrate should be thoroughly cleaned and pretreated to enhance adhesion. During the spin-coating procedure, the gelatin solution is applied while the substrate is rapidly rotated at speeds ranging from 1,000 to 5,000 rpm. The rotation duration is adjusted to achieve the desired coating thickness. Maintaining the solution temperature within the optimal range is crucial to ensure uniform coating. After the coating is applied, the substrate must be kept level to prevent any unwanted movement of the liquid. By adjusting the rotation speed and solution viscosity, the thickness of the gelatin coating can be precisely controlled, ranging from 1 μm to 1 mm.[162] This precise control over coating thickness is a significant advantage of spin-coating, making it a straightforward and reliable process.[163] The thin and smooth films produced by spin-coating are particularly useful in optical applications.[164]

For additional functionality, bioactive particles can be mixed in gelatin solution and spin-coated to impart specific properties to the thin coating. Additionally, by using cyclic spin-coating, multi-material composite coatings can be created. By carefully selecting materials and adjusting spin-coating parameters, the properties and thickness of each layer can be accurately controlled.[165]

Innovative strategies that combine spin-coating with other methods have also emerged. For example, combining spin-coating with electrospinning can produce reinforced gelatin nanofiber coatings,[166] while integrating photolithography with spin-coating enables patterning.[167] These advancements offer precise control over coating characteristics, expanding the applications of spin-coating in electronics, materials science, and ultimately, biomedicine.

3.1.3 Spraying

Spray-coating is a technique that atomizes liquid coating materials into fine droplets and sprays them onto a surface to create a uniform layer.[168, 169] This method is widely used across industries such as automotive, aerospace, electronics, and manufacturing,[170-172] due to its ability to provide even and consistent coatings on complex and irregular surfaces.[173] It is particularly effective for substrates with intricate shapes and hard-to-reach areas, making it a versatile option for various applications.[174] By controlling droplet size and spray parameters, controllable thicknesses can be achieved. Compared to spinning and mold-casting, spray-coating is faster and can quickly cover large areas (scale of serval square meters), thus improving production efficiency. Due to the atomization of fine droplets, the coatings produced have strong adhesion to the substrate, especially when the substrate is pretreated.[175] For the processing of gelatin, spray-coating can help gelatin form microdroplets that are used for surface treatment, such as food packaging and wound dressing.[176, 177] Gelatin spray for wound dressing offers several advantages due to their natural hydrogel composition, which provides favorable biocompatibility and low cytotoxicity, making them compatible with various tissues.[178] The crosslinked network structure of gelatin grants it adaptability with flexibility and elasticity, enabling it to conform to different wound shapes and accommodate complex patient movements.[179] Additionally, the gelatin hydrogel absorbs wound exudate, preventing dehydration and promoting healing. Its high water content can lower wound temperature in emergency situations, while its appropriate adhesion reduces discomfort during dressing changes. The tight mesh structure of the gelatin hydrogel serves as an effective barrier against bacterial infection,[180] and its transparency facilitates easy wound observation, reducing the need for frequent dressing changes.[181]

Beyond forming thin films, gelatin spray-processing is also used for microencapsulation.[182] In this process, gelatin serves as the capsule-wall material by mixed it with bioactive compounds and then processed into microcapsules through spray-drying. The gelatin effectively protects the bioactive compounds, enhances their stability, and controls their release rate, thereby improving their bioavailability.[183] Gelatin microcapsules are highly valuable for their excellent processability, making them broadly applicable in the food, pharmaceutical, and cosmetic industries. These microcapsules contribute to enhanced formulations and provide functional solutions for various products.[184-186]

3.1.4 Mold-casting

Mold-casting is an ancient technique in which liquid materials are poured into molds to create solid objects.[187] Originated thousands of years ago, this method was first used in ancient China and Egypt for casting metals such as copper and bronze.[188] Over time, mold-casting has evolved with advancements in metallurgy and molding technology, becoming a foundational process in modern manufacturing.[189] In the fabrication of gelatin, mold-casting is employed to create various shapes, such as candies and pharmaceutical capsules.[190, 191] The process involves pouring a gelatin solution into a mold and allowing it to set, often with additional thermal or UV treatment, to achieve intricate and precise shapes. This process highlights the technique's versatility and continued relevance. The preparation and functionalization of gelatin for mold-casting follow similar principles as those used in surface-coating techniques, with adjustments in concentration and the incorporation of bioactive compounds offering similar benefits.

While techniques such as dipping, spinning, and spraying are primarily used for applying surface coatings and are not well-suited for creating 3D structures with certain dimensions, mold-casting excels in this aspect. It can easily replicate intricate shapes and details using molds, allowing it to manufacture parts with relatively complicated geometries. With advancements in the 3D printing technology, the cost and time required for mold design and production have been significantly reduced, making mold-casting a more accessible option for creating 3D devices in laboratory settings. For thicker coatings exceeding 10 mm, mold-casting is more efficient than other methods, enabling single-step productions. Additionally, because the process involves pouring the material directly into the mold, it minimizes material waste, unlike techniques such as dipping, spinning, and spraying, which often result in significant material loss, especially when used over large areas. Furthermore, mold-casting generally exerts less mechanical stress on parts during the process, reducing the risk of stress concentrations and cracks in the final coatings or components.

3.1.5 Pore-formation

In the preceding sections, we reviewed several widely used fabrication techniques, including dipping, spinning, spraying, and mold-casting, which play critical roles in shaping gelatin-based materials. However, the microstructural characteristics of gelatin, particularly pore-formation, can be additional key determinants of its performance.[192, 193] The architecture of pore structures profoundly influences the suitability of gelatin for biomedical applications, affecting properties

such as cell spreading, nutrient transport, and mechanical stability.[194, 195] This section provides an overview of the mechanisms of pore-formation and the associated fabrication techniques.

Solvent-casting/particle-leaching is a commonly employed method where a pore-forming agent with controlled particle size is dispersed within a gelatin solution, which is then solidified to form a gelatin-pore agent network.[193] The pore-forming agents are subsequently removed using an appropriate solvent, typically by soaking in water, to leave behind a porous structure. Common pore-forming agents include salts, sugars, water-soluble polymers, and paraffin.[196-198] Among these, biocompatible pore-forming agents, such as polyethylene oxide, polyvinyl alcohol, and dextran, are particularly favored for biomedical applications.[199] By adjusting the size (molecular weight) and concentration of the pore-forming agent, it is possible to adjust the pore size and porosity of the resulting gelatin to meet specific requirements.

Freeze-drying is another widely applied technique, wherein a gelatin solution is rapidly frozen to induce phase-separation, followed by the sublimation of the frozen solvent (typically water) to create a porous structure.[200] The freezing process is pivotal in determining the pore morphology, as rapid freezing leads to the formation of fine ice crystals that shape the pore structure during sublimation. Parameters such as freezing temperature, cooling rate, and sublimation pressure can be optimized to precisely control the pore size, shape, and uniformity.[201] Additionally, this technique is particularly effective for producing gelatin with aligned porous structures through unidirectional freezing.[202].

Gas-foaming is another efficient method for fabricating porous gelatin. It involves introducing gas bubbles into a gelatin solution, typically using chemical foaming agents (such as sodium bicarbonate and ammonium bicarbonate) or by insufflating gases (such as argon, nitrogen, or carbon dioxide).[203, 204] These chemical agents release gas when heated or chemically reacted, forming bubbles that generate pores within the gelatin matrix.[205] This approach is effective in creating interconnected pores. By adjusting the amount of foaming agent and the reaction temperature, the size, shape, and distribution of the pores can be finely tuned. Porous gelatin scaffolds can also be prepared by insufflating inert gases like argon into a concentrated gelatin solution in the presence of surfactants, followed by gelation and purification.[206, 207] Similarly, supercritical carbon dioxide technology provides an alternative gas-foaming method, where

supercritical carbon dioxide dissolves in gelatin under high pressure and is removed by reducing pressure or temperature, forming porous structures.[208, 209]

3.2 Microfluidics

The microfluidic technology involves precise manipulation of fluids at the micrometer scale within microchannels.[210] Traditional methods for fabricating micro- and nanomaterials, such as template-molding, emulsion polymerization, and dispersion polymerization, often encounter challenges in producing materials with high monodispersity, controllable geometric shapes, customizable structures, and complex compositions.[211, 212] To overcome these challenges, the microfluidic technology has emerged as a promising alternative. This technique involves the precise control of small volumes (10^{-9} to 10^{-18} L) of liquids within micron-sized channels. At the microscale, factors such as diffusion, surface tension, and viscosity, primarily influence fluid behaviors. These factors can be carefully controlled through the design and operation of microfluidic chips.[213] Microfluidics allows for high-throughput fabrication of materials with complex and precisely adjustable sizes, shapes, structures, and compositions. By fine-tuning parameters such as flow rate and viscosity, microfluidic platforms have successfully produced materials with good uniformity and tunable geometric shapes, including particles, fibers, and films or bulk materials.[214] Additionally, the microfluidic technology excels in handling the emulsification, encapsulation, and asymmetric solidification of multiple immiscible fluids within confined spaces, driven by the Rayleigh-Plateau instability. This capability not only facilitates the creation of isotropic single-phase materials but also enables the fabrication of materials with capsule/core-shell structures and geometric or compositional anisotropy.[215]

In gelatin-processing, the microfluidic technology is used to fabricate micron-sized gelatin particles,[216] offering high resolution-control and miniaturization for cost-effective and high-throughput experiments.[217, 218] Microfluidic devices typically feature co-flow or flow-focusing channels that create uniformly sized aqueous particles (i.e., droplets) dispersed in an immiscible phase.[219] Using these devices, droplets containing gelatin and other gel-forming molecules can be generated and then subjected to further processing, such as tuning temperature, enzymatic crosslinking, or light treatment, to induce gelation within the droplets and then produce stable microgels.[220-222] The design of microfluidic devices, such as Y-junctions,[223] T-junctions,[224, 225] and flow-focusing systems,[226] allows for the production of particles with various geometries and

compositions.[227] Additionally, the microfluidic technology handles multiphase fluid emulsification and encapsulation, enabling the creation of heterogeneously composed Janus particles and multicompartment particles.[228, 229]

On the other hand, the microfluidic spinning technology shows significant potential in controlling the geometry, structure, and composition of micro-nano fibers.[230] Typical microfluidic spinning devices feature coaxial microchannels through which fluids flow in different laminar layers to form fibers.[231] By adjusting flow rates and fluid viscosities, the diameter and structure of the fibers can be precisely controlled. Microfluidic spinning can produce not only cylindrical microfibers but also fibers with sophisticated geometrical shapes, such as strip-shaped fibers, multi-component fibers, and hollow fibers.[232] It can also generate fibers with specific spatial distributions and complex structures via changing the spinning mold, such as spiral and woven fibers.[233] Some specially designed chips can be further used as templates to create fibrous materials with specific morphologies. For instance, GelMA fibers with precisely defined grooves can be molded from PDMS chips that have grooved surface patterns.[234] These GelMA fibers, with their detailed grooved structures, can effectively guide cell alignment and support the regeneration of anisotropic tissues.

The integration of the microfluidic technology with 3D printing has led to rapid advancements in fabricating customizable two-dimensional (2D) films and 3D bulk materials.[207, 235] Microfluidics enables the creation of films with specific morphologies and structures, such as honeycomb porous films and patterned hydrogel films.[236] Using microfluidic emulsification-templating techniques, uniform films with microcavities can be produced, and multi-layered microfluidic devices can generate multi-component hydrogel films.[237, 238] Further, GelMA hydrogels with adjustable pore sizes have been successfully fabricated by combining the digital light processing (DLP) printing technique with microfluidic chip systems.[207] The microfluidic chip provided precise control over the size and distribution of bubbles within the GelMA hydrogel-precursor. At the flow-focusing junction in the microfluidic chip, gas and GelMA solutions rapidly generated bubbles whose sizes could be finely tuned by adjusting flow rates and surfactant choices. The DLP printer then shaped the bubble-containing GelMA-precursor in 3D. After photocuring, the bubble structure was preserved, resulting in a porous hydrogel scaffold. The notable feature of this technology is its ability to create structures with a wide range of adjustable pore sizes within a single 3D-printed

biocompatible framework. Cellular compatibility studies using fibroblasts demonstrated high cell viability and support for cell proliferation, spreading, and migration, highlighting the potential of this approach in tissue engineering and regenerative medicine.

3.3 3D (Bio)printing

3D printing refers to the additive manufacturing process that creates 3D objects from digital models using various materials, such as plastics and metals.[239] 3D bioprinting is a collection of these sophisticated additive manufacturing techniques that controllably patterns bioinks, to construct structures that aim to replicate biological functions.[240, 241] The key difference between 3D printing and 3D bioprinting is the materials used.[242] 3D bioprinting leverages bioinks to create ELSs and achieve customized bio-functionalities.[243] A bioink typically consists of living cells and supportive substances designed to create biologically functional 3D structures.[244] In the bioink, cells are essential components, oftentimes in the form of single cells or multicellular aggregates (comprising one or more cell types). Cells may also be combined with other biomaterial forms, such as seeded onto microcarriers, embedded in microgels, formulated in physical hydrogels, or mixed with hydrogel-precursors. The primary purpose of a bioink is to form the foundational structure of a tissue during the bioprinting process, with cell interactions usually occurring after fabrication but sometimes during or before as well.

3D (bio)printing can be categorized into four types: zero-dimensional (single-point) methods, typically based on inkjet (bio)printing; one-dimensional (line-at-once) methods, primarily using extrusion techniques; two-dimensional (layer-at-once) methods, such as DLP; and 3D (volume-at-once) methods, exemplified by volumetric (bio)printing, an emerging approach that enables simultaneous patterning of all points within an object (**Figure 8**). These categories highlight the versatility of 3D (bio)printing across different dimensions, driving innovation and advancements in the field. Gelatin and its derivatives are particularly favored as bioinks due to their suitable performances in constructing ELSs. Unlike traditional 2D in vitro studies, 3D-bioprinted structures offer enhanced biological relevance, allowing researchers to more accurately mimic the architectures and behaviors of natural tissues. This feature also enables a more accurate investigations of human physiological and pathological functions in vitro. In this section, we will introduce different 3D printing techniques used for gelatin and its derivatives, discussing the mechanisms and application scopes of these various methods. For clarity, we use the general

terminologies “printing” and “ink” throughout most of the writings, although “bioprinting” and “bioink” may be adopted in specific examples involving cell-based printing.

3.3.1 Inkjet Printing

Inkjet printing is an advanced non-contact manufacture technology that can generate and control tiny volumes of liquid (as low as 1 pL) with high positional accuracy and speed, making it highly suitable for high-resolution graphics production.[245-247] The drop-on-demand process in inkjet printing typically involves the ejection and deposition of droplets smaller than 100 μm.[248] The core of this technology lies in the development of inkjet printheads and continuous innovations in ink chemistry, which not only improve print quality and efficiency but also expand the application range of inkjet printing.[249] Currently, inkjet printing has been successfully applied to emerging markets such as displays, flexible integrated circuits, and wearable sensors.[245, 250] The ability of inkjet printing to precisely deposit liquid materials without contacting the substrate makes it an ideal choice for manufacturing devices that require precise distribution of sensitive active ingredients.[251] Additionally, the high resolution and precise control capabilities of inkjet printing effectively reduce ink waste and manufacturing costs during production, which is especially suitable for biomedical applications.

Inkjet printing can deposit live cells with precision, delivering down to 1-3 cells per droplet. This technology enables arranging structures with the resolution at the level of single-cell size, even with multiple cell types through micro-patterning.[252] The precision of inkjet printing is leveraged to replicate complex tissue structures, such as the successful reproduction of a three-layer alveolar barrier model mimicking the lung tissue, and the creation of full-thickness skin with stratified epidermis.[253] In drop-on-demand inkjet systems, the fluid properties of the ink significantly affect the jetting behavior. High-viscosity inks struggle to pass through the printhead channels and suppress droplet ejection. However, within an optimal viscosity range (1-20 mPa·s), adding a small number of polymers can improve jetting. Low-viscosity inks with high polymer concentrations or molecular weights may form unwanted satellite droplets, and extreme polymer-polymer interactions at high shear rates can prevent jet separation. Due to the large numbers of hydrophilic groups on the gelatin molecular chain, an appropriate amount of gelatin can effectively adjust the rheological properties of the corresponding solution to meet the requirements of inkjet printing.[254]

3.3.2 Extrusion Printing

Extrusion printing is among the most commonly utilized methods due to its relatively low cost and compatibility with a wide range of inks.[242, 255, 256] Gelatin and its derivatives exhibit broad application prospects in biomedicine across various ink types in because of their excellent cytocompatibility and broadly adjustable physical properties. As a base ink for extrusion printing, a 3 wt% gelatin (from pork skin) solution that is pre-gelled physically can be extruded at room temperature to maintain printed 3D structures, demonstrating good printing performance.[257]

The printability is primarily based on the shear-thinning behavior of gelatin.[258] Gelatin, dissolved in the water at room temperature, forms a viscoelastic network that helps maintain the shape of the flow. However, during extrusion printing, the gelatin is forced through a nozzle under high shear conditions. This shear force stretches and aligns the gelatin molecules, disrupting their network structure and reducing the solution's viscosity. As a result, the solution flows more easily along the nozzle, allowing for smooth and precise deposition onto the printing surface. Once extruded, the gelatin's viscosity increases again as the shear force is removed, helping the printed structure solidify and retain its shape. This shear-thinning property is essential for achieving accurate and consistent results in extrusion-based printing. Additionally, the cooling effect can be used to stabilize the printed 3D gelatin architectures.

Several parameters influence the printability of gelatin solutions. As gelatin concentration is increased, the melting point and viscosity also rise. High concentrations improve stability during the printing process and enhance the mechanical properties of the printed structures. However, high viscosities may lead to uneven dispersion of functional ingredients. Tuning the temperature during printing significantly changes the viscosity of the gelatin solution. High temperatures lower the viscosity by disrupting molecular interactions and increasing molecular mobility. The thermal energy at higher temperatures breaks noncovalent bonds (such as hydrogen bonds and electrostatic interactions) that hold the gelatin network together, allowing the molecules to move more freely. This increased molecular movement reduces the resistance to flow, resulting in lower viscosity. Moreover, higher temperatures can partially denature the gelatin molecules, further disrupting the structure and decreasing viscosity.

The pH of a gelatin solution significantly affects its viscosity due to changes in the molecular structure and interactions of gelatin molecules.[259] At the isoelectric point (pH 4.8-9.4, depending

on the type of gelatin), gelatin molecules have no net charge, leading to aggregation and high viscosity.[260] Below the isoelectric point (acidic pH), gelatin molecules become positively charged, causing repulsion and reducing viscosity. Above the isoelectric point (basic pH), gelatin molecules are negatively charged, also resulting in repulsion and lower viscosity. Thus, deviations from the isoelectric point lead to decreased viscosity due to increased electrostatic repulsion and reduced hydrogen bonding between gelatin molecules. By adjusting the type of gelatin, tuning the temperature, and selecting appropriate additives, it is possible to precisely control viscosity, gelation speed, and printability, thereby adapting gelatin to different printing needs.

In addition to being used as ink for extrusion printing, gelatin can also function as a suspension bath for extrusion printing.[240] It enhances the printability of complex materials by stabilizing and maintaining the shape of inks that are otherwise difficult to print, such as low-viscosity or slow-curing inks.[261, 262] This technique enables the creation of non-self-supporting 3D structures, particularly in biofabrication, where biocompatible gelatin provides a supportive, temperature-controlled environment for printing soft or fluid inks, facilitating the formation of gradient and multi-material constructs.[263] After printing, the gelatin bath can be conveniently removed without damaging the delicate structures, making it a versatile and essential tool for expanding the range of printable materials in additive manufacturing. Alternatively, when printing the bioink, the suspension medium can be preserved through subsequent crosslinking, allowing it to serve as a 3D cell culture matrix. This matrix not only supports the printed structures, such as vascular channels or cell clusters, but also promotes the maturation and development of engineered tissues.

3.3.3 DLP Printing

DLP printing is an additive manufacturing technology that uses a digital light modulator to cure ink layer-by-layer, creating highly detailed and intricate 3D objects.[264, 265] In this process, each layer of the ink undergoes photopolymerization through light projection and adheres to the previously cured layer. By projecting a digital image of each layer onto the ink, the entire layer is solidified simultaneously, resulting in faster print times compared to methods like stereolithography.[266] DLP is known for its high resolution across all three dimensions, particularly along the Z-axis, where it produces smoother surfaces with minimal stepping effects compared to extrusion printing. While the DLP technology excels in precision and speed, it is

typically limited to photopolymer resins and often requires post-processing to achieve the desired properties, and the costs can be higher due to the need for specialized equipment and materials.

To achieve direct DLP printing with gelatin, it typically needs to be modified with photo-crosslinkable moieties that allow it to solidify upon exposure to light, which was discussed in Section 2.4.1.2. The performances of photocurable gelatin-based hydrogels, such as swelling capacity, modulus, strength, and toughness, are closely tied to the crosslinking dynamics that occur during the photopolymerization process. Several strategies can be employed to enhance the mechanical strengths of gelatin hydrogels, including adjusting the concentration of prepolymers,[267] tuning the degree of substitution of photocrosslinkable groups on the gelatin chains,[268, 269] and varying the curing parameters.[270, 271] The degree of substitution of photocrosslinkable groups is a key factor that directly influences the final performances of gelatin hydrogels. As the degree of substitution is increased, the swelling capacity of the hydrogels decreases, while their mechanical properties improve. For example, GelMA hydrogels with varying degrees of methacryloyl-substitution were prepared by adjusting the molar excess of methacrylic anhydride relative to the free amino groups on the gelatin chains.[272] The swelling ratios of these hydrogels ranged from 770% to 194%, with storage moduli varying between 5 and 368 kPa, while the degrees of functionalization increased from 68.5% to 100%.

During the photopolymerization process, factors such as light intensity, exposure time, and choice of photoinitiators are critical in determining the behaviors of these gelatin hydrogels. Under stable light intensity, longer exposure times lead to higher mechanical strengths in the hydrogels. It was found that the compressive modulus of 30 wt% GelMA hydrogels crosslinked for 10 minutes was more than twice that of samples crosslinked for 5 minutes.[124] Moreover, the type and concentration of photoinitiators have substantial impacts on the mechanical properties of the resulting hydrogels (**Table 2**). Common water-soluble photoinitiators for gelatin hydrogels include ruthenium/sodium persulfate (RU/SPS),[273] 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (I2959),[274] lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP),[258] 2,2'-azobis[2-methyl-*N*-(2-hydroxyethyl)propionamide] (VA-086),[275] and eosin Y/triethanolamine.[276] The influence of the photoinitiator on the performance of gelatin hydrogels mainly results from the polymerization efficiency. The type and concentration of the photoinitiator determines how effectively the gelatin hydrogel polymerizes under light exposure. A higher

concentration or a more efficient photoinitiator can lead to faster and more complete crosslinking, resulting in a robust and stable gelatin network. Typically, the initiator concentration is selected between 0.01-1 wt%. Different photoinitiators and concentrations respond to different wavelengths of light. Notably, the cytotoxicity of the photoinitiator is a critical factor, especially for cell-laden and cell-related inks. When printing bioinks, it is essential to avoid toxic photoinitiators. For inks that meet cells after printing, it is important to implement post-treatment to remove any unreacted toxic molecules or residues left after the reaction. For example, VA086 is a more biocompatible photoinitiator compared to I2959 when used with Saos-2 cells in tissue engineering applications.[274] Selecting a photoinitiator with the appropriate concentration allows for precise control over the polymerization process, enabling the creation of hydrogels with tailored properties. Overall, by adjusting these parameters, it is possible to precisely control the performance of gelatin hydrogels to meet the requirements of various applications.

Besides the modifications of gelatin, combining photopolymerizable polymers with gelatin can also be used to shape the gelatin-containing materials via DLP.[277, 278] In these cases, the gelatin mainly plays a role as a physically crosslinked network, which serve to tune the behaviors of the synthetic polymer networks. The parameters and their effects on printing, discussed above, also apply to these photopolymerizable polymer systems that contain gelatin.

3.3.4 Volumetric Printing

Volumetric printing is a newer 3D printing technology that uses advanced optical principles to quickly and accurately fabricate biological materials.[279, 280] Unlike traditional layer-by-layer printing methods such as DLP, volumetric printing creates entire 3D structures within a single volume, eliminating the need for ink-renewal and significantly improving speed and efficiency.[281] It uses intensity-modulated light patterns combined with rotational projections to precisely control the light dosages on a vat of light-sensitive ink.[282] Photopolymerization occurs only where the accumulated light energy exceeds the crosslinking threshold, resulting in the solidification of the desired geometry. This process, known as tomographic additive manufacturing, allows for rapid, precise, and one-step 3D structural formation.[283]

The advantages of this technology include a significant reduction in manufacturing time, often allowing for the creation of centimeter-scale structures with micron-level features in no more than tens of seconds.[284] Additionally, the cell-friendly light doses used in volumetric printing, such as

those involving Ru/SPS with green light activation, help maintain cell viability and functionality during and after printing.[279] Compared to traditional methods, such as the line-by-line extrusion-based or the layer-by-layer DLP printing, volumetric printing minimizes the time required for printing the same volumes and eliminates the need for support materials in complex structures. Furthermore, volumetric printing offers remarkable design flexibility, easily replicating intricate features found in natural tissues, such as suspended parts, movable components, and complex porous networks.[285, 286] This ability to recreate such detailed structures without additional support materials highlights the technology's emerging potential in biomedicine and tissue engineering.[287]

Since the curing principle is photopolymerization, gelatin systems suitable for DLP (including gelatin modified with photopolymerizable moieties and those mixed with other reactive monomers) can mostly be used for volumetric printing after appropriate parameter adjustments. Notably, the optical properties of the inks are critical in determining the resolution in volumetric printing, especially concerning the uniform light transmission once cells are incorporated. Volumetric printing depends on precise control of light dosage and the kinetics of photopolymerization.[288] Factors such as the spatial coherence of the light source, light projection resolution, pattern-generation algorithms, and the ink's absorbing or scattering properties all influence this process. While hardware and software control the first three, the absorbing and scattering properties of inks, especially with cells present, can cause photon-attenuation or scattering, reducing printing resolution. For example, scattered light can blur the projected tomographic images, resulting in increased light dosage in the volumetric regions of adjacent parts that are being printed. Recently, iodixanol was incorporated into GelMA-based bioinks to adjust their optical properties, further improving volumetric printing resolution.[287] Iodixanol was chosen for its water compatibility and low toxicity to cells, effectively matching the refractive indices of cells and GelMA to reduce light scattering. As the concentration of iodixanol was increased, the refractive index of the bioink rose from 1.352 to 1.3783. With higher concentrations of iodixanol, light scattering decreased to better match the refractive index of the cells and organoids, leading to improved forward light guidance.

Table 2. Common photoinitiators for gelatin and its derivatives in light-based (bio)printing.

Photoinitiator	Chemical Structure	Activation Wavelength	Biocompatibility	Notes

Irgacure 2959	2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methylpropan-1-one	350-380 nm	Biocompatible (<0.3 wt%), toxic (>0.9 wt%)	Efficient in UV region; low efficiency in visible light spectrum
LAP	Lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate	360-410 nm	Biocompatible (<0.5 wt%), toxic (>0.7 wt%)	High efficiency in visible light spectrum
Eosin Y	2',4',5',7'-tetrabromo-3',6'-dihydroxy-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one	520-560 nm	Biocompatible (<0.05 wt%), toxic (>0.1 wt%)	Effective under visible light
Ru/SPS	Tris-bipyridyl-ruthenium (II) hexahydrate/Sodium persulfate	400-450 nm	Biocompatible (<1 wt%)	Effective under visible light; able to crosslink gelatin directly
VA-086	2,2'-(diazene-1,2-diyl)bis(*N*-(2-hydroxyethyl)-2-methylpropanamide)	345-380 nm	Biocompatible (<1.5 wt%)	
Rose Bengal	Sodium 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodo-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-bis(olate)	520-560 nm	Biocompatible (<0.1 wt%)	Effective under visible light
Camphorquinone	1,7,7-trimethylbicyclo [2.2.1]heptane-2,6-dione	360-510 nm	Biocompatible (<1 wt%)	Low efficiency when used alone; high efficiency with tertiary amine as co-initiator
Riboflavin	7,8-dimethyl-10-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)benzo[g]pteridine-2,4(3H,10H)-dione	300-670 nm	Biocompatible (<3 wt%)	Low efficiency

3.3.5 Acoustic Printing

Most 3D printing technologies mentioned above rely on light to induce material shaping, which limits manufacturing strategies to conditions where optical accessibility is possible.[242, 289, 290] In contrast, ultrasound (<10 MHz) can penetrate various materials, offering the potential to deposit energy deep within materials to trigger ink curing.[291] This penetrating capability has long been used in clinical diagnostic imaging and therapy.[292, 293] Using ultrasound for material curing holds promise for applications within the human body, enabling deep printing by penetrating through bodily tissues.

Ultrasound can generate active oxygen (such as hydroxyl and peroxide radicals) through cavitation in water using ultrasound baths or horn-shaped reactors, causing vinyl monomers to polymerize into shapes within minutes to hours.[294, 295] Additionally, using a focused ultrasound transducer, ultrasound waves can be concentrated into small volumes. In principle, sound waves can be focused within optically opaque media with a volume of several centimeters, achieving a spatial resolution of approximately 100 μm.[296] Focused transducers produce alternating positive and negative pressure sound waves at MHz-frequencies, propagating along the depth direction, allowing high acoustic energies to be precisely delivered to the focal area. At the focal point within the material, acoustic cavitation and chemical reactions cause the liquid resin to cure and deposit onto a platform or atop previously deposited and cured areas. This process occurs within a microscale region centered on the focal point. Previously, cavitation-based ultrasound printing has been successfully used to cure active inks based on polydimethylsiloxane.[297] The point-by-point printing method creates only one pixel at a time, significantly slowing down the printing speed. Drawing on the evolution from point-based to surface-based techniques in stereolithography and DLP, acoustic holography has been introduced into 3D printing. This technology can create specific images on the printing cross-section and generate acoustic pressure to cure an entire surface simultaneously. Unlike the focused points in traditional DSP methods, this new approach achieves simultaneous curing by covering the entire pressure pattern and desired area with polymerization induced by cavitation bubbles.[298] Because the intense acoustic streams generated by high sound pressures can disrupt the local ink in the focal area, these technologies can only print relatively simple geometries.

To address these issues, the design of the ink is crucial. Phase-change viscoelastic sono-inks have accordingly been developed to achieve deep acoustic penetration, low acoustic streaming, and rapid thermoacoustic-induced radical polymerization, enabling deep acoustic volumetric printing.[299] This type of sono-inks typically use multi-vinyl monomers (such as poly(ethylene glycol)-diacrylate or GelMA) as the base component, agar particles as rheological modifiers, poly(*N*-isopropylacrylamide) as self-reinforcing acoustic absorbers, and ammonium persulfate as a thermal initiator. The key to the sono-ink's effectiveness is its ability to avoid cavitation and use of the heat generated by the ultrasound to drive the reaction. At the ultrasound focus, when the initial temperature is slightly increased, the thermosensitive polymers in the sono-ink rapidly undergo phase-transition to form a gel, which reduces flow and maintains the clarity of thermally driven chemical voxels. The gel effectively absorbs ultrasound and converts it into additional heat, enhancing constraint and accelerating polymerization. As a result, voxels of approximately 1 mm can be printed at depths of several centimeters, which is only two to three times the wavelength of the ultrasound, allowing for the formation of complex geometries. By adding different components, phase-change viscoelastic ultrasound inks can be highly customized. For instance, incorporating GelMA as an active polymerizing monomer into the sono-ink enables the creation of biocompatible scaffolds with minimum cytotoxicity using the ultrasound printing technology. Furthermore, it was demonstrated that ultrasound printing can be used to fill bone defects by in situ manufacturing of artificial bone or to treat atrial fibrillation by occluding the left atrial appendage. These demonstrations were performed in ex vivo tissues injected with the sono-inks. If further optimized and validated in in vivo animal models, such a method would represent a promising approach to transforming open surgeries into minimally invasive therapies.

In addition to directly inducing ink-curing, sound can also shape the ink through acoustic wave patterns, followed by a secondary curing mechanism to maintain the patterns in planar and 3D structures.[300, 301] Acoustic wave-induced patterning is a rapid, precise, and non-contact method for controlling the spatial distributions of cells, where it has been widely applied to bioprinting gelatin-based bioinks.[302-304] Due to post-curing requirements, modified gelatin with chemically crosslinkable groups, such as GelMA, is particularly suitable.[305] For example, surface acoustic waves were used to quickly align cells within the GelMA hydrogel-precursor solution.[302] These waves were generated on the substrate and transfer their energy into the GelMA gel, enabling rapid, non-contact cell alignment in less than 10 seconds. Upon exposure to UV light, the GelMA

hydrogel-precursor underwent photocrosslinking, fixing the encapsulated cells in place and achieving cellular patterning. Patterned cardiomyocytes within the GelMA hydrogels expanded rapidly after alignment and exhibit beating activity after 5-7 days of culture. This acoustic assembly method not only allowed precise control over the spatial distribution of cells within 3D structures but also preserved the viability and functionality of the patterned cells (e.g., the contractions of cardiomyocytes).

3.3.6 Others

In addition to the major printing techniques discussed previously, certain other additive manufacturing technologies can be applied to gelatin as well. Two-photon polymerization (TPP), also known as two-photon or multi-photon lithography, has emerged as a cutting-edge technique for fabricating intricate micro- and nano-structured materials.[306] This technology leverages the two-photon absorption process, which is induced under near-infrared radiation, to create highly precise and customizable 3D structures.[307] The integration of advanced nano-positioning stages and galvo scanners ensures the efficiency of TPP in fabricating intricate structures in gelatin. With TPP, photosensitive materials are selectively cured exclusively within the focal region of a laser beam, enabling sub-micron resolutions in the fabrication of complex architectures.[308] For gelatin-based systems, the material is often functionalized with photopolymerizable groups, such as methacryloyl, to make it responsive to the polymerization process.[309-311] TPP employs a pulsed near-infrared laser to cure these photosensitive gelatin inks, with the energy density required for polymerization confined precisely to the laser's focal point.[312] This enables the fabrication of high-resolution, micron-scale 3D gelatin structures. By adjusting exposure time and laser scanning patterns, gelatin-based constructs with tailored geometries and surface features can be produced. Furthermore, the photon energy applied during the process can be finely tuned by modifying the laser power, allowing precise control over the mechanical properties of the cured gelatin. In summary, TPP provides a versatile and powerful platform for engineering the structural and mechanical characteristics of gelatin-based hydrogels with micron-scale precision, meeting the requirements of specific applications. However, achieving such precision often comes with the drawbacks of lengthy fabrication times and limited scalability for large-scale structures, which remain significant limitations of current TPP technology, despite that newer innovations may partially address some of these issues.[313]

In addition to TPP, laser-induced forward transfer (LIFT) is another technology well-suited for gelatin microfabrication.[314, 315] LIFT is a digital printing technique that also uses pulsed laser beams to transfer materials from a donor film to a receiving substrate, depositing them voxel by voxel.[316] This approach accommodates both solid and liquid donor films, greatly expanding the range of printable materials and surpassing other digital techniques, such as inkjet printing, in versatility.[317] LIFT supports a broader range of ink viscosities and particle sizes, and enables single-step fabrication of multilayer structures and complete devices directly from solid films, including applications in 3D printing. Through LIFT, gelatin arrays can be successfully fabricated, enabling the deposition of patterned gelatin films with spatial precision and resolution on the scale of tens of micrometers.[315] This technique is compatible with various substrate materials and can dispense volumes smaller than 100 pL, as well as transfer solid tissues, demonstrating significant potential for micron-scale manufacturing applications. When applied to gelatin, LIFT for example demonstrated a neuroblastoma and astroglial cell survival rate of 65% to 70% over both short- and long-term periods, highlighting its potential utility in cell-based applications.[318] However, LIFT remains in the prototype stage with limited commercial availability and throughput, making it less suitable for large-scale structural fabrication.

Table 3. Comparison of different printing technologies for gelatin towards ELS fabrication, outlining their advantages and challenges.

Printing type	Advantages	Challenges
Inkjet printing	·High resolution (5-50 μm) ·High cell viability (>90%)	·Slow printing speed ·Limited 3D construction ability ·Limited cell density (10^6-10^7 cells mL^{-1}) ·Low viscosity of inks required (<10 mPa·s)
Extrusion printing	·High material compatibility ·High structural strength ·High cell densities (10^8-10^{12} cells mL^{-1})	·Slow printing speed ·Limited resolution (50-200 μm) ·High shear forces lead to low cell viability ·High viscosity of inks required (>10 mPa·s) ·Support required for bridge and cavity structures
DLP printing	·High resolution (20-50 μm) ·Suitable for most photocurable materials	·Modification required for gelatin ·Limited materials for high cell viability ·Support required for bridge and cavity structures

Volumetric printing	·Fast printing speed (3-30 s) ·Minimal mechanical stress on cell	·Complexity in calibration ·Modification required for gelatin ·Limited materials for high cell viability
Acoustic printing	·Non-contact printing	·Limited resolution ·Sophisticated instrumentation
TPP	·Sub-micron resolution ·High customization for complex 3D architectures	·Long fabrication time ·High cost of equipment and operation ·Limited throughput for large-scale structures
LIFT	·High resolution (5-50 μm) ·Versatile material compatibility	·Prototype stage, limited commercial availability ·Limited throughput for large-scale structures

4 ELSs based on Gelatin

This section provides an in-depth exploration of ELSs based on gelatin, highlighting its wide-ranging applications across various biomedical fields (**Figure 9**). The discussion begins with a thorough examination of gelatin's pivotal role in tissue engineering, particularly in the regeneration and repair of organs such as the skin, liver, heart, and bone, demonstrating its essential functions in tissue restoration. We then expand on gelatin's use in cell therapy, focusing on its role as carriers or scaffolds to support cell proliferation and tissue repair. Furthermore, the development of gelatin-based bioadhesives is explored, offering advancements for wound healing and surgical procedures. The section continues with a discussion on gelatin's potential in biorobotics, showcasing its applicability in the creation of biomimetic systems. Finally, we introduce the emerging use of gelatin in biosensor technology. Overall, this section comprehensively illustrates the versatility and importance of gelatin in contemporary biomedical engineering.

4.1 Tissue Engineering

Tissue engineering integrates biology, materials science, and engineering to develop scaffolds with cells, biomaterials, and bioactive molecules that restore or replace damaged tissues by mimicking the native environments for regeneration or augmentation.[319] To this end, gelatin exhibits several key properties that make it favorable as a scaffolding system in tissue engineering, including its biocompatibility, biodegradability, and non-cytotoxicity. Its adjustable physicochemical properties allow for broad applicational flexibility. The versatility of gelatin is further enhanced by various processing techniques, enabling cross-scale preparation (from micrometer to centimeter)

through advanced fabrication methods. A range of cell types can adhere to and proliferate on gelatin matrices, and cells can be encapsulated within gelatin hydrogels while maintaining high viability.[320-322] Overall, the multifunctionality of gelatin makes it highly adaptable for addressing specific requirements in tissue engineering. This section will explore the various applications of gelatin across broad areas of tissue engineering.[71]

4.1.1 Skin

The skin is the largest organ covering the entire body, providing physical and chemical protection against harmful factors such as heat and microorganisms.[323] It comprises two main layers: the epidermis (outer layer), which constantly regenerates, and the dermis (inner layer), which provides mechanical support to the dermis.[324] The epidermis comprises four distinct layers: the basal layer, the spinous layer, the granular layer, and the stratum corneum. Keratinocytes undergo differentiation and stratification as they move from the basal layer to the stratum corneum, with changes in shape, structure, and functions.[325] This layered structure of the epidermis is crucial for its role as a protective barrier.[326]

Due to their numerous advantages, gelatin-based scaffolds have been widely used for constructing the skin tissues, exhibiting good affinity.[267, 327, 328] Gelatin concentration is critical to scaffold performances; high concentrations strengthen scaffolds but reduce porosity, impairing cell growth. For example, GelMA (Type A, derived from porcine skin) was used in skin tissue engineering because of its highly tunable mechanical and degradative properties (**Figure 10a**).[267] By adjusting the concentration of GelMA hydrogels, the mechanical modulus can be fine-tuned from several to hundreds of kPa, and degradation times can be controlled to last from days to months. These hydrogels also demonstrated excellent cell viability (>90%) across all concentrations, with higher concentrations promoting greater cell adhesion and proliferation. Additionally, GelMA hydrogels effectively support the growth, differentiation, and stratification of keratinocytes, resulting in the formation of a functional, multilayered epidermis with barrier properties. To further enhance cell growth in high-concentration gelatin scaffolds, basic fibroblast growth factor is often incorporated,[329] which accelerates skin regeneration.[330] In contrast, low gelatin concentrations may lead to low cell adherence. Some biocompatible fillers are blended with gelatin to solve this issue, such as cellulose, poly(ε-caprolactone), chitosan, and hyaluronic acid.[331, 332]

Aside from being used as the scaffolding material, gelatin can also serve as the sacrificial phase to assist in 3D extrusion printing for reconstructing reliable full-thickness skin models.[333, 334] In constructing full-thickness skin models, the use of gelatin as a sacrificial phase enabled the uniform coverage of the 3D dermis with a confluent cellular monolayer, facilitating epidermal differentiation and stratification (**Figure 10b**).[335] Gelatin-assisted extrusion printing employed a 4 wt% gelatin solution (Type A, derived from porcine skin) combined with cells to produce layers with a thickness of 400 μm. Following the printing process, the construct underwent heated incubation at 37 °C for 3 hours to facilitate the effective attachment of the keratinocyte layer by dissolving the gelatin bioink. In comparison to traditional manual seeding, the keratinocyte monolayer produced through gelatin-assisted printing exhibited tight connections in both central and peripheral regions, significantly reducing uneven cell aggregation and voids, which in turn influences gene expressions and promotes cell differentiation. Additionally, the keratinocytes bioprinted with gelatin were placed onto a dermis composed of GelMA and decellularized extracellular matrix derived from the dermis, successfully establishing a full-thickness skin model.

Another application of gelatin-based skin tissue engineering is skin regeneration, commonly referred to as wound healing. GelMA has been utilized in the development of sprayable bilayered dressing materials designed to facilitate scarless healing of extensive wounds (**Figure 10c**).[336] In this approach, photocrosslinkable hydrophilic GelMA was combined with hydrophobic poly(lactide-co-propylene glycol-co-lactide)-dimethacrylate, forming a bilayered structure through rapid auto-phasing driven by water/oil separation. The GelMA-based bottom layer promoted rapid hemostasis by releasing calcium ions, while the poly(lactide-co-propylene glycol-co-lactide)-dimethacrylate top layer maintained a moist, breathable, and sterile environment, which helps suppress the inflammatory tumor necrosis factor-α pathway and promotes M2 macrophage polarization. This dynamic system further coactivated the cyclic guanosine monophosphate/protein kinase G-Wnt/Ca^{2+} signaling pathways, enhancing vascular reconstruction and supporting scarless healing. The sprayability of the ink, combined with its strong tissue adhesion and adaptability to joint movement, represented a significant advancement over traditional wound dressings. Additionally, GelMA's photocrosslinking properties enabled robust bonding between the two layers, enhancing therapeutic efficacy by accelerating hemostasis and promoting angiogenesis, making this approach a promising innovation in wound care.

4.1.2 Liver

Liver tissue engineering aims to develop a bioartificial liver by reconstructing the liver's highly complex microstructures, which include various cell types and a microvascular network.[337] These intricate structures are essential for the liver's ability to perform critical physiological functions such as metabolism, detoxification, protein synthesis, and bile secretion.[338] Understanding and replicating these microstructures are crucial in advancing liver tissue engineering.[339]

Gelatin was employed as the primary material to fulfill the mechanical and 3D printing performance requirements of the ink, while also creating a conducive microenvironment for the proliferation and functional maturation of liver cells (**Figure 11a**).[340] By combining gelatin with sodium alginate and liver decellularized matrix, the resulting bioink demonstrated favorable fluidity and moderate softness at a concentration range of 1 to 10 wt% and a pH of 7.0 at room temperature. When used in isolation, liver decellularized matrix exhibited a soft texture but lacked the structural support necessary for the fabrication of liver constructs. However, by combined with gelatin and sodium alginate, an ink was developed that provided adequate mechanical support, rendering it suitable for 3D bioprinting of liver structures. In this context, gelatin was utilized to integrate in vitro expanded primary liver cells that retained essential liver functions. The optimized 3D bioprinting materials facilitated the creation of a bioartificial liver. Following culture, the 3D bioprinted liver exhibited mature liver functional phenotypes, including glycogen storage and drug metabolism. Upon transplantation into Fah-deficient tyrosinemic mice or mice subjected to 90% liver resection, the bioprinted liver rapidly established connections with the host vasculature via capillaries, successfully restoring liver functions, alleviating liver damage, and significantly extending the lifespan of the liver failure mice. Furthermore, the bioprinted liver, equipped with artificial blood vessels, demonstrated the capability to transport large biomolecules and glucose, underscoring the potential for direct vascular connection, a critical requirement for orthotopic liver transplantation.

In addition, gelatin/chitosan-based biomaterials, known for their biocompatibility and mechanical integrity, are suitable for creating tissue scaffolds with hierarchical channel networks.[341] Chitosan and gelatin solutions were prepared and mixed, followed by crosslinking with glutaraldehyde. After casting, the hydrogel was frozen and freeze-dried to create micropores within the scaffold. Cell culture experiments have demonstrated that HepG2 cells successfully attach to the micropores

and microchannels within the gelatin/chitosan scaffolds. The proliferation rate of HepG2 cells was substantially higher on scaffolds with channel networks compared to those without, highlighting the importance of these structural features in liver tissue engineering. To facilitate the liver differentiation in vitro, liver-derived serum was introduced into gelatin-based microporous scaffolds.[342] This combination exhibited a synergistic effect, effectively guiding liver colonization of patient-derived bone marrow mesenchymal stem cells. By using liver-derived serum and bone marrow mesenchymal stem cells from the same individual, it was possible to generate hepatocyte-like cells supported by gelatin-based microporous scaffolds, offering promising potential for liver regeneration therapies.

Moreover, GelMA was used to create in vitro liver-on-a-chip platforms designed for the long-term culture of 3D human HepG2/C3A spheroids, which are used for drug toxicity assessment (**Figure 11b**).[343] In this case, GelMA (Type A, derived from porcine skin) was utilized to print dot arrays in a hydrogel solution mixed with spheroids fabricated through micro-molding. The dimensions of the central cell culture chamber were optimized for compatibility with the direct write bioprinter. When integrated with a microfluidic bioreactor, these liver spheroids exhibited increased expression of liver-specific markers and reduced metabolic activity in response to the hepatotoxic drug acetaminophen. The liver-on-a-chip design permitted direct access for in situ monitoring without disrupting operations. GelMA's functionality could be assessed by measuring the secretion rates of key proteins and through immunostaining for hepatocyte markers, effectively modeling toxic responses similar to those observed in animal studies.

4.1.3 Heart

Cardiac tissue engineering holds significant importance in medicine, particularly in addressing the growing severity of cardiovascular diseases.[344, 345] As the number of heart disease patients increases, the demand for effective treatments for common conditions such as myocardial infarction, cardiomyopathy, and coronary artery disease is becoming increasingly urgent. Although heart transplantation is one effective treatment for these conditions, the shortage of donors and the side effects of immunosuppressive therapy post-transplant limit its widespread application.[346] Consequently, cardiac tissue engineering has gained increasing attention as an emerging therapeutic approach.[347, 348] Gelatin is a favorable material choice in cardiac tissue engineering due to its natural cell adhesion without the need for additional extracellular matrix

attachment steps.[349, 350] For example, micro-molding gelatin effectively aligned cardiomyocytes, thereby inducing anisotropy in cardiac tissue (**Figure 12a**).[351] Gelatin (Type A, derived from porcine skin) was used to fabricate micro-molded hydrogels that mimic the structure and mechanical properties of ventricular tissue. By crosslinking with microbial transglutaminase, the elastic modulus, adhesiveness, thickness, and morphology of gelatin can be controlled. Laser-cutting was used to pre-cut cantilevers, ensuring uniform film thickness and minimizing intervention before cell culture. When neonatal rat ventricular cardiomyocytes were seeded on non-micro-molded gelatin and cultured for 4 days, they developed an isotropic monolayer. In contrast, cells seeded on micro-molded gelatin formed an anisotropic monolayer with uniform sarcomere alignment. Cardiomyocytes cultured on gelatin demonstrated a higher spare respiratory capacity compared to those on fibronectin-coated PDMS, suggesting that enhanced metabolic function may contribute to an extended culture lifespan.

By generating cardiac tissues directly from pluripotent stem cells (iPSCs) rather than assembling them from pre-differentiated cells, multiple cell-handling steps can be eliminated, thereby enhancing the potential for process-automation and large-scale production. GelMA hydrogels were used as scaffolds to support the differentiation of iPSCs into 3D engineered cardiac tissues (**Figure 12b**).[352] With the photoinitiators, low-density GelMA (Type B, from bovine) could quickly form hydrogels, successfully encapsulating iPSCs while maintaining high cell viability. The GelMA-based scaffold supported tissue growth and dynamic remodeling, leading to efficient cardiac differentiation (>70%). On the 8^{th} day of differentiation, the GelMA-based cardiac tissue began to spontaneously contract, with the synchronicity, frequency, and velocity of contractions increasing over time, and exhibiting time-dependent gene expression changes that align with developmental stages. The cardiomyocytes within the tissue showed clear sarcomere boundaries and organized alignment, and they responded appropriately to drug treatments (including the β-adrenergic agonist isoproterenol and antagonist propranolol) as well as external pacing at frequencies up to 3.0 Hz. The developed method showed promise for the efficient and scalable production of functional, bioprintable human cardiac tissues for therapeutic and drug testing purposes. In summary, GelMA is an enabling biomaterial for generating physiologically relevant cardiac tissues through diverse biofabrication strategies.

Additionally, gelatin plays a crucial role as a support bath in cardiac bioprinting, enabling the effective fabrication of high-resolution collagen structures (**Figure 12c**).[261] By combining

acidified collagen with gelatin microparticles (Type B, from bovine), gelatin provided thermoreversible support, allowing collagen to self-assemble under precise pH changes. After bioprinting, the gelatin melted away at 37 °C, leaving behind precisely formed collagen-based constructs. The introduction of gelatin microparticles significantly enhanced bioprinting resolution, achieving accurate bioprinting of collagen filaments with diameters ranging from 20 to 200 μm. Optimized gelatin microparticles formed uniform spheres and possessed tunable storage modulus, improving the polydispersity and particle size of the support bath, thereby enhancing the bioprinting performance of the ink. This improvement allowed complex printed structures to better maintain their geometric shape while promoting cell infiltration and vascularization, particularly in conjunction with pro-angiogenic molecules. In the bioprinting of the cardiac left ventricular model, gelatin served as the primary structural material, ensuring the integrity of the model while facilitating cell growth and functional recovery. After 28 days of culture, the bioprinted ventricle demonstrated good contractility and electrophysiological characteristics, further confirming the importance of gelatin in cardiac tissue engineering.

4.1.4 Bone

Bone tissue engineering aims to reconstruct functional bone tissue in the laboratory or in vivo to repair or replace damaged bone, utilizing scaffolds, cells, and growth factors.[353] These scaffolds, made from biocompatible materials like natural polysaccharides, proteins such as gelatin and collagen, or synthetic polymers such as PLA and PEG, provide structural support for cells, promote their growth, and facilitate nutrient exchange and waste removal.[354] In bone tissue engineering, commonly used cells include osteoblasts, osteocytes, chondrocytes, mesenchymal stem cells, and iPSCs, among others, each playing a distinct role in promoting bone regeneration.[355] When combined with appropriate scaffolds and growth factors, these cells facilitate the successful regeneration of the bone tissue in engineering applications. The most common one is mesenchymal stem cells, due to their self-renewal capability and multipotent differentiation potential, enabling them to differentiate into osteoblasts, chondrocytes, and other cell types under the designed conditions.[356]

To emulate the structure of natural bone, gelatin was processed into aligned fibers via unidirectional freezing (**Figure 13a**).[357] Specifically, a 5 wt% solution of GelMA (Type A, derived from porcine skin) was utilized to print a 3D lattice scaffold. This freeze-dried scaffold

was subsequently immersed in a solution containing 0.18 wt% GelMA and 0.02 wt% gelatin. The soaked scaffold was then transferred to a pre-cooled stainless-steel plate at -80 °C for 24 hours, undergoing unidirectional freezing and chemical crosslinking. The resulting scaffold exhibited a colorless and transparent lattice structure, with the treated scaffold displaying a comparable morphology but containing voids within the lattice pores. Following freeze-drying, the pores of the treated scaffold were filled with aligned filaments. The aligned gelatin fibers significantly enhanced cell recruitment and migration, outperforming random fibers. The incorporation of oriented gelatin fibers into 3D-printed scaffolds further facilitated granulation tissue formation and promotes new bone development through endochondral ossification.

Moreover, autolyzed antigen-extracted allogenic bone matrix gelatin was used as scaffold materials for bone tissue engineering.[358, 359] In vitro, these scaffolds effectively induced the differentiation of mesenchymal cells into chondrocytes.[360] When implanted in vivo, the scaffolds were rapidly absorbed, releasing bone morphogenetic proteins and other growth factors that promote a robust healing response. Thanks to its osteoinductive properties, bone matrix gelatin supported the formation of new cartilage on its surface, serving a dual role as both a scaffold for cartilage formation and a bone substitute. In vitro experiments demonstrated that chondrocytes grown on bone matrix gelatin develop transparent cartilage caps with characteristics closely resembling those of natural joint surfaces. This integrated bone-cartilage structure would hold significant potential for repairing small osteochondral defects in load-bearing joints.

To enhance its mechanical properties, gelatin is often combined with various inorganic materials to compensate for its natural limitations.[361] Common inorganic materials used in bone repair include hydroxyapatite, bioactive glass, silver nanoparticles, and black phosphorus nanosheets, all of which possess excellent mechanical properties and osteoinductive activity.[362] For example, when hydroxyapatite was combined with gelatin, it not only promoted cell adhesion and growth but also improved the biodegradability and stability of the material, making it suitable for bone and dental substitutes.[363, 364] Additionally, bioactive glass can slowly release beneficial ions to promote bone repair and angiogenesis,[365, 366] while gelatin composites incorporating silver nanoparticles enhanced antimicrobial properties and osteoinductive activity, as well as mechanical strength.[367] Black phosphorus nanosheets, due to their excellent electrical conductivity and

biodegradability, are suitable for creating conductive scaffolds that allow for electrical stimulation of cells.[368]

Besides being used as scaffolds, gelatin plays a crucial role in the coatings of metal implants by enhancing their biological activity through multiple mechanisms, thereby improving implant functionality (**Figure 13b**).[369] Gelatin was covalently bonded to the titanium surface using silane-based coupling agents, creating a stable coating. Amorphous calcium phosphate was incorporated into the gelatin matrix for the first time, with the amorphous phases shown to facilitate calcium-release as confirmed by titration methods. This coating enhanced the implant's biocompatibility and promoted calcium phosphate mineralization, closely mimicking the structure of biological hydroxyapatite, thereby improving bone conductivity. The incorporation of gelatin significantly increased the roughness and surface area of the coating, providing an ideal 3D scaffold for cell adhesion, which in turn promotes the activity and proliferation of osteoblasts. A marked increase in the expression of osteogenic genes within the coating was confirmed, further demonstrating gelatin's positive impact on bone formation. Moreover, gelatin's excellent water solubility and cost-effectiveness made it a suitable choice for applications requiring rapid bone integration and initial bone stabilization.

4.1.5 Others

In addition to the previously mentioned fields, gelatin is also widely used in vascular, neural, ophthalmic, gastrointestinal, and muscular tissue engineering.[370] Gelatin is utilized to construct vascular scaffolds,[371] effectively promoting the growth of endothelial cells and the formation of vascular lumens, thereby repairing or replacing damaged blood vessels.[372] In neural tissue engineering, gelatin is often combined with bioactive materials to create nerve conduits or scaffolds that supported the growth and regeneration of nerve cells, aiding in the treatment of nerve injuries or neurodegenerative diseases.[373, 374] In ophthalmology, gelatin is employed to fabricate corneal scaffolds or repair materials,[375] supporting the growth of corneal cells and promoting corneal tissue regeneration, thereby aiding in vision restoration.[376] Gelatin is also used in gastrointestinal tissue engineering, where it formed scaffolds that support the proliferation and differentiation of gastrointestinal cells, facilitating the repair of gastrointestinal tissue.[377] Similarly, gelatin scaffolds are applied in muscular tissue engineering to support the growth and functional recovery of muscle cells,[378, 379] showing significant effects, particularly in the treatment of muscle injuries or degenerative diseases.[380] These applications demonstrate the

adaptability and functionality of gelatin across various complex biological systems, providing strong support for tissue repair and regeneration.

4.2 Cell Therapy

Cell therapy promotes healing by injecting, transplanting, or implanting live cells, such as to aid wound healing.[381, 382] However, one of the major challenges is effectively delivering these cells to the target site and ensuring their survival and integration within the body.[383] To fully unlock the potential of cell therapies, it is important to develop and refine biomanufacturing techniques to produce high-quality cell products.[384] Traditional cell therapies typically involve suspending cells in a liquid and injecting them directly into the target area via a needle or catheter. Although this method is straightforward, clinical outcomes are often suboptimal, partly because the injected cells do not survive well at the target site due to their easy mobility. Therefore, reliable modules maintaining cell structures in place have emerged as a promising alternative. Common carriers include injectable amorphous hydrogels, microgels, and porous polymer microspheres loaded with cells, among other structured biomaterials.[385, 386] In this context, gelatin and its derivatives are frequently selected to provide a supportive environment that enhances cell survival, integration, and functions at the target site, owing to their unique material characteristics and versatile processing capabilities.[387] Gelatin offers superior biocompatibility and biodegradability, closely mimicking the natural extracellular matrix, due to its animal tissue source. Additionally, its tunable biodegradation and ability to incorporate bioactive molecules make it particularly well-suited for customized cell therapy applications.

Stem cell therapy is considered the most promising among various cell therapies due to its abilities for self-renewal, multi-lineage differentiation, and tissue repair. However, inadequate cell retention and survival can significantly reduce the effectiveness of injected stem cells. To improve regenerative outcomes, gelatin microspheres have been used as microcarriers to enhance the delivery and efficacy of ischemic myocardial cardiac progenitor cells (CPCs) (**Figure 14a**).[388] The preparation of gelatin microspheres entailed the incorporation of 10 wt% solution of gelatin (Type B, from bovine) into heated olive oil while continuously stirring and cooling the mixture. This was followed by the addition of chilled acetone for filtration, the collection and sieving of microspheres sized 50-75 μm, crosslinking with a glutaraldehyde solution, and termination of the reaction using glycine. These gelatin microspheres can effectively adhere to CPCs, preserving their

cardiac progenitor potential. In a mouse model of myocardial infarction, these gelatin microcarriers significantly enhanced cellular engraftment in the heart, resulting in a tenfold increase in the number of CPCs in the myocardium.

Additionally, mesenchymal stem cells can also be loaded into gelatin microspheres for the regeneration of musculoskeletal and soft tissue defects (**Figure 14b**).[389] Gelatin microspheres were prepared by dispensing drops of an 8 wt% gelatin-hydroxyphenyl propionic acid /horseradish peroxidase solution onto a superhydrophobic substrate. H_2O_2 was added to crosslink the gelatin-hydroxyphenyl propionic acid, forming microspheres with precise size control. After gelation, the microspheres were transferred into a cylindrical mold with 2 wt% gelatin-hydroxyphenyl propionic acid as the matrix phase, creating a dual-phase hydrogel system. The inter-sphere spaces (~350 μm) allowed for cell infiltration, attachment, and proliferation. Gelatin microspheres provided suitable mechanical strengths and degradation-resistant compartments to prolong their in-body functionality, while slowly releasing repair factors, including growth factors. These effects can promote the recruitment, proliferation, and differentiation of endogenous repair cells, thereby enhancing therapeutic outcomes.

Cell-loaded gelatin microspheres can be used to create tissue-engineered structures with high cellular vitality through bioassembly techniques, enhancing their application in cell therapy. Chondrocyte-precursor cells, ATDC5 (a chondrocyte cell line), and bone marrow-derived mesenchymal stem cells were incorporated into porous gelatin microspheres to replicate the characteristics of cartilage tissue, including a rich extracellular matrix and low cell density (**Figure 14c**).[390] The gelatin microspheres were then suspended in a photocurable GelMA solution, and Faraday waves drove the cell-loaded microspheres into a patterned structure. This structure was fixed by curing the GelMA solution (8% wt/vol in phosphate buffered saline containing 0.25% wt/vol LAP) under blue light. After the bioassembly process, the cells retained high vitality and proliferation. Following incubation and cartilage induction, the engineered cartilage structures were formed and thoroughly evaluated both in vitro and in vivo. In vivo studies showed that these structures could effectively promote the repair of cartilage defects.

In addition to stem cells, chimeric antigen receptor T cells (CAR-T) can also be combined with gelatin, showing significant clinical efficacy in treating B-cell malignancies.[391] CAR-T therapy involves genetic engineering, enabling patient-derived T cells to recognize tumor antigens.

Injectable, photocurable GelMA hydrogels (Type A, derived from porcine skin) are able to serve as a reservoir for CAR-T cells, facilitating their effective delivery. Briefly, the hydrogel was prepared by mixing GelMA, cells, cytokines, and LAP as the photoinitiator, then injected into the tumor site and solidified with blue light irradiation, allowing for the continuous release of CAR-T cells to ablate tumor cells. With the protection of GelMA, CAR-T cells could proliferate, release gradually, and maintain their anti-tumor activity in vitro. Compared to local or intravenous injection of CAR-T cell solutions, injecting GelMA hydrogels containing CAR-T cells around the tumor significantly enhanced anti-tumor effects and notably extends the survival of mice.

4.3 Bioadhesives

The use of gelatin as an adhesive dates back over 3,300 years to ancient Egypt, where it was used in crafting furniture and murals.[392] Its application continued through the Greek, Roman, and Chinese civilizations for repairing pottery, applying veneers, and preserving artworks.[393, 394] By the 20th century, gelatin adhesives had become a global industry, now valued in billions, with applications in footwear, clothing, construction, automotive, and paper products.[23] The effectiveness of gelatin as an adhesive stems from its unique protein structure, which offers multiple functional groups capable of forming hydrogen or covalent bonds, enhancing adhesive strength. These functional groups enable gelatin-based adhesives to bond with a wide range of materials, including wood, leather, paper, and even metals. Furthermore, gelatin's ability to melt when heated and solidify upon cooling allows it to conform to surfaces and fill gaps, forming strong bonds. As a naturally derived, eco-friendly, and cost-effective material, gelatin is almost ideal for diverse adhesive applications across biomedical and industrial sectors.

In the biomedical field, the biocompatibility and biodegradability of gelatin make it particularly suitable for medical applications, allowing it to adhere to biological tissues without triggering adverse reactions.[395] Its hydrophilic nature facilitates adhesion in aqueous environments, making it effective for bonding hydrogels, elastomers, and even human tissues. However, one challenge with gelatin-based adhesives is insufficient adhesive strength and functionality. Various chemical modifications have been developed to overcome this to enhance gelatin's mechanical properties, such as thiolation, methacrylation, catechol conjugation, and amination.[396] For instance, glycidyl methacrylate has been grafted onto gelatin through a chemical modification process involving epoxide ring-opening reactions and visible light crosslinking.[397] The resulting hydrogel

demonstrated remarkable elasticity, stretching up to four times its original length and withstanding tensile stresses up to 1.95 MPa, while maintaining compressive strains up to 80%. This hydrogel achieved approximately 60 kPa of adhesion to various biological tissues, including the cornea, aorta, heart, muscle, kidney, liver, and spleen.

In another study, incorporating 2 wt% dopamine moieties into the 20 wt% GelMA (Type A, derived from porcine skin) prepolymer solution, followed by dopamine oxidation, significantly improved the mechanical and adhesive properties of GelMA-based hydrogels (**Figure 15a**).[398] The method produced crosslinked patches with 140% stretchability and a toughness of 19 kJ m^{-3}, showing 5.7- and 3.3-time improvements, respectively, over unmodified GelMA. The oxidized dopamine significantly enhanced adhesive properties, yielding a fourfold increase in tensile adhesion and a sevenfold increase in shear adhesion due to the presence of reactive oxidized quinone species. Similarly, gelatin combined with tannic acid can be used to prepare adhesives (**Figure 15b**).[399] By employing a stepwise immersion method, a gelatin/tannic acid hydrogel was produced, effectively preventing severe coagulation of gelatin and tannic acid in water. In this process, gelatin hydrogel (10% wt/vol, Type A, derived from porcine skin) was first immersed in a tannic acid solution (1% wt/vol) to form a pre-gel, followed by a second treatment in either hot water or a urea solution. During the first step, strong hydrogen bonds formed between gelatin and tannic acid, creating a sturdy yet non-adhesive hydrogel. The adhesive properties could then be activated through heating or urea treatment, with adhesive strength adjustable up to 1,500 kPa, making it suitable for various substrates.

In addition to serving as a source of adhesion, gelatin can also provide a structural scaffold for adhesive materials.[400] A double-network hydrogel composed of snail glycosaminoglycan and GelMA can be used as a bioadhesive (**Figure 15c**). The snail glycosaminoglycan, a major bioactive component of snail mucus, imparted adhesive properties, while GelMA provided structural support, mimicking the proteins in snail mucus. This composite hydrogel, prepared by incorporating photopolymerized glycosaminoglycan (1.5 wt%) into GelMA (8 wt%) using EDC/NHS activation, contained both covalent amide and C-C bonds, as well as non-covalent hydrogen and ionic bonds. The resulting biodegradable hydrogel exhibited strong tissue adhesion, potent anti-inflammatory effects, and excellent biocompatibility, substantially accelerating the

healing of chronic wounds in streptozotocin-induced type 1 diabetic rat and db/db mouse models after a single treatment.

Despite these advancements, functionalizing next-generation gelatin-based bioadhesives presents a continuing challenge. This is a common issue across diverse types of bioadhesives, especially in developing smart, stimuli-responsive adhesives or achieving controllable deactivation of adhesive properties for delicate applications, such as on the sensitive skin of diabetic patients or infants. Additionally, incorporating features such as electrical conductivity could expand their applications as interfaces for bioelectronic devices and sensors that interact with moist tissues and organs in the body.

4.4 Biorobots

Gelatin-based soft materials, known for their biodegradability, have been applied to the manufacturing of biodegradable soft robots and electronic devices.[401-403] Compared to other soft materials, gelatin offers superior biocompatibility and environmental sustainability, making it particularly suitable for biorobots. Furthermore, its excellent processability and customizable functionalization enable precise customization for specific robotic functions. By incorporating properties such as self-healing, stretchability, and high mechanical performances, gelatin-based hydrogels can meet the demands of soft robotics for durability.

As an example, soft robots based on gelatin (Type A, derived from porcine skin) were fully degradable in wastewater, yet could maintain their mechanical properties for over a year under normal conditions, with actuators capable of operating over 330,000 cycles (**Figure 16a**).[404] Furthermore, the 3D printing technology has also been utilized in the production of these degradable robots. Using a fused deposition modeling process, fully biodegradable gelatin ink (Type A, derived from porcine skin) were printed directly into dimensionally stable, 3D robots (**Figure 16b**).[405] This technique enabled the rapid and cost-effective fabrication of elastic soft robots from gelatin hydrogels with zero waste, as the materials are fully recyclable. The printed pneumatic actuators responded swiftly, executing omnidirectional movements in less than a second. Through 3D printing, stretchable waveguides were integrated into the design, allowing the robots to sense both internal and external stimuli. These soft robots were equipped with dynamic real-time control capabilities, enabling them to autonomously navigate and remove obstacles. They

could be reprinted multiple times or safely disposed of at the end of their lifecycle, paving the way for a sustainable future in soft robotics.

In addition to macro-biorobots, gelatin is also well-suited for the development of functional micro-biorobots.[406-408] Magnetic GelMA micro-robots can be fabricated using straightforward microfluidic techniques (**Figure 16c**).[409] These GelMA micro-robots were specifically engineered for stem cell delivery and employ droplet-based microfluidic devices to rapidly produce a large quantity of tiny droplets, with droplet size modifiable by adjusting the flow rates of the two immiscible fluids. Leveraging gelatin's exceptional biological properties, these GelMA micro-robots were capable of directly delivering stem cells. Upon completion of their tasks, the robots could be fully dissolved through enzymatic degradation. For micro-robots, gelatin can also serve as an effective encapsulating material, ensuring the edibility of robots.[410] This innovative edible micro-robot was protected and released by a gelatin capsule, which enabled it to unfold within the gastrointestinal tract and navigate to the target location, particularly the stomach, using magnetic guidance. Upon reaching the target site, the robot adhered to the gastric mucosa and delivered programmable electrical pulses for prolonged stimulation through near-field coupling. In this design, gelatin encapsulation was critical. Initially, the robot was encased in a gelatin capsule, which dissolved in the gastrointestinal tract, releasing the folded micro-robots. This encapsulation not only preserved the stability and integrity of robots within the body but also ensured their proper deployment and functionality. The dissolving properties of the capsule facilitated the robot's smooth unfolding and precise positioning, thereby greatly enhancing the device's practicality and therapeutic efficacy.

4.5 Biosensors

Biomarkers in biological fluids such as blood, urine, saliva, and sweat reflect physiological processes and disease states, and are widely used in medical diagnostics and health monitoring.[411] Lightweight, wearable biosensors can enable in situ, continuous, and non-invasive detection of biomarkers (including glucose, metabolites, electrolytes, vitamins, and amino acids, among others),[412] and hold significant promise for advancing health monitoring.[413] Gelatin offers distinct advantages in biosensor applications due to its good biocompatibility and biodegradability, as well as ease of processing, which benefit the preparations and performances of biosensors.

Additionally, its versatility in incorporating functional groups allows for easy integration of bioactive molecules, making it an ideal material for sensitive and efficient biosensors.

To effectively manage diabetes, rapid and accurate monitoring of blood glucose levels is essential. Among the various electronic devices available, glucose oxidase-based sensors are widely utilized for blood glucose monitoring due to their low production costs, fast response times, high sensitivities, and excellent selectivity.[414, 415] However, a significant challenge in developing highly sensitive glucose sensors is selecting the appropriate matrix for immobilizing glucose oxidase.[416] A novel glucose biosensor based on gelatin (Type A, derived from porcine skin) has been developed, where glucose oxidase was immobilized on a gelatin electrode coating.[417] Gelatin, as the primary immobilization material, not only provided stable support for the enzyme but also exhibited favorable biocompatibility and functionality. The gelatin-based sensor demonstrated a linear response to glucose concentrations ranging from 6.30 to 20.09 mM and maintains stability over 2 weeks. Additionally, it showed no response to 0.5-mM ascorbic acid, urea, acetaminophen, pyruvate, or lactate, highlighting its potential for accurate glucose measurement in human serum samples.

Additionally, gelatin has extensive applications in sweat sensors.[418, 419] Gelatin-based sensors can safely contact the skin without causing adverse reactions. Their high water content and flexibility ensured stability in sweat environments. By adjusting the crosslinking degree of gelatin and incorporating conductive materials, it is possible to enhance the sensitivity and stability of biosensors, improving their ability to detect electrolytes and metabolites in sweat (**Figure 17a**).[420] Black phosphorus was incorporated into gelatin films to mitigate the negative effects of the hydration layer on wet tissue and enhance adhesion strength. Compared to films without black phosphorus, the blended gelatin films demonstrate improved swelling capacity when adhering to wet tissue. This property allows the material to efficiently absorb excess fluids, promote blood coagulation, and seal wounds for rapid hemostasis. Moreover, the conductivity of black phosphorus nanosheets makes the composite an excellent candidate for wearable biosensors, enabling the monitoring of physiological activities in living organisms. These characteristics make gelatin-based sweat sensors highly promising for applications in health monitoring, sports physiology, and early disease detection.

A gelatin-based electroactive hydrogel was developed as a 3D electronic skin scaffold, integrating electrical stimulation to achieve multifunctional motion sensing and accelerated skin wound healing.[421] Specifically, poly(3,4-ethylenedioxythiophene):polystyrene sulfonate and multi-walled carbon nanotubes-COOH were introduced into the gelatin backbone to enhance the conductivity and mechanical properties of the gelatin hydrogel, while crosslinking EDC to form amide bonds further stabilized the gelatin networks. This hydrogel demonstrated high sensitivity and excellent motion-monitoring capabilities, enabling real-time tracking of wound healing. When combined with electrical stimulation applied through the hydrogel, it showed favorable repair effects in a full-thickness skin defect model in rats. Additionally, its electroactivity has been shown to enhance the in vitro proliferation of hamster lung cells.

Also, discreet and immediately ingestible biosensors have the potential to replace traditional invasive procedures, such as endoscopy, especially for monitoring epithelial barrier disruptions caused by gastrointestinal diseases like reflux disease, irritable bowel syndrome, and eosinophilic esophagitis.[422] An edible biosensor based on gelatin was reported to monitor epithelial barrier functions through electrochemical impedance measurements (**Figure 17b**).[423] The sensor was created by combining gelatin with water, glycerol, and genipin, where genipin reacted with the primary amines in gelatin to form a covalently crosslinked polymer network. This provided precise control over the material's mechanical and degradation properties. The ingestible impedance-sensing capsule was constructed by attaching an uncured gelatin film electrode array to a gelatin capsule. While components such as Parylene-C and metal wire films are not biodegradable, they can safely pass through the gastrointestinal tract as the gelatin matrix dissolves. Additionally, materials like Parylene-C and metals (including gold and platinum) are biocompatible and are not expected to induce adverse reactions during transit. A key design feature of this electronic capsule is that, even if it became lodged, it would not cause gastrointestinal obstruction or tissue perforation. In practical in vivo applications, maintaining reliable contact between the electrodes and the gastrointestinal epithelium is essential. With a diameter of approximately 1 cm, the capsule was similar in size to the average digestive tract diameter (2-4 cm). Peristaltic pressure (approximately 20 mmHg) ensured continuous contact between the capsule and epithelial tissue as it moved through the digestive system. In vitro tests using pig esophageal tissue further demonstrated the sensor's practical effectiveness.

5 Conclusions and Perspectives

5.1 Conclusions

Over the past several decades, gelatin has emerged as a highly versatile and invaluable material in biomedical applications, particularly in the context of ELSs. As a naturally derived polymer, gelatin offers distinct advantages over synthetic alternatives, including superior biocompatibility, biodegradability, and customizable mechanical properties, establishing it as a fundamental material for numerous biomedical uses. This review has provided an in-depth examination of gelatin, encompassing its structural characteristics, sources, production processes, mechanical properties, and biodegradability, among other aspects.

The modification strategies and processing techniques discussed underscore gelatin's adaptability to meet the specific requirements of biomedical applications. In addition, the review has extensively covered the fabrication of gelatin, including fabrication methods, microfluidic technologies, and various 3D printing approaches. The contributions of gelatin to tissue engineering, cell therapy, bioadhesives, biorobots, and biosensors, among others, were thoroughly examined, demonstrating its pivotal role in advancing modern biomedical engineering. With its structural versatility and biological compatibility, gelatin presents substantial potential for future innovations in next-generation biomedical devices and engineered tissues. Its continued development will play a crucial role in shaping the future of biomedical technologies.

5.2 Perspectives

Despite the remarkable progress in gelatin-based ELSs, challenges remain, particularly in optimizing its properties and enhancing fabrication methods to meet the demands of more complex applications. Nonetheless, ongoing research and development in this field are paving the way for innovative breakthroughs, reinforcing gelatin's potential as an indispensable material in the future of biomedical technologies.

Although gelatin has long been regarded as a biocompatible material and is well-known for its favorable biocompatibility, the issue of endotoxins cannot be overlooked.[424] Endotoxins, also known as lipopolysaccharides, are toxins found in the outer membrane of Gram-negative bacteria and may be present in all biological materials, whether contaminated or not. Currently, there is limited research on the effects of endotoxins carried by gelatin on cells or living organisms.

However, given that gelatin is one of the most widely used biomaterials, this issue requires comprehensive investigations to confirm or refute its implications.

Cell-Integration in Gelatin Bioinks

In biofabrication, the integration of living cells into gelatin-based biomaterials presents unique challenges. The formulation of these biomaterials requires precise control over initiators, monomers, and curing conditions such as temperature and activation wavelength. Unfortunately, many efficient additives, such as potent photoinitiators or crosslinkers, are unsuitable due to their cytotoxic effects, forcing researchers to carefully balance the optimization of gelatin's properties with the preservation of cell viability. To address this, scientists are actively exploring milder, cell-compatible additives and advanced gelatin formulations that enhance both the performance of bioinks and their compatibility with cells.

Hybrid Bioprinting Technologies for Enhanced ELSs

Each (bio)printing and processing technology comes with distinct advantages and limitations. Some excel in resolution, speed, or material compatibility, while others face challenges such as limited functionality, slow production times, or excessive costs. Consequently, no single technology is able to meet the diverse demands of complex biomedical applications. Hybrid approaches, combining multiple processing technologies, have emerged as a more effective solution.[425, 426] For example, integrating the microstructural control of microfluidics with the high resolution of DLP printing enables the fabrication of complex, cross-scale, and multi-material structures.[207] This hybrid strategy significantly enhances the complexity and functionality of engineered products, unlocking new possibilities for tissue engineering and regenerative medicine. Similarly, combining extrusion printing with light-based volumetric printing can also achieve the complementarity of the two technologies and thus prepare multifunctional and high-precision ELSs.[427] Incorporating pre-shaped scaffolds into volumetric printing allows for further customization of the printed item's functions, including mechanical, electrical, and optical properties.[285]

Vascularization in Gelatin Matrix for Human-Sized Bioprinted ELSs

Another primary challenge in bioprinting, large human-sized structures is difficult to achieve, which requires the efficient transport of nutrients and the removal of metabolic waste.[428, 429] As

these structures increase in complexity and scale, maintaining adequate nutrient flow and waste elimination becomes increasingly difficult, yet these are crucial for the survival of the engineered tissues.[430] Current solutions mostly rely on pre-designed channels within the structures, which can serve as blood vessels during later stages of cultivation, facilitating nutrient delivery.[431] However, due to the unpredictable nature of tissue differentiation, pre-set channels may not perfectly meet the needs for tissue development in later stages. As cell cultures progress, the ability to dynamically adjust the diameter and distribution of blood vessels can enhance the success rate of tissue construction. Gelatin, as a material, can undergo controlled degradation through various chemical and enzymatic techniques, enabling precise manipulation of the structure of the constructed materials.[432] Highly efficient and controllable degradation methods can selectively erode specific regions of gelatin, thereby dynamically creating channels or cavities. These channels or cavities can then be filled with blood vessels or other tissue components. This level of precise control is crucial for replicating the complex structures of natural tissues and may be key to overcoming the current vascularization challenges that limit the clinical application of large-scale engineered tissues.

Gelatin's Role in Long-Term Cell Culture

In tissue engineering, one of the ongoing challenges is the ability to achieve high-density, multicellular bioprinting with precise control over the spatial arrangement of cells.[433] Gelatin, often regarded as a supporting material, has broader applications beyond simple scaffolding. Under various external stimuli, gelatin molecules can adopt different conformations, and their microstructures can be finely controlled. With good cytocompatibility, gelatin offers significant potential in orchestrating cellular behaviors at the microscopic level. While much of the focus is on controlling gelatin's performances during the initial stages of cell seeding, its role during long-term culture is often overlooked. Gelatin's functional groups and adjustable properties can play a critical role across various stages of cell development and differentiation. By dynamically tuning gelatin's properties, the scaffold can better meet the evolving needs of cells, emphasizing the importance of gelatin throughout the entire process of tissue cultivation.

Artificial Intelligence (AI) for Gelatin-Based Biofabrication

AI has recently been employed to optimize organoid construction, significantly improving research reproducibility and scalability by enhancing the precision and efficiency of cell culture

protocols.[434, 435] AI is equally promising in advancing gelatin-based biofabrication including bioprinting.[436] Through AI-driven material-optimizations, ideal formulations for specific applications can be rapidly designed and predicted, while dynamically adjusting critical printing parameters such as temperature, flow rate, and crosslinking intensity to ensure the precision and stability of gelatin. Looking ahead, AI integration is poised to propel gelatin-based bioprinting technologies toward standardization and automation, reducing costs, enhancing efficiency, and accelerating their adoption in ELS applications. These proposed bioprinting solutions will empower research laboratories without specialized expertise to seamlessly adopt the technology, enabling them to focus on their own core disciplines and foster progress in the interdisciplinary field.

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Conflict of Interest

YSZ consulted for Allevi by 3D Systems, and sits on the scientific advisory board and holds options of Xellar, neither of which however, participated in or biased the work. The relevant interests are managed by the Brigham and Women's Hospital. The other authors declare no conflict of interest.

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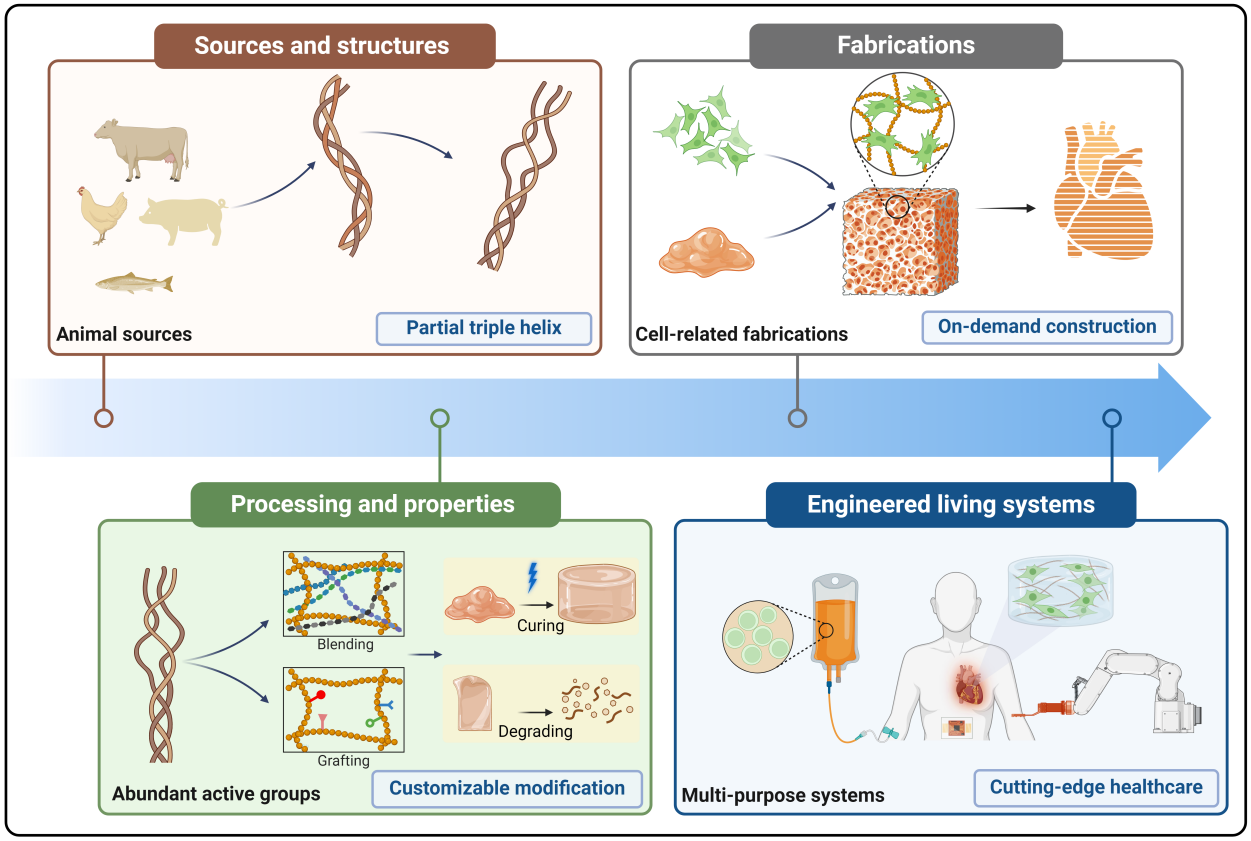

Figure 1. ELSs based on gelatin: from sources, structures, processing, properties, fabrication to multipurpose ELSs.

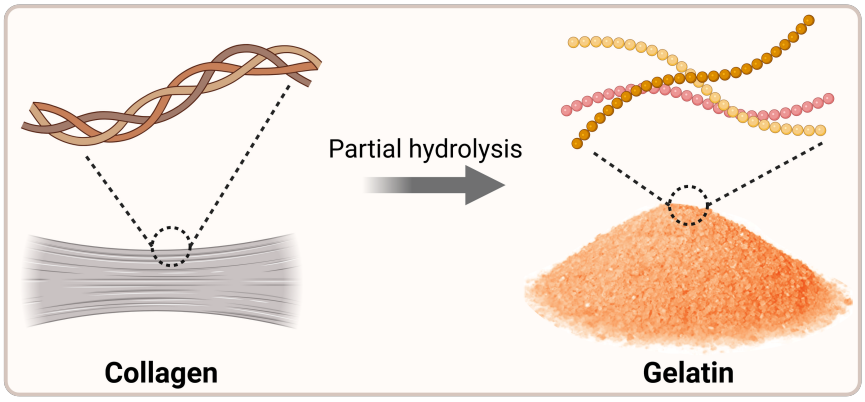

Figure 2. Gelatin, derived from the partial hydrolysis of collagen.

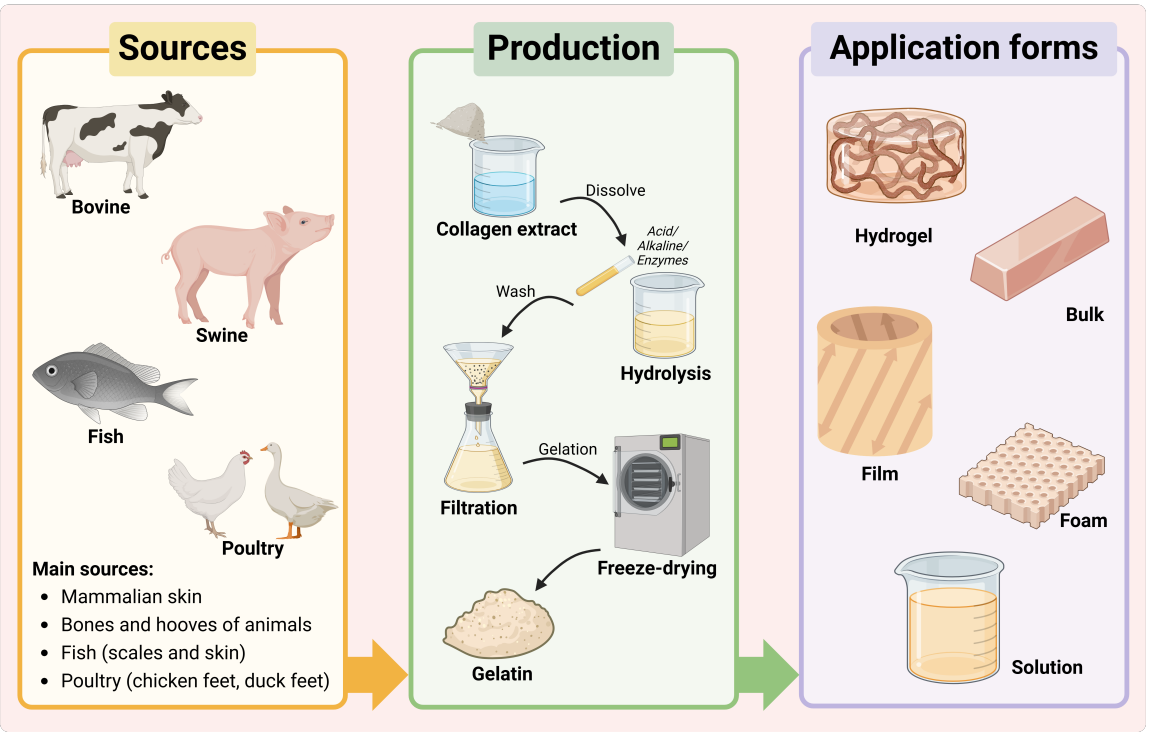

Figure 3. Schematic of the production of gelatin, including sources, production, and application forms.

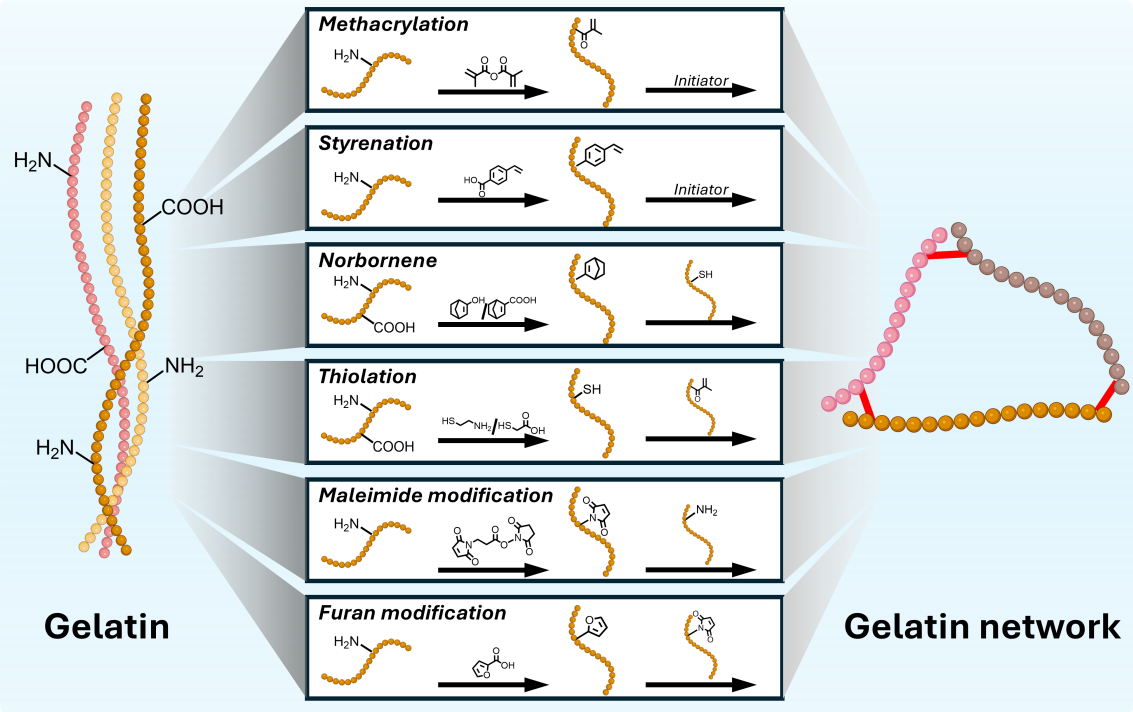

Figure 4. Common chemical modification strategies of gelatin for forming gelatin networks.

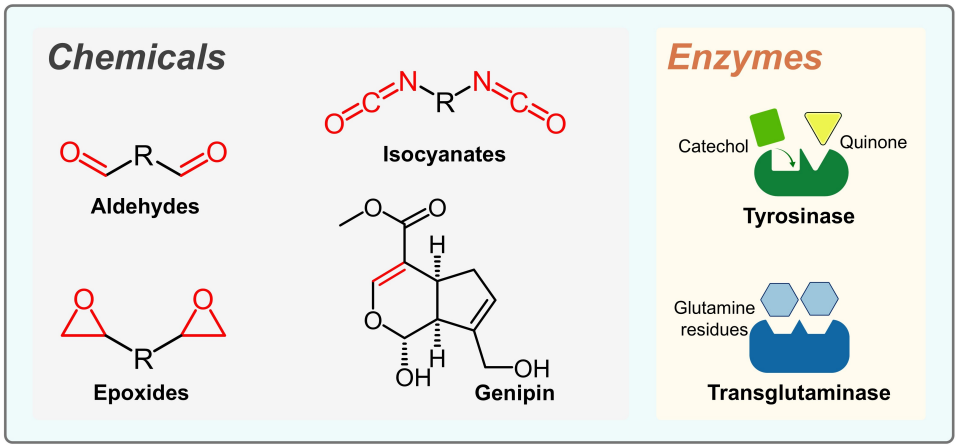

Figure 5. Common crosslinkers for gelatin without prior modification.

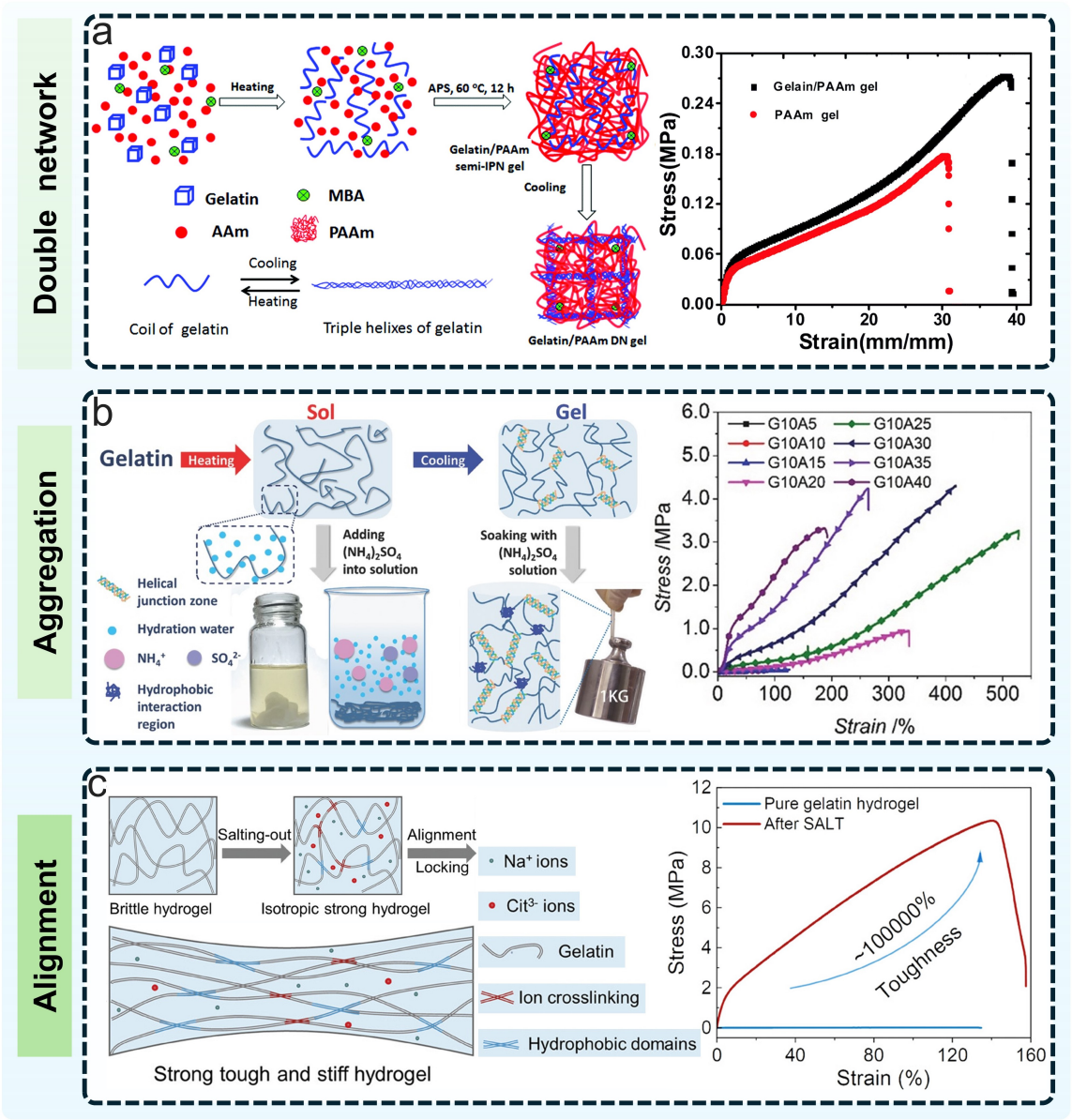

Figure 6. Network design strategies for tough gelatin hydrogels include double networks, aggregation, and alignment. a) Synthesis of gelatin/polyacrylamide double-network hydrogels. Right: tensile stress-strain curves comparing double-network and single-network hydrogels. Reproduced with permission.[127] Copyright 2017, Royal Society of Chemistry. b) Strengthening gelatin hydrogels by soaking in the $(NH_4)_2SO_4$ solution. Right: tensile stress-strain curves for gelatin hydrogels (10 wt%) treated with varying concentrations of $(NH_4)_2SO_4$. Reproduced with permission.[135] Copyright 2017, John Wiley and Sons. c) Gelatin hydrogel processed via "salting out-alignment-locking". Right: tensile stress-strain curves of gelatin hydrogels, with and without processing. Reproduced with permission.[139] Copyright 2024, John Wiley and Sons.

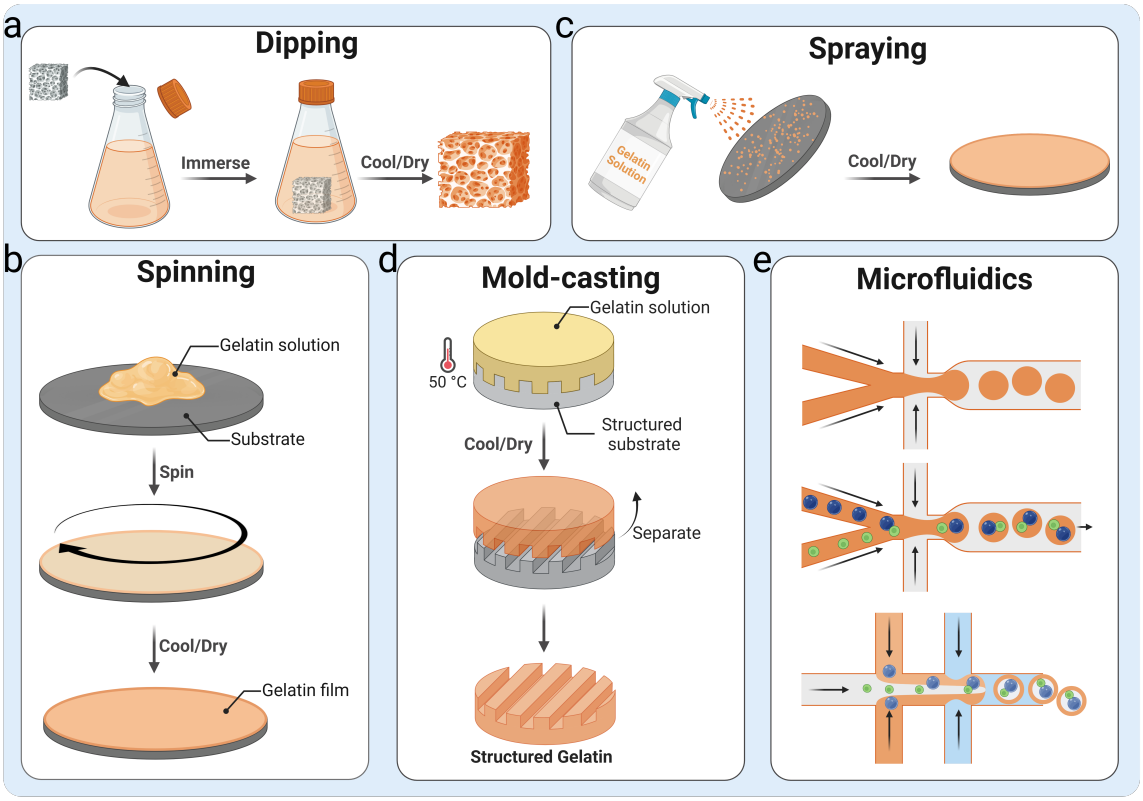

Figure 7. Various fabrication techniques for gelatin, including (a) dipping, (b) spinning, (c) spraying, (d) mold-casting, and (e) microfluidics.

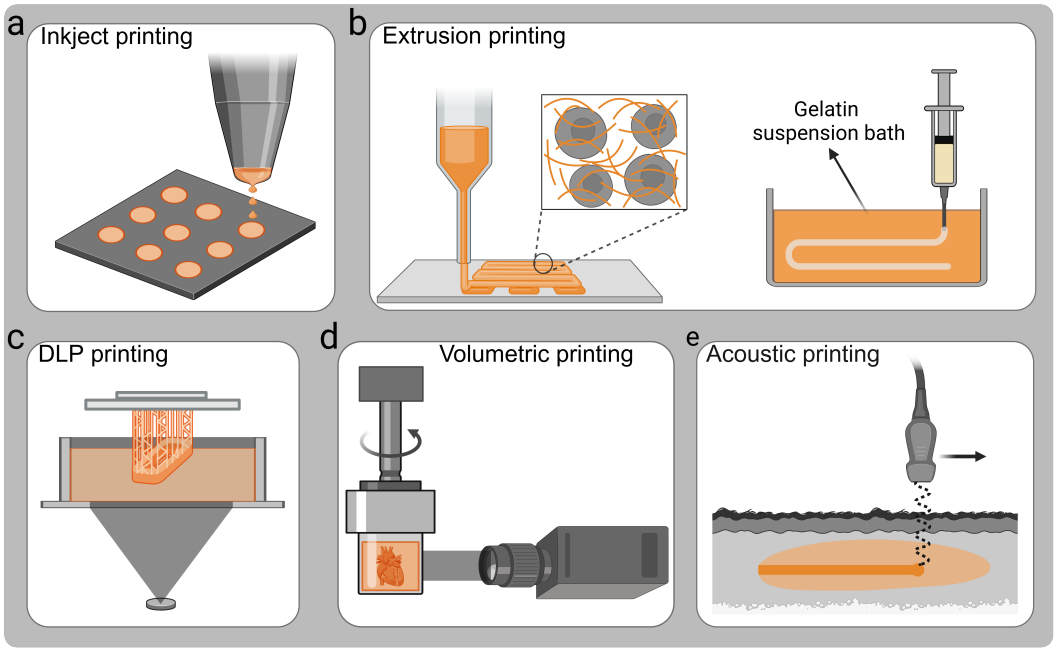

Figure 8. Schematics of key 3D (bio)printing techniques for fabrication with gelatin and its derivatives, including (a) inkjet, (b) extrusion, (c) DLP, (d) volumetric, and (e) acoustic (bio)printing.

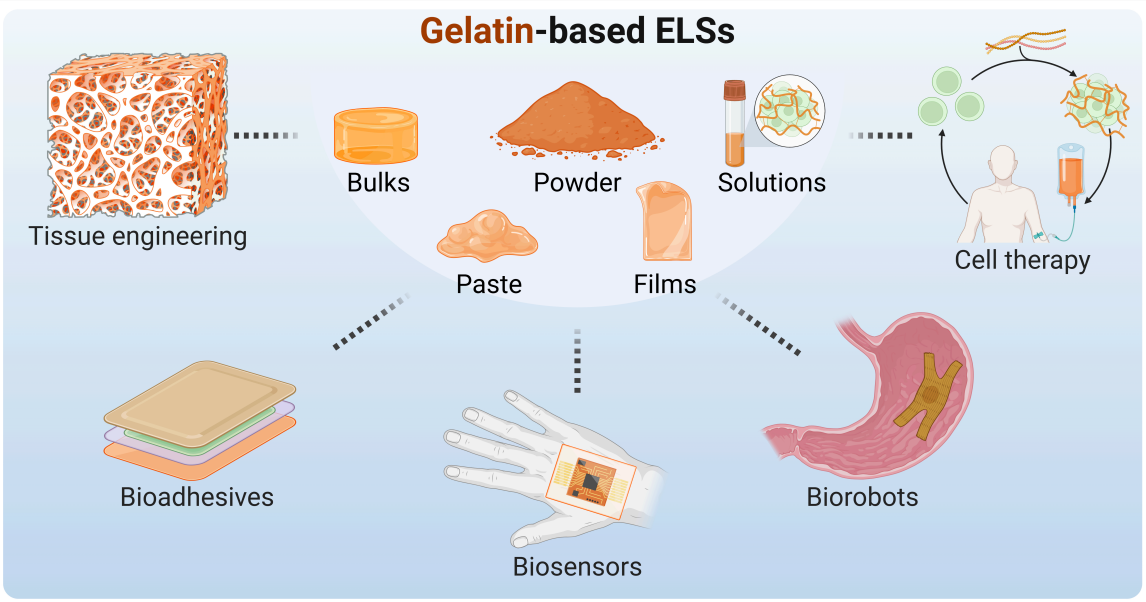

Figure 9. Various gelatin-based ELSs, including tissue engineering, cell therapy, bioadhesives, biorobots, and biosensors.

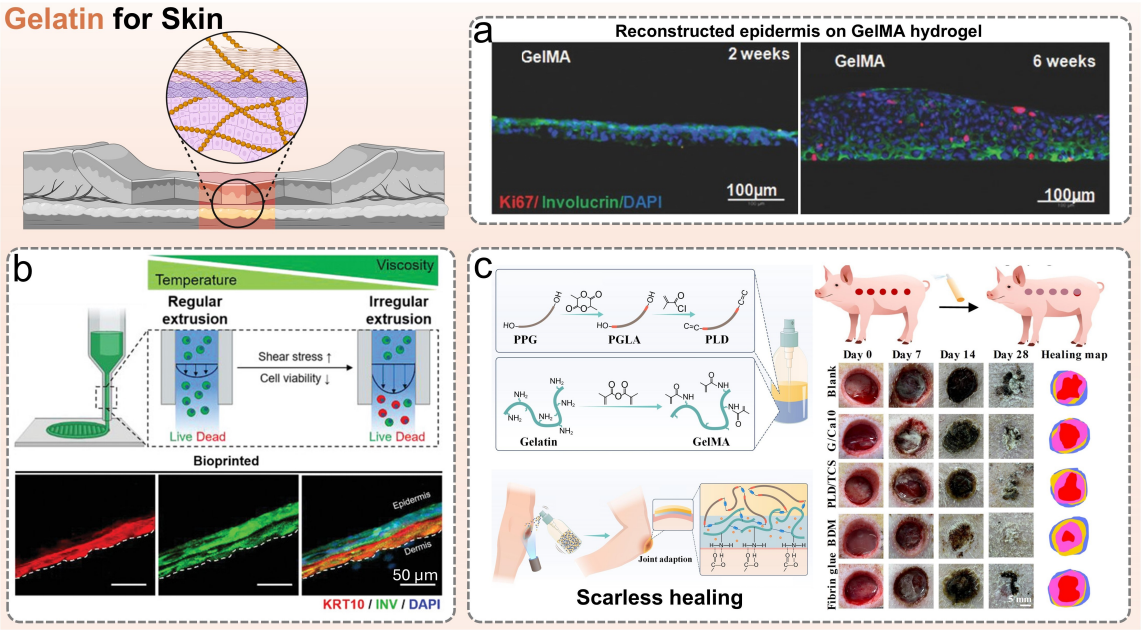

Figure 10. Gelatin for skin tissue engineering. a) Biomarker expressions of reconstructed epidermis on GelMA hydrogel scaffolds. Reproduced with permission.[267] Copyright 2016, John Wiley and Sons. b) The schematic illustrates how extrusion bioprinting impacts cell viability due to high shear stress during nozzle passage. Bottom: immunofluorescence staining of early (green) and late (red) epidermal differentiation markers, with the dashed white line indicating the dermis-epidermis boundary. Reproduced with permission.[335] Copyright 2023, John Wiley and Sons. c) Schematic illustration depicting the construction of a *Staphylococcus aureus*-infected full-thickness porcine skin wound model and representative wound images following treatment with different wound masks, utilizing sprayable GelMA-based ink for scarless healing. Reproduced with permission.[336] Copyright 2023, The American Association for the Advancement of Science.

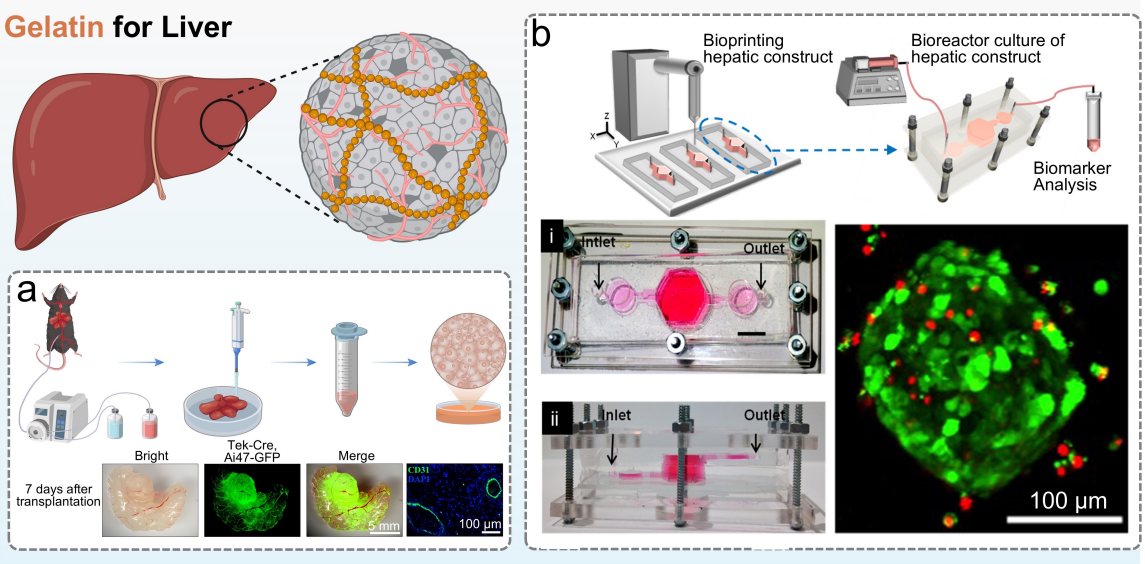

Figure 119. Gelatin for liver tissue engineering. a) Revitalizing liver functions in mice with liver failure through the transplantation of 3D-bioprinted gelatin-based livers. Vascularization was observed in the mesentery of mice at 7 days after transplantation, where immunofluorescence staining of CD31 in 3D-bioprinted livers at 7 days post-transplantation in mice. Reproduced with permission.[340] Copyright 2024, The American Association for the Advancement of Science. b) Schematic of the hepatic bioreactor platform with bioprinted liver microtissues, where the photographs show top and side views of the bioreactor. Bottom right: Live/Dead staining of spheroids after 5 days of culture. Reproduced with permission.[343] Copyright 2016, IOP Publishing.

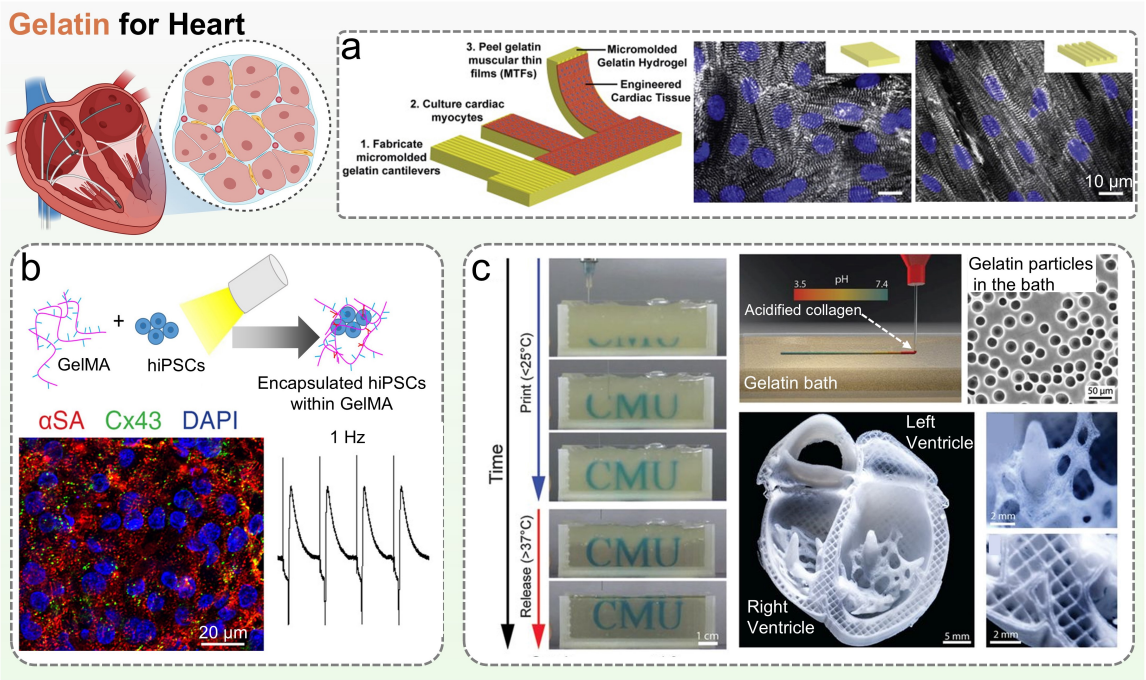

Figure 1210. Gelatin for cardiac tissue engineering. a) Schematic showing films made from cardiac tissues cultured on micro-molded gelatin hydrogel cantilevers. Right: neonatal rat ventricular myocytes formed isotropic or anisotropic monolayers after 4 days of culture on flat or micro-molded gelatin hydrogels. White: α-actinin, blue: nuclei.[351] Copyright 2014, Elsevier Ltd. b) GelMA-based direct fabrication of human cardiac tissues using iPSCs encapsulation, showing spontaneous contractions. Reproduced with permission.[352] Copyright 2016, American Chemical Society. c) Utilizing gelatin as a support bath for 3D bioprinting of collagen to reconstruct human heart components. Left: time-lapse sequence of printing the letters "CMU" using a gelatin support bath. Middle: acidified collagen rapidly gelled into filaments in the pH-7.4 gelatin support bath. Top right: image of gelatin microparticles in the bath. Bottom right: cross-sectional views of the heart printed with gelatin support bath showing the left and right ventricles and their internal structures (high-fidelity images of left ventricular trabeculae and the septal wall). Reproduced with permission.[261] Copyright 2019, The American Association for the Advancement of Science.

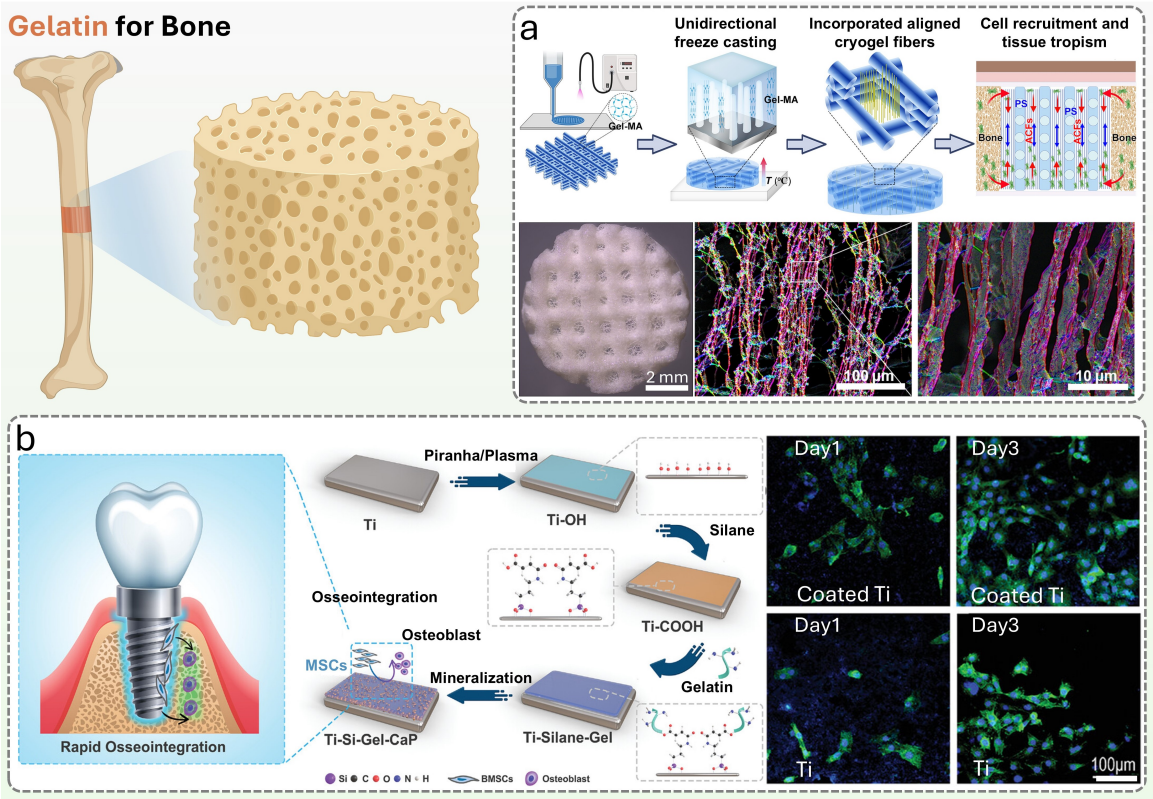

Figure 13. Gelatin for bone tissue engineering. a) 3D-printed scaffolds with aligned gelatin fibers boost bone regeneration by enhancing cell recruitment and functions (top). Gross view of printed scaffolds after lyophilization (bottom left), with false-color images showing angle mapping of fiber orientations (bottom right). Reproduced with permission.[357] Copyright 2024, The American Association for the Advancement of Science. b) Gelatin coating on titanium implants for bone regeneration. Right: immunofluorescence images of MC3T3 cells cultured on coated and uncoated Ti after 1 and 3 days. Reproduced with permission.[369] Copyright 2023, John Wiley and Sons.

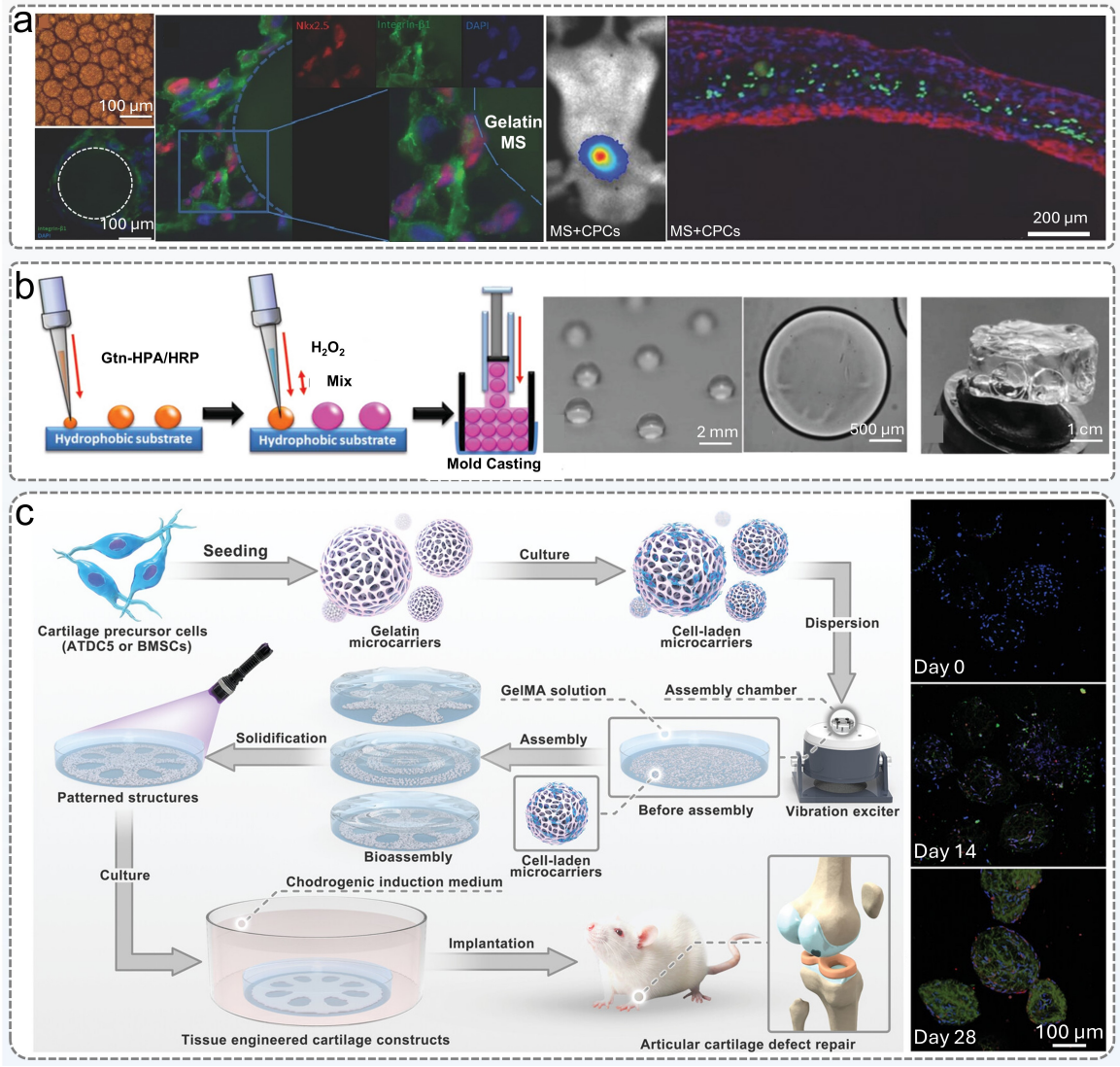

Figure 14. Gelatin for cell therapy. a) Gelatin microspheres as carriers for delivering cardiac progenitor cells to the myocardium. Middle: cryosections showed cells on the microsphere surface, expressing early cardiac markers. Right: histology at 1 month showing improved long-term cell engraftment in mice receiving cell-loaded gelatin microspheres. Reproduced with permission.[388] Copyright 2016, John Wiley and Sons. b) Injectable biphasic bead-reinforced gelatin microspheres allowing mesenchymal stem cells, infiltration for musculoskeletal soft tissue repair. Reproduced with permission.[389] Copyright 2021, John Wiley and Sons. c) Gelatin microcarriers assembled via faraday wave for the tissue-engineered cartilage constructs. Right: immunofluorescence images show cell-laden gelatin microcarriers after 0, 14, and 28 days of chondrogenic differentiation. Reproduced with permission.[390] Copyright 2024, John Wiley and Sons.

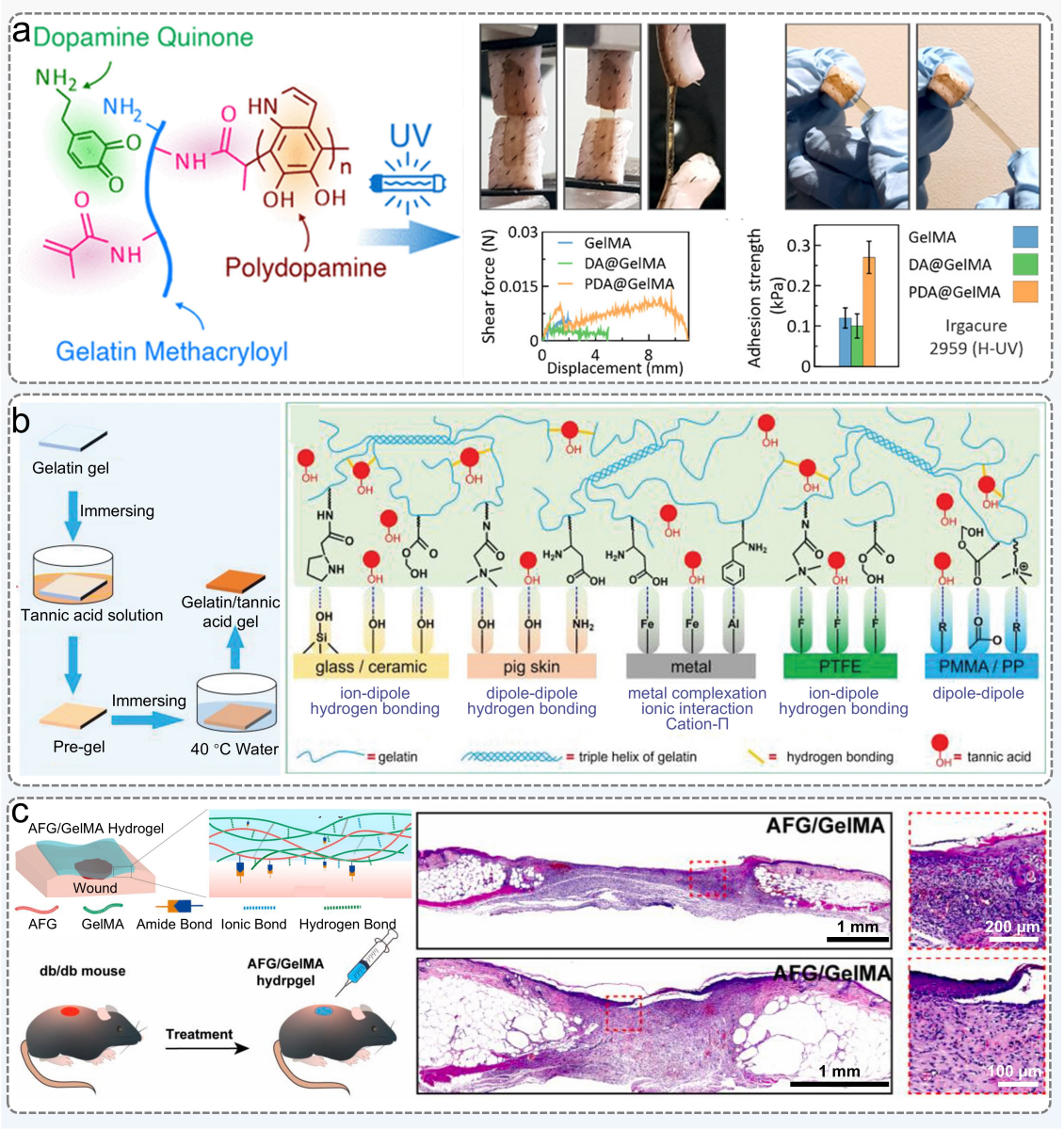

Figure 15. Gelatin for bioadhesives. a) GelMA-based hydrogels with *in-situ* dopamine polymerization as stretchable adhesives. Reproduced with permission.[398] Copyright 2021, American Chemical Society. b) Gelatin-tannic acid hydrogel prepared from a stepwise immersion method for underwater adhesion. Right: the bonding between hydrogel and various substrates included ion-dipole, dipole-dipole, and metal complexation. Reproduced with permission.[399] Copyright 2024, John Wiley and Sons. c) GelMA/glycosaminoglycan adhesives for diabetic wound healing. Right: hematoxylin and eosin staining images show the effects of GelMA adhesives on mouse skin wound healing at postoperative days 6 and 14. Reproduced with permission.[400] Copyright 2023, Elsevier Ltd.

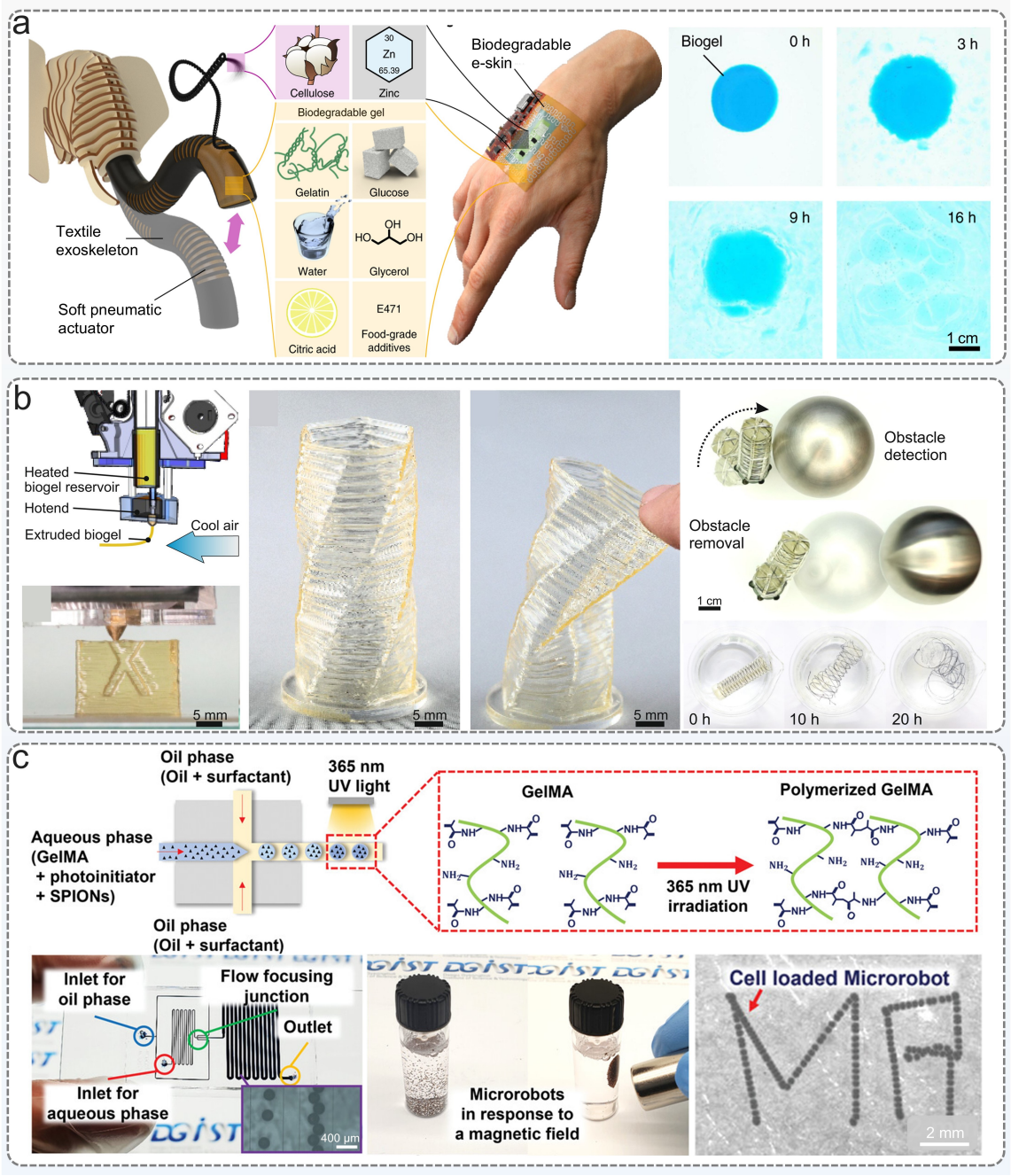

Figure 16. Gelatin for biorobots. a) Degradable gelatin-based soft robots and electronics with entire degradability. Reproduced with permission.[404] Copyright 2024, Springer Nature Limited. b) Omnidirectional and exteroceptive soft actuators based on gelatin fabricated by 3D printing. The 3D-printed actuators could be degraded in 20 h. Reproduced with permission.[405] Copyright 2022, American Association for the Advancement of Science. c) GelMA-based biodegradable magnetic microrobots for precise delivery. Reproduced with permission.[409] Copyright 2022, John Wiley and Sons.

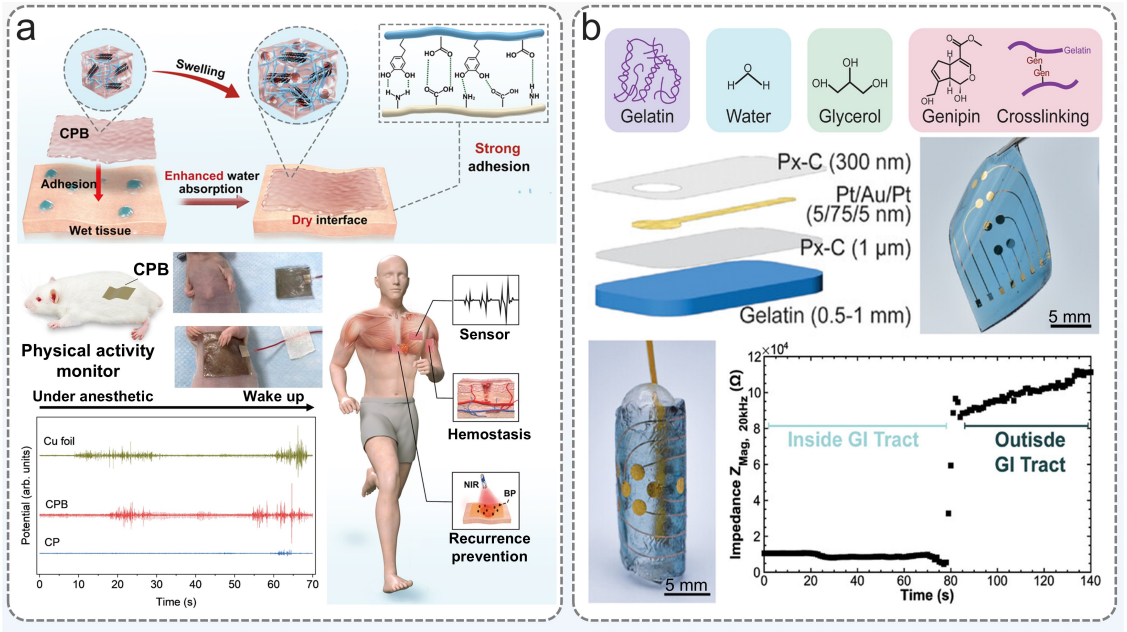

Figure 17. Gelatin for biosensors. a) Gelatin/black phosphorus sensors for physical activity monitoring on a nude mouse with enhanced adhesion. Reproduced with permission.[420] Copyright 2024, Springer Nature Limited. b) Gelatin-based edible biosensor for monitoring epithelial barrier functions via electrochemical impedance measurements. Reproduced with permission.[423] Copyright 2024, John Wiley and Sons.